GENOTYPE-PHENOTYPE CORRELATION IN PARKINSONIAN CONDITIONS

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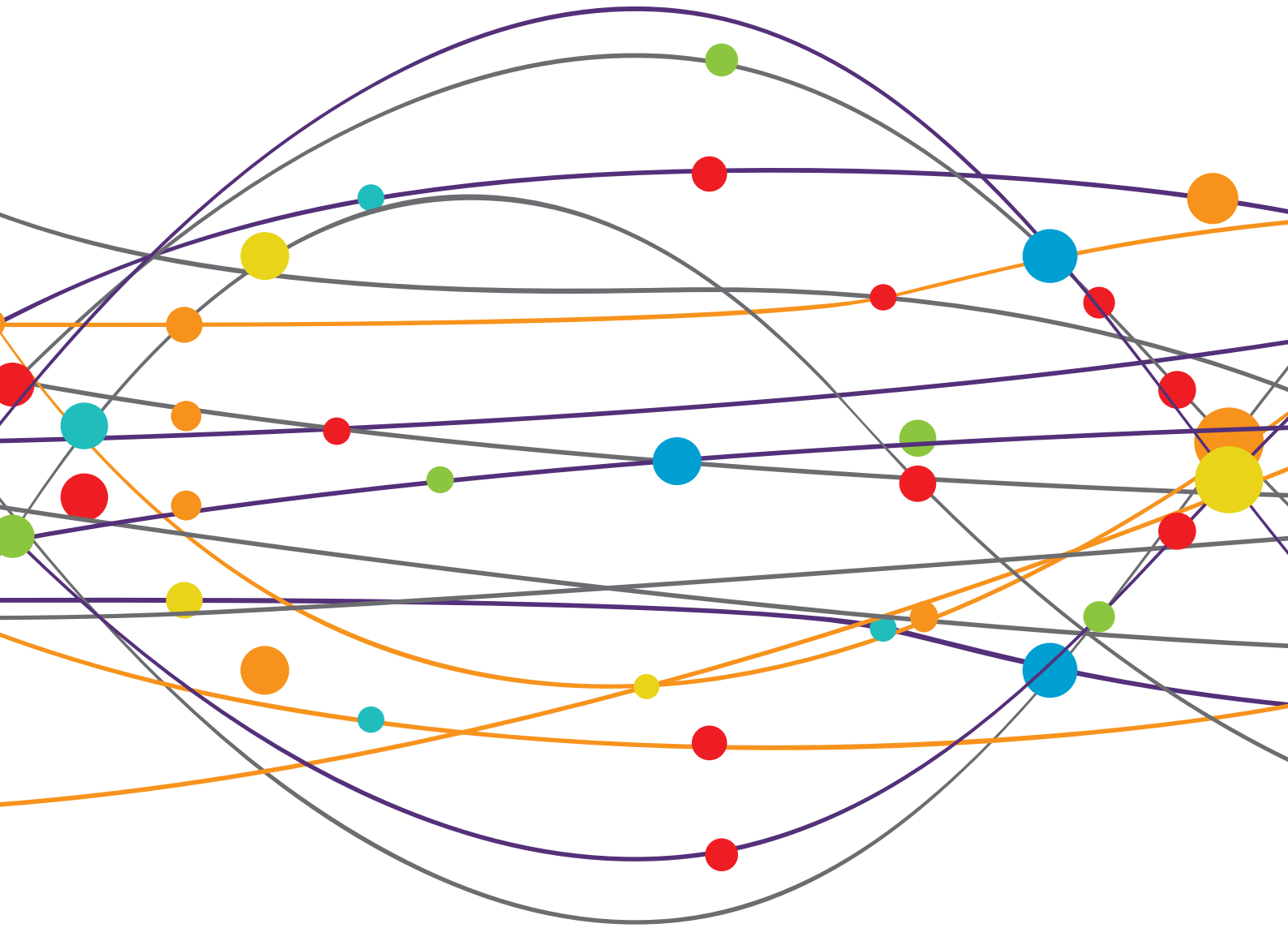

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GENOTYPE-PHENOTYPE CORRELATION IN PARKINSONIAN CONDITIONS

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EDITORIAL
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Editorial: Genotype-Phenotype Correlation in Parkinsonian Conditions

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Editorial on the Research Topic

Genotype-Phenotype Correlation in Parkinsonian Conditions

With the diffusion of cost-effective genetic analyses, an increase in the spectrum of reported genetic variants associated with sporadic Parkinson's disease (sPD) (e.g., glucocerebrosidase—*GBA*) and monogenic parkinsonisms (dominant, recessive, and atypical forms) has been achieved. Each single variant may be associated to distinct prominent phenotypic characteristics helpful for diagnostic and prognostic purposes, thus ushering the era of precision medicine for movement disorders (1). This special issue was designed to explore the genotype-phenotype correlation of parkinsonian conditions extensively. Of the 18 papers initially submitted to the journal by international researchers, 14 were considered suitable for publication after a thorough peer-review process. These included three original research articles, five reviews, five brief research reports, and one mini-review. The following is a short summary of the main results of each of these manuscripts.

Guadagnolo et al., provide a detailed review of genotype-phenotype correlation in monogenic parkinsonisms. Their clinical presentations may range from cases indistinguishable from sPD to very early-onset (childhood) conditions with several associated neurological features. Despite the broad clinical spectrum and the multiple genes involved, the phenotype of these conditions is strictly related to the altered cell function and inheritance pattern. Genotype-phenotype studies in genetic parkinsonisms may help in the earlier identification and in the development of disease-modifying treatments based on precision medicine strategies. Menozzi and Schapira, in their mini-review, focus on the genotype-phenotype correlation of *GBA*, one of the most important known risk factors for sPD (2). *GBA*-PD patients show more severe motor and non-motor symptoms, rapid disease progression, and reduced survival than non-carriers. However, the impact of *GBA* variants on clinical phenotype may significantly vary. Homozygous, compound heterozygous, and the "complex" (recombinant) and "severe" heterozygous variants' carriers display aggressive phenotypes with faster disease progression. Differently, the so-called "mild" and "risk" (not causative) variants have slower and more benign disease courses. The stratification of *GBA* carriers in the prodromal and manifest phase of PD is fundamental for patients' counseling, prognosis, and a better understanding of the possible efficacy of advanced treatments.

The disease spectrum of genetic parkinsonian conditions is constantly expanding. Salles et al., reviewed the full spectrum of clinical manifestations of *ATP1A3* mutations. Pathogenic variants in this gene have been implicated in several phenotypes: (a) rapid-onset dystonia-parkinsonism; (b) alternating hemiplegia of childhood; and (c) cerebellar ataxia, *pes cavus*, optic atrophy, and sensorineural hearing loss (CAPOS syndrome). Since the original descriptions of these

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disease entities, a growing number of cases showing overlapping features have been reported. We can consider *ATP1A3*-related disorders as a clinical continuum rather than distinct entities, with clinical features ranging from early-life epilepsy (in the most severe extreme side of the spectrum) to rapid-onset dystonia-parkinsonism (in the milder side of the spectrum). Lesage et al., investigate the clinical variability of *SYNJ1*-associated early-onset parkinsonism. They describe two cases carrying previously unreported biallelic mutations of *SYNJ1* and two siblings with the recurrent homozygous p.R258Q mutation. The patients studied show different clinical symptoms ranging from epilepsy, intellectual disability, and progressive parkinsonism (biallelic mutations) to a parkinsonian syndrome with no atypical features and slow disease progression (p.Y832C mutation).

The most frequent forms of autosomal dominant monogenic parkinsonism are those related to mutations in the *LRRK2* gene (3). Vinagre-Aragón et al. in their review, explored the role of the *LRRK2*-R1441G variant in the Basque population. These cases appear to be associated with a homogeneous, less aggressive, "pure" motor phenotype, which may resemble sPD. Genetics constitute a relevant aspect, as it may significantly influence the phenotype, with differences according to the mutation within the same gene, not only in familial but also in sPD. Thus, expanding our understanding of genetic parkinsonisms implies an extension of knowledge regarding sPD, which may be relevant for future therapeutic implications of all parkinsonisms. Leija-Salazar et al., in their original research article investigate single nucleotide variants (SNVs) in brains from patients with sporadic synucleinopathies and a monozygotic twin carrying the *LRRK2*-G2019S variant. Somatic SNVs in coding regions of genes associated with synucleinopathies could contribute to these disorders, according to the number of affected cells and mechanisms of diffusion of the causal agent (4). The authors did not detect disease-relevant somatic SNVs, although their presence at the initial stages of neurodegeneration is postulated. These results suggest that, while coding somatic SNVs in neurodegeneration are rare, other somatic variants may have a pathogenic role in synucleinopathies.

Genome-wide association studies (GWAS) have suggested the possible role of several genetic variants as risk factors for the development of sPD (4–6). Szwedo et al., explore the impact of *SNCA* polymorphisms on a large longitudinal population-based cohort of sPD patients. Their results show that the variant rs356219 has a minor effect on modifying disease progression, whereas no differences were associated with other variants. These results imply that *SNCA* variants associated with sPD risk are not central factors responsible for the clinical heterogeneity of sPD. Tunold et al., describe a significant effect of *APOE* and *MAPT* genetic variants on dementia in pathologically confirmed sPD. These results support the critical role of *APOE*-ε4 and *MAPT*-H1 haplotypes in the etiology of sPD-related dementia, with potential relevance for patient selection for future clinical trials of disease-modifying therapies. Moran et al., explore the motor, cognitive, psychiatric, and olfactory functioning between carriers and non-carriers of *GBA* variants (both groups without PD). *GBA* mutation carriers show reduced performance on executive functioning, and carriers with hyposmia have lower cognition scores than those without hyposmia. Hence, pre-manifest features of PD may not be found in *GBA* mutation carriers; however, a difference in executive functioning among some non-manifesting *GBA* mutation carriers is present. These findings, combined with hyposmia, should be further investigated as possible biomarkers for pre-manifest and pre-clinical *GBA*-related sPD. Markopoulou et al., analyze single nucleotide polymorphisms (SNPs) identified in previous GWAS studies, together with low frequency and rare variants at parkinsonism-associated genes from the MDSgene database, in sPD patients. They suggest that genetic risk factors for sPD may variably affect the phenotypic presentation and that genes associated with sPD risk are also differentially associated with individual phenotype at baseline.

Torrealba Acosta et al., and Milanowski et al., investigated monogenic parkinsonism-associated variants in some underrepresented populations. Torrealba Acosta et al., studied the genetic and clinical underpinnings of PD patients of Costa Rican origin. Although they do not identify a direct relationship between the genes tested and PD, they find six rare *LRRK2* variants located in the C-terminal-of-ROC (COR) domain, non-synonymous *GBA* variants (p.T369M, p.N370S, p.L444P) in three healthy individuals and one PD patient carrying a pathogenic *GCH1* variant (p.K224R). They also show that physical activity and education are correlated with better motor and cognitive outcomes, respectively, while hallucinations, falls, mood disorders, and coffee consumption correlate with worse cognitive performance. This is the first study clinically and genetically characterizing a cohort of Costa Rican PD patients, thus expanding the genomic research in the Latino population. Milanowski et al., screen 109 patients with PD from Nigeria finding 22 variants [*PRKN*, 9 (40.9%); *PINK1*, 10 (45.5%); and *DJ-1*, 3 (13.6%)]. They identify three (13.6%) rare, non-synonymous variants, without finding any homozygous/compound heterozygous carriers. This study underlines how, although more studies are needed in sub-Saharan African countries, population-specific variation may contribute to a better knowledge of the genes involved in PD in the local population and also to the interpretation of results from larger studies performed in European or Asian populations.

Finally, several lines of evidence have recently suggested a possible role of inflammation and lysosomal activity on the pathogenesis of parkinsonian conditions (7–9). Magnusen et al., review data from genetics, immunology, and *in vivo* and *ex vivo* functional studies showing that genetic defects associated with monogenic parkinsonisms might contribute to microglial cell activation and generation of pro-inflammatory cytokines and chemokines, responsible for neurodegeneration. Understanding the involvement of various immune mediators, their source, and the target could provide a better knowledge of PD pathogenesis for diagnostic and prognostic purposes. Abe and Kuwahara, review the roles of parkinsonian gene products implicated in the lysosomal pathway, namely *LRRK2*, *VPS35*, *ATP13A2*, and *GBA*, providing an overview of their disease-associated functions and their cooperative actions

in the pathogenesis of sPD, based on the evidence from cellular and animal models. This study suggests that targeting lysosomal activation could represent a possible strategy to treat neurodegeneration.

In conclusion, the editors wish to thank all the authors, the reviewers, and the editorial board members for contributing to this special issue. We hope that this special issue might inspire future and novel research approaches in the genetics of parkinsonian conditions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Bandres-Ciga S, Diez-Fairen M, Kim JJ, Singleton AB. Genetics of Parkinson's disease: an introspection of its journey towards precision medicine. *Neurobiol Dis*. (2020) 137:104782. doi: 10.1016/j.nbd.2020.104782
2. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* (2009) 361:1651–61. doi: 10.1056/NEJMoa0901281
3. Bekris LM, Mata IF, Zabetian CP. The genetics of Parkinson disease. *J Geriatr Psychiatry Neurol.* (2010) 23:228–42. doi: 10.1177/0891988710383572
4. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genetics.* (2009) 41:1303–7. doi: 10.1038/ng.485
5. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci USA*. (2005) 102:16842–7. doi: 10.1073/pnas.0507360102
6. Leija-Salazar M, Piette CL, Proukakis C. Somatic mutations in neurodegeneration. *Neuropathol Appl Neurobiol.* (2018) 44:267–85. doi: 10.1111/nan.12465
7. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
8. Caggiu E, Arru G, Hosseini S, Niegowska M, Sechi G, Zarbo IR, et al. Inflammation, infectious triggers, and Parkinson's disease. *Front Neurol.* (2019) 10:122. doi: 10.3389/fneur.2019.00122
9. Nguyen M, Wong YC, Ysselstein D, Severino A, Krainc D. Synaptic, mitochondrial, and lysosomal dysfunction in Parkinson's disease. *Trends Neurosci.* (2019) 42:140–9. doi: 10.1016/j.tins.2018.11.001

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ORIGINAL RESEARCH
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Investigation of Somatic Mutations in Human Brains Targeting Genes Associated With Parkinson's Disease

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Background: Somatic single nucleotide variant (SNV) mutations occur in neurons but their role in synucleinopathies is unknown.

Aim: We aimed to identify disease-relevant low-level somatic SNVs in brains from sporadic patients with synucleinopathies and a monozygotic twin carrying *LRRK2* G2019S, whose penetrance could be explained by somatic variation.

Methods and Results: We included different brain regions from 26 Parkinson's disease (PD), one Incidental Lewy body, three multiple system atrophy cases, and 12 controls. The whole SNCA locus and exons of other genes associated with PD and neurodegeneration were deeply sequenced using molecular barcodes to improve accuracy. We selected 21 variants at 0.33–5% allele frequencies for validation using accurate methods for somatic variant detection.

Conclusions: We could not detect disease-relevant somatic SNVs, however we cannot exclude their presence at earlier stages of degeneration. Our results support that coding somatic SNVs in neurodegeneration are rare, but other types of somatic variants may hold pathological consequences in synucleinopathies.

Keywords: SNCA, synuclein, Parkinson's disease, somatic mutation, targeted sequencing, synucleinopathies, molecular barcodes

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INTRODUCTION

Synucleinopathies are disorders characterized by the pathological aggregation of α-synuclein (1). Among synucleinopathies, Parkinson's disease (PD) is the commonest disorder and is characterized predominantly by neurodegeneration of dopaminergic neurons in substantia nigra (SN) (2, 3). Somatic variation occurs in human brain and its role in neurodegeneration has started to be explored (4). Current estimations of the occurrence of somatic variants in human brains suggest that single nucleotide variants (SNVs, or "point mutations") could be the most prevalent form (5, 6). Somatic SNVs are reported to increase with age, where large genes or transcriptionally active genomic regions appear to be susceptible (7). Somatic SNVs in coding regions of genes associated with synucleinopathies could contribute directly to these disorders, depending on the amount of affected cells and mechanisms of spread of the aetiological agent [see review (8)]. The study of somatic SNVs has been facilitated by the latest technological improvements. Compared to single-cell studies, bulk-sequencing offers a cost-effective strategy to study somatic variation across

tissues and brain regions of multiple individuals. The error rate of bulk-sequencing at low allele frequencies (AF) can be reduced by using molecular barcodes (9). In this study, we used targeted sequencing in PD-associated genes from post-mortem human brains aimed for the detection of pathogenic somatic SNVs.

METHODS

Samples were obtained from the Parkinson's UK and Queen Square brain banks. Patients gave informed consent and the study was approved by the local ethics committee. We evaluated 66 samples from multiple brain regions and three matched-blood samples, derived from 42 individuals with the following conditions: 26 PD, 12 control, three Multiple system atrophy (MSA), and one Incidental Lewy Body case (**Supplementary Table 1**). PD cases were sporadic, except for case 18, a manifesting *LRRK2* G2019S carrier, whose identical twin was non-penetrant (10) and somatic variation was suggested as an explanation for the discordance in the development of PD (11). We did not include other monogenic cases, as we did not have access to their brain tissue. The mean and standard deviation for onset age was 62.0 ± 11.1 years, and for disease duration 10.1 ± 7.0 years. This calculation excludes case 18, whereas these were not available.

We used a previously reported protocol for genomic DNA extraction (12) and the Haloplex^{HS} method to prepare sequencing libraries. Details about the generation of artificial mosaics, the sequencing panel design, the customization of library preparation and bioinformatic analysis are provided in **Supplementary Table 2** and **Supplementary Figure 1**.

For amplicon sequencing, primers were designed with Primer3Plus (13) to generate amplicons larger than 300 bp, targeting the variants of interest at >50 bp away from the primer annealing sites. Amplicons belonging to the same sample were pooled together before Nextera XT library preparation, following manufacturer instructions. Samples were pooled equimolarly before sequencing using a MiSeq v3 kit (600 cycles). The bioinformatic analysis is described in **Supplementary Figure 2**. Droplet digital PCR (ddPCR) assays were designed using Primer3plus, according to manufacturer. Bulk DNA from putamen, occipital, frontal cortex, and cingulate gyrus was used for this analysis. The ddPCR conditions are described in **Supplementary Table 3**. Data analysis was performed in QuantaSoft Pro v1.0 following Bio-Rad guidelines.

RESULTS

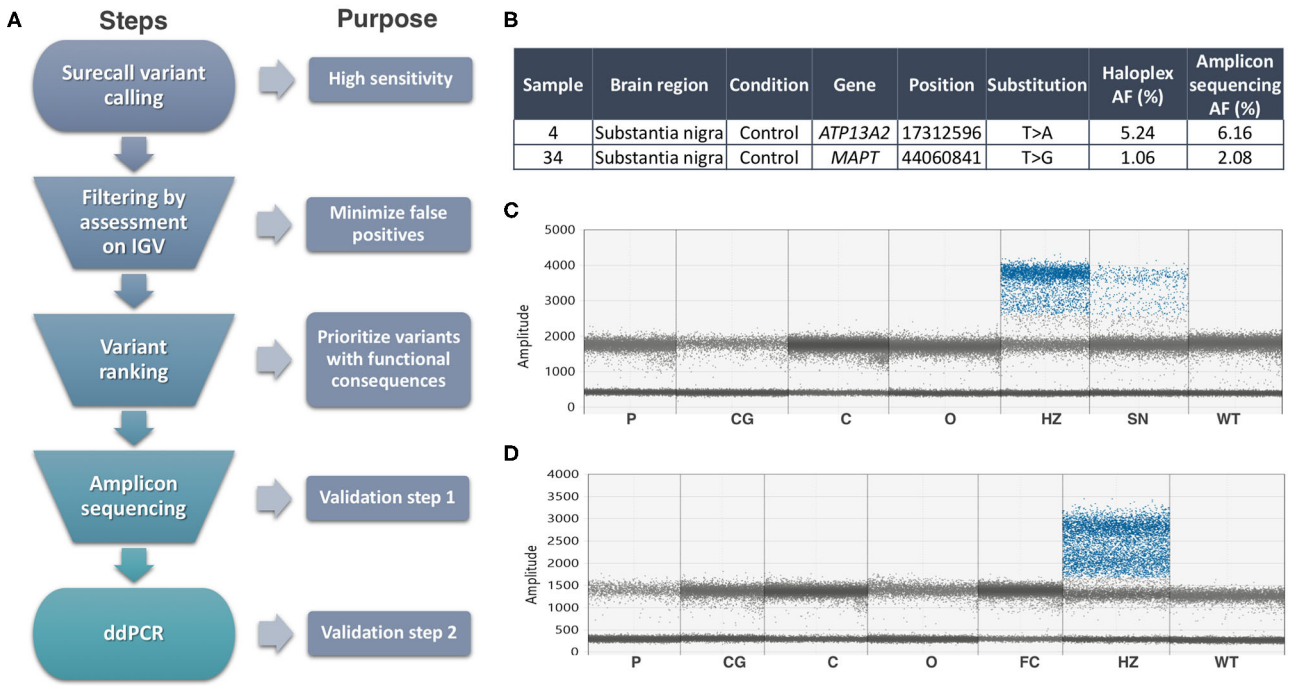
Validation of the Methodology

"Artificial mosaics" were used to estimate the variant detection limit, sensitivity and false positive and negative rates. We were expecting 37 variants to be present within regions covered in artificial mosaics. We detected 95% of these variants at 1% AF and 87% at 0.5% AF (supplementary results).

We aimed to reduce to a minimum false positives at lower AF levels. We firstly counted 'Potential false positives' (PFP) in artificial mosaics at different AF thresholds. PFP comprised SNVs not recorded as expected mosaic variants, nor reported in dbSNP (14). We observed 1.2× more PFP when the minimum AF was lowered from 1% to 0.5% (**Supplementary Figure 3**). Surecall showed greater sensitivity when compared to other variant callers (**Supplementary Figure 3**). To increase the specificity of our variant calling analysis, we filtered false positives visually, using fixed criteria to discard errors (**Supplementary Figure 4**). Surecall variants in mosaic 0.25% (at AF = 0.25–5%) were analyzed on IGV. From the 114 variants analyzed, visual analysis could not discard 4 false positives. The highest AF was reported as 0.32%, therefore we set our detection limit at 0.33%. This filter allowed us to discard numerous false positives, but also increased the false negative rate. In the artificial mosaic sample carrying variants at 0.5%, Surecall detected 78% of the expected variants. After visual inspections, 46% of the expected variants remained, and false positives were completely discarded. The most common reason to filter real variants was their presence in only one paired-read orientation (strand-bias; **Supplementary Figure 4B**).

Sample Analysis

We focused on the substantia nigra, and sequenced DNA from 42 samples (including 12 controls). Where available, we also analyzed DNA from other sources from the same individuals (**Supplementary Table 1**): frontal cortex (13, including two controls), cerebellum (11, including 1 control), and blood from three. An explanation of our analysis is summarized in **Figure 1A**. On the Haloplex^{HS} step, all samples were sequenced at an average 2,541×. We focused on the detection of coding SNVs not reported before as common SNPs (population frequencies < 1%), to reduce the risk of calling low-level variants arising due to contamination. Thirty-one variants in 23 samples passed the filtering step, but most of the variants detected (24 out of 31) had an average AF of 0.45%, close to the detection limit of our analysis. Twenty-one variants in 18 samples were prioritized for validation, based on a ranking scale to select variants with a predictable role in disease (**Supplementary Table 4**). We generated amplicons to target the prioritized variants and sequenced those at even higher coverage (mean= 14,883×). To account for possible sequencing errors at the genomic positions of interest, we compared the amplicons from the interrogated sample with amplicons from controls (a commercial reference DNA and six samples showing a candidate variant in other parts of the genome). Two variants in samples 4SN and 34SN were validated, as these were detected at AFs close to the original analysis, and significantly different from the sequencing errors in controls (**Figure 1B**). The variants were further confirmed by Mutect2 paired-analysis, using the reference DNA as a normal sample. However, these variants corresponded to rare heterozygous SNPs present in samples from our study. SN tissue was not available for further validation, but the AF at which the variants were detected was an indicative that the variants might be present in other brain regions when real (15). ddPCR did not reveal the variants in the brain regions tested (**Figures 1C,D**). In one of the assays, the presumably contaminated DNA was still available and the variant was confirmed only in this sample (**Figure 1C**). To further examine cross-contamination, we recorded all mosaic variants from Surecall in 4SN, 34SN and control 1 (a sample used for demonstration purposes) at



Sample	Brain region	Condition	Gene	Position	Substitution	Haloplex AF (%)	Amplicon sequencing AF (%)
4	Substantia nigra	Control	*ATP13A2*	17312596	T>A	5.24	6.16
34	Substantia nigra	Control	*MAPT*	44060841	T>G	1.06	2.08

FIGURE 1 | Summary of methods and results. **(A)** Somatic variant calling workflow explained step by step. **(B)** Validated variants by Amplicon sequencing (AF, allele frequency). **(C)** ddPCR assay for the *ATP13A2* variant did not reveal its presence in additional brain regions of sample 4, nor in control DNA (WT). Sample 32SN (presumable contaminant) showed the variant at heterozygous levels (HZ). The presumably contaminated sample 4SN used in Haloplex^{HS} and Amplicon sequencing assays showed a mutant signal at AF ∼6%. **(D)** ddPCR assay for the *MAPT* variant did not reveal its presence in additional brain regions of sample 34, nor in control DNA (WT). Sample 22SN (presumable contaminant) showed the variant at heterozygous levels (HZ). Codes for brain regions tested: SN, substantia nigra; P, putamen; CG, cingulate gyrus; C, cerebellum; O, occipital.

AF similar to the variants of interest. The mosaic variants were compared to germline variants from samples where the contamination was suspected to come from (in the case of control one, a non-related sample or control two). While control one showed fewer mosaic variants, not matched with control two germline variants, the presumably contaminated samples showed numerous mosaic variants matched with germline variants from samples where the contamination came from ($p < 0.0001$, linear regression; **Supplementary Figure 5**).

DISCUSSION

Previous work from our group could not detect somatic SNVs in *SNCA* exons at AF above 5% in cerebellum, frontal cortex and SN of sporadic PD patients (16). In this study, we expanded our search to other PD-genes. We excluded as many cases as possible with long disease duration and late-onset, as somatic variants playing a role in disease are hypothesized to be less likely to occur in these cases (16, 17). We included a patient carrying a *LRRK2* G2019S mutation, who had a phenotypically discordant monozygotic twin and where somatic variation could have played a role in penetrance (11). We used a highly sensitive approach to detect low-level variants in the genes of interest, by firstly combining deep sequencing coverage and molecular barcodes, followed by amplicon sequencing at higher coverage and ddPCR as validation steps (18). We could not detect somatic SNVs in PD-associated genes at AF higher than 0.33%. Similar to our results, a recent report could not identify somatic SNVs at AF above 0.5% in familial PD-genes from brains with Lewy body disorders ($n = 20$), using similar methodologies and higher sequencing coverage (19). Previous studies using Haloplex^{HS} reported variant detection at AF above 0.2%, further supporting that our analysis was close to the detection limits of this methodology (19–21). We focused on refining the analysis to mainly discard false positives. Our filtering criteria were tailored to discard sequencing artifacts, similar to other studies using Haloplex and common sequencing datasets (22–26). Advantages of visual analysis are the comprehensive analysis for each variant, easy implementation across datasets; however, it can become labor-intensive. Our results demonstrate the difficulties of SNV detection at low AF, due to low-level contamination and false positives, even when using molecular barcodes.

Challenges of somatic variant studies are not only technical, but also related to the stochastic nature of the variants. According to a previous hypothesis where neurons carrying somatic variation may be the most vulnerable and first to degenerate, we selected for patients with disease duration as short as possible (∼10 years) (16). When studying neurodegeneration in post-mortem brains, only the latest stages of the disease are being portrayed and, perhaps, events involved in disease development are missed. Conversely, if somatic SNVs arise post-mitotically in an age-dependent manner (4), detailed studies at different age groups are required. Furthermore, as we focused on the SN,

and only had access to DNA from other brain regions or blood in a few cases, we have not provided a detailed assessment of these. The use of patient-derived cell lines or animal models could also be considered. We are not aware of studies of somatic mutations in such samples, but PD patient fibroblasts have inefficient DNA repair, specifically the nucleotide excision repair (NER) pathway, and mice with a mutation compromising NER have dopaminergic pathology (27).

Our data combined with work discussed above, suggest that coding somatic SNVs in PD-associated genes are uncommon. In Alzheimer's disease, two brain somatic SNVs were found in 72 sporadic AD-patients (28). When using molecular barcodes, two brain somatic SNVs were found in AD-associated genes of 98 patients (29), whereas no somatic SNVs in familial AD-genes were found in 20 patients (19). Somatic SNVs in *APP* were reported in AD in the context of the novel mechanism of recombination leading to "genomic cDNA" (30). Recently, 14 out of 52 AD-patients analyzed by deep exome sequencing harbored exonic somatic mutations in genes involved in tau phosphorylation, but not familial AD genes (31). This contrasts with somatic CNVs, with *SNCA* gains in PD nigral dopaminergic and cortical neurons (32, 33).

In summary, our study could not detect coding somatic SNVs at AF above 0.33% when analyzing PD-associated genes from brain samples. Reaching lower AF to detect late somatic variant events using bulk-tissue requires an even larger sequencing effort, and it is complicated by the common presence of contamination and sequencing errors. Sequencing of dopaminergic single-nuclei should give enough resolution to describe somatic variants in cells mainly affected by PD (dopaminergic neurons). Additinal studies can be aimed to explore other types of somatic variations or other mechanisms by which somatic SNVs outside PD-associated genes could play detrimental roles in neurodegeneration.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/ena, PRJEB36518.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NRES Committee central—London. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ML-S conducted the experiments, analyzed the data, wrote, revised, and submitted this manuscript. AP participated in the experimental design and data analysis. KM participated in the experimental design and performed initial experiments. HM and AS participated in the design of the study. CP conceived and designed the study, contributed to interpret the data and revised the final version of the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.570424/full#supplementary-material

REFERENCES

1. Goedert M, Jakes R, Spillantini MG. The synucleinopathies: twenty years on. *J Parkinsons Dis.* (2017) 7:S51–69. doi: 10.3233/JPD-179005
2. Rocca WA. Comment the burden of Parkinson's disease: a worldwide perspective. *Lancet Neurol.* (2018) 17:928–9. doi: 10.1016/S1474-4422(18)30355-7
3. Mullin S, Schapira AHV. Pathogenic mechanisms of neurodegeneration in Parkinson disease. *Neurol Clin.* (2015) 33:1–17. doi: 10.1016/j.ncl.2014.09.010
4. Lodato MA, Walsh CA. Genome aging : somatic mutation in the brain links age-related decline with disease and nominates pathogenic mechanisms. *Hum Mol Genet.* (2019) 28:R197–206. doi: 10.1093/hmg/ddz191
5. McConnell MJ, Moran JV, Abyzov A, Akbarian S, Bae T, Cortes-Ciriano I, et al. Intersection of diverse neuronal genomes and neuropsychiatric disease: the brain somatic mosaicism network. *Science.* (2017) 356:eaal1641. doi: 10.1126/science.aal1641
6. Bae T, Tomasini L, Mariani J, Zhou B, Roychowdhury T, Franjic D, et al. Different mutational rates and mechanisms in human cells at pregastrulation and neurogenesis. *Science.* (2017) 555:eaan8690. doi: 10.1126/science.aan8690
7. Lodato MA, Rodin RE, Bohrson CL, Coulter ME, Barton AR, Kwon M, et al. Aging and neurodegeneration are associated with increased mutations in single human neurons. *Science.* (2018) 359:555–9. doi: 10.1126/science.aao4426
8. Leija-Salazar M, Piette CL, Proukakis C. Somatic mutations in neurodegeneration. *Neuropathol Appl Neurobiol.* (2018) 44:267–85. doi: 10.1111/nan.12465

9. Salk JJ, Schmitt MW, Loeb LA. Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations. *Nat Rev Genet.* (2018) 19:269–85. doi: 10.1038/nrg.2017.117
10. Xiromerisiou G, Houlden H, Sailer A, Silveira-Moriyama L, Hardy J, Lees AJ. Identical twins with Leucine rich repeat kinase type 2 mutations discordant for Parkinson's disease. *Mov Disord.* (2012) 27:1323–4. doi: 10.1002/mds.24924
11. Schneider SA, Johnson MR. Monozygotic twins with LRRK2 mutations: genetically identical but phenotypically discordant. *Mov Disord.* (2012) 27:1203–4. doi: 10.1002/mds.24991
12. Nacheva E, Mokretar K, Soenmez A, Pittman AM, Grace C, Valli R, et al. DNA isolation protocol effects on nuclear DNA analysis by microarrays, droplet digital PCR, and whole genome sequencing, and on mitochondrial DNA copy number estimation. *PLoS ONE.* (2017) 12:e0180467. doi: 10.1371/journal.pone.0180467
13. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Res.* (2007) 35:W71–4. doi: 10.1093/nar/gkm306
14. Sherry ST. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* (2001) 29:308–11. doi: 10.1093/nar/29.1.308
15. Lodato MA, Woodworth MB, Lee S, Evrony GD, Mehta BK, Karger A, et al. Somatic mutation in single human neurons tracks developmental and transcriptional history. *Science.* (2015) 350:94–8. doi: 10.1126/science.aab1785
16. Proukakis C, Houlden H, Schapira AH. Somatic alpha-synuclein mutations in Parkinson's disease: hypothesis and preliminary data. *Mov Disord.* (2013) 28:705–12. doi: 10.1002/mds.25502
17. Nicolas G, Veltman JA. The role of de novo mutations in adult-onset neurodegenerative disorders. *Acta Neuropathol.* (2019) 137:183–207. doi: 10.1007/s00401-018-1939-3
18. Abyzov A, Tomasini L, Zhou B, Vasmatzis N, Coppola G, Amenduni M, et al. One thousand somatic SNVs per skin fibroblast cell set baseline of mosaic mutational load with patterns that suggest proliferative origin. *Genome Res.* (2017) 27:512–23. doi: 10.1101/gr.215517.116
19. Keogh MJ, Wei W, Aryaman J, Walker L, van den Ameele J, Coxhead J, et al. High prevalence of focal and multi-focal somatic genetic variants in the human brain. *Nat Commun.* (2018) 9:4257. doi: 10.1038/s41467-018-06331-w
20. Hirsch P, Tang R, Abermil N, Flandrin P, Moatti H, Favale F, et al. Precision and prognostic value of clone-specific minimal residual disease in acute myeloid leukemia. *Haematologica.* (2017) 102:1227–37. doi: 10.3324/haematol.2016.159681
21. de Kock L, Wang YC, Revil T, Badescu D, Rivera B, Sabbaghian N, et al. High-sensitivity sequencing reveals multi-organ somatic mosaicism causing DICER1 syndrome. *J Med Genet.* (2016) 53:43–52. doi: 10.1136/jmedgenet-2015-103428
22. de Leng WWJ, Gadellaa-van Hooijdonk CG, Barendregt-Smouter FAS, Koudijs MJ, Nijman I, Hinrichs JWJ, et al. Targeted next generation sequencing as a reliable diagnostic assay for the detection of somatic mutations in tumours using minimal DNA amounts from formalin fixed paraffin embedded material. *PLoS ONE.* (2016) 11:e0149405. doi: 10.1371/journal.pone.0149405
23. Beyens M, Boeckx N, Van Camp G, Op de Beeck K, Vandeweyer G. pyAmpli: an amplicon-based variant filter pipeline for targeted resequencing data. *BMC Bioinform.* (2017) 18:554. doi: 10.1186/s12859-017-1985-1
24. Nishioka M, Bundo M, Ueda J, Katsuoka F, Sato Y, Kuroki Y, et al. Identification of somatic mutations in postmortem human brains by whole genome sequencing and their implications for psychiatric disorders. *Psychiatry Clin Neurosci.* (2018) 72:280–94. doi: 10.1111/pcn.12632
25. Deshpande A, Lang W, McDowell T, Sivakumar S, Zhang J, Wang J, et al. Strategies for identification of somatic variants using the ion torrent deep targeted sequencing platform. *BMC Bioinform.* (2018) 19:5. doi: 10.1186/s12859-017-1991-3
26. Barnell EK, Ronning P, Campbell KM, Krysiak K, Ainscough BJ, Sheta LM, et al. Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples. *Genet Med.* (2019) 21:972–81. doi: 10.1038/s41436-018-0278-z
27. Sepe S, Milanese C, Gabriels S, Derks KWJ, Payan-Gomez C, van IJcken WFJ, et al. Inefficient DNA repair is an aging-related modifier of Parkinson's disease. *Cell Rep.* (2016) 15:1866–75. doi: 10.1016/j.celrep.2016.04.071
28. Sala Frigerio C, Lau P, Troakes C, Deramecourt V, Gele P, Van Loo P, et al. On the identification of low allele frequency mosaic mutations in the brains of Alzheimer's disease patients. *Alzheimer's Dement.* (2015) 11:1265–76. doi: 10.1016/j.jalz.2015.02.007
29. Nicolas G, Acuña-Hidalgo R, Keogh MJ, Quenez O, Steehouwer M, Lelieveld S, et al. Somatic variants in autosomal dominant genes are a rare cause of sporadic Alzheimer's disease. *Alzheimer's Dement.* (2018) 14:1632–9. doi: 10.1016/j.jalz.2018.06.3056
30. Lee M-H, Siddoway B, Kaeser GE, Segota I, Rivera R, Romanow WJ, et al. Somatic APP gene recombination in Alzheimer's disease and normal neurons. *Nature.* (2018) 563:639–45. doi: 10.1038/s41586-018-0718-6
31. Park JS, Lee J, Jung ES, Kim MH, Kim I Bin, Son H, et al. Brain somatic mutations observed in Alzheimer's disease associated with aging and dysregulation of tau phosphorylation. *Nat Commun.* (2019) 10:3090. doi: 10.1038/s41467-019-11000-7
32. Mokretar K, Pease D, Taanman J-W, Soenmez A, Ejaz A, Lashley T, et al. Somatic copy number gains of α-synuclein (SNCA) in Parkinson's disease and multiple system atrophy brains. *Brain.* (2018) 141:2419–31. doi: 10.1093/brain/awy157
33. Perez-Rodriguez D, Kalyva M, Leija-Salazar M, Lashley T, Tarabichi M, Chelban V, et al. Investigation of somatic CNVs in brains of synucleinopathy cases using targeted SNCA analysis and single cell sequencing. *Acta Neuropathol Commun.* (2019) 7:219. doi: 10.1186/s40478-019-0873-5

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Early-Onset Parkinson Disease Screening in Patients From Nigeria

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Introduction: Nigeria is one of the most populated countries in the world; however, there is a scarcity of studies in patients with age-related neurodegenerative diseases, such as Parkinson disease (PD). The aim of this study was to screen patients with PD including a small cohort of early-onset PD (EOPD) cases from Nigeria for *PRKN*, *PINK1*, *DJ1*, *SNCA* multiplication, and LRRK2 p.G2019S.

Methods: We assembled a cohort of 109 Nigerian patients with PD from the four main Nigerian tribes: Yoruba, Igbo, Edo, and Hausa. Fifteen cases [14 from the Yoruba tribe (93.3%)] had EOPD (defined as age-at-onset <50 years). All patients with EOPD were sequenced for the coding regions of *PRKN*, *PINK1*, and *DJ1*. Exon dosage analysis was performed with a multiplex ligation-dependent probe amplification assay, which also included a *SNCA* probe and LRRK2 p.G2019S. We screened for LRRK2 p.G2019S in the entire PD cohort using a genotyping assay. The PINK1 p.R501Q functional analysis was conducted.

Results: In 15 patients with EOPD, 22 variants were observed [*PRKN*, 9 (40.9%); *PINK1*, 10 (45.5%); and *DJ1*, 3 (13.6%)]. Three (13.6%) rare, nonsynonymous variants were identified, but no homozygous or compound heterozygous carriers were found. No exonic rearrangements were present in the three genes, and no carriers of *SNCA* genomic multiplications or LRRK2 p.G2019S were identified. The PINK1 p.R501Q functional analysis revealed pathogenic loss of function.

Conclusion: More studies on age-related neurodegenerative diseases are needed in sub-Saharan African countries, including Nigeria. Population-specific variation may provide insight into the genes involved in PD in the local population but may also contribute to larger studies performed in White and Asian populations.

Keywords: Nigerian population, MLPA, Sanger sequencing, *LRRK2*, *PRKN*, *PINK1*, *DJ1*, Parkinson disease

INTRODUCTION

Sub-Saharan Africa has one of the highest birth rates in the world. In 2019, the population of Nigeria exceeded 200 million inhabitants, divided into 250 ethnic groups, with ∼7 million Nigerians aged 65 years or older (1). The largest tribes in Nigeria are Hausa (30.0%), Yoruba (15.5%), and Igbo (15.2%) (2). The increasing number of aging Nigerians has prioritized studies evaluating the epidemiology and causes of Parkinson disease (PD), the prevalence of which is estimated at 10 to 235/100,000 people (3). However, there is still a lack of studies in this population. Most reports have concentrated on the prevalence of PD in Nigeria, environmental risk factors for PD in Nigeria, other diseases mimicking the clinical features of PD, and biochemical or pathological findings (3); The first Nigerian National PD Registry was just published in 2020 (4).

Genetic factors influence PD occurrence, especially in patients with positive family history or early-onset PD (EOPD; defined as age-at-onset <50 years) (5). In White populations, about 5–10% have monogenic forms of PD. The most common gene associated with PD is *LRRK2* (6). Missense mutation and multiplications have been reported in *SNCA* (7). *PRKN*, *PINK1*, and *DJ-1* are the three most common genes reported in EOPD (6). Functionally, PINK1 and PRKN protein together orchestrate the degradation of selectively damaged mitochondria via the autophagy-lysosome system, while DJ-1 operates in parallel to the PINK1-PRKN mitophagy pathway (8).

While most of these genes have been extensively examined only in White and Asian populations, three studies have included Nigerian patients for genetic analysis. Sanger sequencing was performed in *LRRK2*, *PRKN*, and *ATXN3* in 57 Nigerian patients with PD from Yoruba, Igbo, and Edo tribes (12.3% with EOPD) but did not identify any pathogenic mutations (9). The LRRK2 p.G2019S screening of 126 patients with PD was also negative (10). Fourteen Nigerian patients with PD were screened for 16 genes associated with PD. However exon dosage and *SNCA* multiplications analysis were never performed in this population (11).

There is also little data in the literature on PD in other sub-Saharan populations. In 39 Zambian patients with PD, a new potentially pathogenic mutation in LRRK2 p.A1464G and compound heterozygous mutations in *PRKN* were described (12). In a Ghanese study, no *LRRK2* variants were revealed (13). In a South African population, no LRRK2 p.G2019S mutations were identified in patients with African ancestry (14); however, in another study, two South African patients with EOPD had compound heterozygous mutations in *PRKN* (15).

Due to the lack of data on mutations in previously reported genes, analysis in the Nigerian population is warranted. We report data from the screening of apparently sporadic cases of PD from Nigeria [Yoruba (n = 86), Igbo (n = 2), Hausa (n = 19), and Edo (n = 2)] for *LRRK2* in all patients with PD and *PRKN*, *PINK1*, *DJ1*, and *SNCA* multiplications in patients with EOPD.

FIGURE 1 | Map of Nigeria. Nigeria has 250 different tribes. We obtained blood samples from patients from Edo (n = 2), Hausa (n = 2), Igbo (n = 19), and Yoruba (n = 86) tribes. The estimated populations of these tribes are 5, 60, 30.4, and 31 million, respectively. Hausa is the largest Nigerian ethnic group that inhabits mostly the northern part of Nigeria. A large population lives also in the south of Niger. Yoruba tribe is located in the area of Lagos, the previous capital city of Nigeria. Igbo people inhabit the southern part of Nigeria, in the Biafra region. Edo people live in the Atlantic Ocean coastal areas. All samples were collected in Lagos, the largest port of Nigeria, with an estimated population of 8 million. The entire Nigerian population is over 200 million.

MATERIALS AND METHODS

Blood specimens from a series of 109 clinical patients with PD were collected and characterized by movement disorder specialists (OO and SO) in the Division of Neurology at Lagos State University Teaching Hospital, Lagos, Nigeria. The study protocol was reviewed and approved by the Institutional Review Board of Lagos State University Teaching Hospital. Written informed consent for participation was not required for this study in accordance with the Nigerian national legislation and the institutional requirements. The Mayo Clinic IRB Committee approved this international collaboration. Although all patients were from Nigeria, their specific tribal origins were as follows: Yoruba, 86 (79.0%); Igbo, 19 (17.4%); Edo, 2 (1.8%); and Hausa, 2 (1.8%) (**Figure 1**). The collected blood specimens were then shipped to Mayo Clinic Florida in Jacksonville via international courier service. Diagnosis of PD was based on the UK Brain Bank diagnostic criteria for PD (16).

All patients with EOPD were Sanger sequenced for *PRKN* (exons 1–12), *PINK1* (exons 1–8), and *DJ1* (exons 1–6). Polymerase chain reaction products were purified using Mag-Bind TotalPure NGS and Mag-Bind SeqDTR chemistry (Omega Bio-tek, Inc) on the Biomek FX Automated Workstation (Beckman Coulter, Inc). Purified products were analyzed using a 3730*xl* DNA analyzer (Applied Biosystems), and sequences were analyzed using SeqScape Software v3.0 (Applied Biosystems). The identified variants were labeled according to appropriate reference sequences: *PRKN* (NM_004562), *PINK1* (NM_032409), and *DJ1* (NM_007262) (17). Mutations were referred to data from the Human Gene Mutation Database (18)

TABLE 1 | *PINK1*, *DJ1*, and PRKN Variants in Nigerian Patients With Early-Onset Parkinson Disease ($n = 15$) and *LRRK2* G2019S in the Total Study Cohort ($N = 109$)[a].

rs number	AA	Alleles	Molecular consequences	Genotypes (major:het:minor)	MAF	MAF African (gnomAD)	MAF European (non-Finnish) (gnomAD)	CADD score
PINK1								
rs537679886	E55E	c.165G>A	Synonymous	AA:AG:GG (14:1:0)	3.333%	0.59% ($n = 77$)	<0.01% ($n = 1$)	6.3
rs45530340	L63L	c.189C>T	Synonymous	CC:CT:TT (14:1:0)	3.333%	6.39% ($n = 959$)	19.91% ($n = 13,063$)	9.4
rs2298298		c.388-7A>G	Intronic	GG:AG:AA (8:7:0)	23.33%	76.22% ($n = 18,979$)	87.73% ($n = 23,369$)	2.7
rs142183624	L316L	c.948C>T	Synonymous	CC:CT:TT (13:2:0)	6.667%	1.29% ($n = 321$)	<0.01% ($n = 3$)	9.7
rs3131713		c.960-5G>A	Intronic	AA:AG:GG (7:8:0)	26.67%	76.50% ($n = 18,991$)	87.72% ($n = 113,035$)	5.8
rs774946874	**N410N**	**c.1230C>T**	**Synonymous**	**CC:CT:TT (14:1:0)**	**3.33%**	**0.00% ($n = 0$)**	**0.02% ($n = 21$)**	**15.2**
rs2298300		20516T>C	Intronic	CC:CT:TT (0:3:12)	90.00%	5.08% ($n = 1,266$)	0. 09% ($n = 109$)	2.1
rs115477764	E476K	c.1426G>A	Missense	AA:AG:GG (14:1:0)	3.33%	4.00% ($n = 999$)	0.01% ($n = 17$)	14.3
rs61744200	R501Q	c.1502G>A	Missense	AA:AG:GG (14:1:0)	3.333%	3.25% ($n = 811$)	<0.01% ($n = 811$)	32
rs1043424	N521T	c.1562A>C	Missense	AA:AC:CC (8:7:0)	23.33%	26.45% ($n = 6,596$)	27.79% ($n = 9,265$)	14.2
DJ1								
rs11548933		c.-22 C>T	5′UTR	CC:CT:TT (7:7:1)	30.00%	13.22% ($n = 2,933$)	0.04% ($n = 43$)	2.9
rs11548937	G78G	c.234C>T	Synonymous	CC:CT:TT (11:4:0)	13.33%	11.13% ($n = 2,765$)	0.05% ($n = 61$)	11.5
rs72854882		c.323-14A>G	Intronic	AA:AG:GG (6:8:1)	33.33%	18.84% ($n = 4,692$)	0.08% ($n = 98$)	2.3
PRKN								
rs112155221		**c.-76-427G>A**	Intronic	**CC:CT:TT (14:1:0)**	**3.333%**	**0.0% ($n = 0$)**	**0.01% ($n = 6$)**	**11.5**
rs77795533	P37P	c.111G>A	Synonymous	AA:AG:GG (14:1:0)	3.333%	10.61% ($n = 2,649$)	0.04% ($n = 56$)	2.6
rs2075923		c.171+25T>C	Intronic	CC:CT:TT (10:5:0)	16.667%	38.88% ($n = 9,681$)	23.28% ($n = 29,681$)	5.5
rs1801474	S167N	c.500G>A	Missense	AA:AG:GG (9:6:0)	20.00%	7.17% ($n = 7,807$)	1.84% ($n = 2,369$)	15.7
rs9456735	M192L	c.574A>C	Missense	AA:AC:CC (13:2:0)	6.667%	5.94% ($n = 1,483$)	0.03% ($n = 35$)	20.7
rs9456711	L261L	c.783A>G	Synonymous	AA:AG:GG (9:6:0)	20.000%	17.79% ($n = 4,464$)	0.06% ($n = 78$)	7.7
rs114696251	Y267H	c.799T>C	Missense	TT:TC:CC (14:1:0)	3.333%	0.18% ($n = 45$)	<0.01% ($n = 2$)	27.8
rs144340740	G319G	c.957T>C	Synonymous	TT:TC:CC (14:1:0)	3.333%	3.45% ($n = 859$)	<0.01% ($n = 7$)	0.0
rs1801582	V380L	c.1138G>C	Missense	CC:CG:GG (8:4:3)	33.333%	17.48% ($n = 4,359$)	16.13% ($n = 20,838$)	11.2
LRRK2								
rs34637584	G2019S	c.6055G>A	Missense	GG:AG:AA (109:0)	0.000%	0.01% ($n = 3$)	0.03% ($n = 33$)	31

5′UTR, 5′ untranslated region; AA, amino acid; gnomAD, CADD-Combined Annotation Dependent Depletion; Genome Aggregation Database; Het, heterozygous; MAF, minor allele frequency.

[a]*Bold rows indicate variants not previously reported in African populations; gray rows, variants found more often in African populations than in European (non-Finnish) in gnomAD.*

and Genome Aggregation Database (gnomAD) (19). Multiple ligation-dependent probe amplification probemix P051 was used to screen for gene dosage (MRC Holland). This screening was performed using an 3730*xl* DNA analyzer, and the data were analyzed using Coffalyser. Net software (MRC Holland). The LRRK2 p.G2019S variant was also genotyped using TaqMan SNP Genotyping Assay (Applied Biosystems), and genetic analysis was completed using SDS2.2.2 software (Applied Biosystems). The potential pathogenicity of discovered variants was predicted with Combined Annotation Dependent Depletion (CADD) scores (CADD score >20).

For functional testing, *PINK1* cDNA was cloned into a V5-tagged expression vector (pcDNA6A *PINK1*-V5/His). The p.R501Q mutant was introduced by site-directed mutagenesis, and the presence of the mutation was confirmed by sequencing. PINK1 WT or p.R501Q were then expressed at or near-endogenous levels in previously established Hek293 PINK1 knockout (KO) cells (20). To mimic endogenous expression levels, 500 ng *PINK1*-V5 cDNA was diluted with 3,500 ng carrier DNA (pCMV-GST HA vector) and mixed with 5 μl Lipofectamine 2000 (Thermo Fisher). Cells were then transfected according to the manufacturer's instructions and were further treated the next day with 20 μM Carbonyl cyanide m-chlorophenyl hydrazine (CCCP) or with 10 μM MG132 (both Sigma) and harvested after 4 h. Cells were lysed in RIPA buffer with protease and phosphatase inhibitors (Roche). Protein (25 μg) was loaded onto 8–16% Tris-glycine gels, transferred onto PVDF (polyvinylidene fluoride) membranes, and probed with antibodies against PINK1 (#6946, Cell Signaling Technology, 1:2,000) and vinculin (V9131, Sigma, 1:100,000) as a loading control.

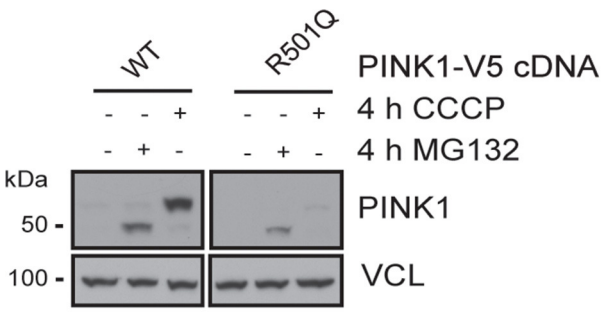

FIGURE 2 | PINK1 p.R501Q is unstable and poorly accumulates on the outer mitochondrial membrane upon stress. PINK1 KO Hek293 cells were transfected with *PINK1*-V5 tagged cDNA using special conditions to mimic endogenous expression levels, and protein lysates were analyzed by western blot. PINK1 WT is almost undetectable at basal conditions but is swiftly stabilized as a full-length protein (~63 kDa) following mitochondrial damage (CCCP treatment). However, the PINK1 p.R501Q mutant remains unstable and only poorly accumulates on the outer mitochondrial membrane upon stress. Yet, the cleaved forms of PINK1 WT and p.R501Q (~52 kDa) can be stabilized in the cytosol upon proteasome inhibition (MG132 treatment), confirming the transient expression of both variants.

RESULTS

Of the 109 patients with PD, 15 had EOPD (13.8%; 14 from Yoruba and 1 from Igbo). In the whole study group, mean (SD) age of onset was 60.5 (9.1) years, and 77 participants (70.6%) were men. In the EOPD group, mean (SD) age of onset was 44.5 (5.0) years, and 10 participants (66.7%) were men. The cardinal symptoms of PD, such as bradykinesia, rigidity, and asymmetrical rest tremor, were observed in all patients. Postural instability was observed in 17 patients [15.6%; Yoruba, 14 (82.4%); Igbo, 2 (11.8%); and Edo, 1 (5.8%)]. There was no difference in postural instability occurrence between EOPD and late-onset PD [3 (20.0%) vs. 14/94 (14.9%); Fischer exact test, $P = 0.70$] (**Table 1**). One man with late-onset PD from Igbo had positive family history (0.9%). No LRRK2 p.G2019S mutation carriers were detected in our cohort (**Table 1**).

In 15 patients with EOPD, 22 variants were discovered in three genes [*PRKN*, 9 (40.9%); *PINK1*, 10 (45.5%); and *DJ1*, 3 (13.6%)]. In all genes, there were six intronic variants (27.3%), one in 5′ untranslated region (4.5%), eight coding synonymous (36.4%), and seven coding non-synonymous (31.8%). rs774946874 in *PINK1* and rs112155221 in *PRKN* have not been observed in African populations but have been reported in European (non-Finnish) ancestries. Two variants in *PRKN*, three in *DJ1*, and three in *PINK1* occur more often in African than in European (non-Finnish) populations in gnomAD. We found three (13.6%; two from Yoruba and one from Igbo) rare, non-synonymous variants (defined as minor allele frequency <5% in gnomAD), but no homozygous or compound heterozygous carriers were present. No exonic rearrangements were observed (**Table 1**).

PINK1 and PRKN together orchestrate a stress-induced mitochondrial quality control pathway that can be probed at multiple steps along its sequence to functionally assess the pathogenicity of genetic variants (8). While both *PRKN* variants have been analyzed earlier (21, 22), to our knowledge the pathogenicity of the *PINK1* variant has never been tested before. As part of its surveillance, PINK1 WT is constitutively imported into healthy mitochondria, where it is N-terminally cleaved, exported to the cytosol, and degraded by the proteasome. Upon mitochondrial damage, PINK1 can no longer be imported and thus locally accumulates as a full-length protein on the outer mitochondrial membrane where it initiates mitophagy through the activation and recruitment of PRKN. To assess the functionality of the identified variant, PINK1 KO Hek293 cells were transfected with either WT or p.R501Q mutant *PINK1* cDNA (**Figure 2**). Using conditions that result in near-endogenous expression levels, full-length PINK1 WT was only detectable upon mitochondrial depolarization (4 h CCCP), whereas PINK1 p.R501Q appeared highly unstable and only poorly accumulated even following CCCP treatment. Transient, though successful, expression of either *PINK1* variant in cells was confirmed after proteasome inhibition (4 h MG132) which in both cases stabilized the N-terminally cleaved form of PINK1.

DISCUSSION

Genetic studies of PD in sub-Saharan African countries are sparse. We comprehensively screened a small ($n = 15$) series of patients with EOPD from Nigeria for the most commonly

associated genes (*PRKN*, *PINK1*, and *DJ1*, *SNCA* multiplication) and screened a larger series ($n = 109$) of Nigerian patients with PD for LRRK2 p.G2019S. No pathogenic mutations were revealed. Two observed variants have been found previously only in non-African populations, and 15 have been reported more often in African than European (non-Finnish) populations in gnomAD. In the EOPD group, seven discovered variants were non-synonymous coding variants.

Clinical characteristics in the analyzed patients were consistent with typical PD symptoms in other populations. *LRRK2* variants are commonly found in the Mediterranean area and northern African countries. However, similar to other sub-Saharan African study groups, no LRRK2 p.G2019S variants were detected (9, 10, 23). The percentage of patients with EOPD in our cohort was similar to previous reports (9, 10). There were no homozygous or compound heterozygous EOPD mutations carriers in genes causing autosomal recessive EOPD.

In a previous study of Nigerian patients with PD, only *PRKN* was sequenced, with 10 variants reported, but no pathogenic mutations (24). In our study, two potential pathogenic heterozygous substitutions were discovered in PRKN (p.M192L, CADD score = 20.7 and p.Y267H, CADD score = 27.8) and one in PINK1 (p.R501Q, CADD score = 32). PRKN p.M192L and p.Y267H have been reported in a previous Nigerian study, which analyzed *PRKN* mutations in Yoruba, Igbo, and Edo tribes (9). Both variants are most frequently reported in Black African populations in gnomAD.

The herein identified PRKN mutants p.M192L and p.Y267H had been previously analyzed in cell-based mitophagy paradigms and using different functional readouts. No obvious defect was found for PRKN p.M192L (or p.M192V), and as such this variant was functionally classified as benign (21). However, PRKN p.Y267H showed an early delay in translocation to damaged mitochondria compared to PRKN WT but perhaps more importantly a significant reduction in ubiquitin charging of its active site (22). This defect is reflective of overall reduced enzymatic activity and thus is supportive of a pathogenic *PRKN* loss of function.

Similarly, the PINK1 p.R501Q variant that we functionally tested here likely results in a pathogenic loss of function. Compared to PINK1 WT, p.R501Q was unstable and only very poorly accumulated upon stress on the outer mitochondrial membrane. Although we have used special conditions to mimic near-endogenous expression, the results are based on transient transfections and as such need to be verified. However, we recently identified another PINK1 variant (p.I368N) with a similar phenotype that was unstable as a full-length protein but could be stabilized as a cleaved form upon proteasome inhibition in patients' fibroblasts (25).

To our knowledge, this is the first study in which multiple ligation-dependent probe amplification was performed in a Nigerian population and the first time patients from Nigeria's largest tribe, Hausa, were screened for LRRK2 p.G2019S. Although we did not find any exonic rearrangements, they have been discovered in another sub-Saharan population (15). In White and Asian patients, 43.2% of *PRKN* mutations may be structural variants (26). These data suggest that exon dosage analysis should always be performed in potential *PRKN* mutation carriers.

Our study has several limitations. Our small study cohort may not reflect the prevalence of reported PD genes in all tribes analyzed. We also had limited clinical characteristics for our study population. Genetic factors are usually present in populations with EOPD or family history of PD, so including these groups into analysis increases the chance of reporting positive results (27).

Further analyses are urgently needed to characterize the genetic variation in Nigeria. Our study is the first step in genetic characterization of known PD genes in four tribes in Nigeria. Future studies should include larger cohorts with better clinical characterization. Known genes should be analyzed first, then a genome-wide association study on a population of non-carriers may lead to discovery of unique loci responsible for PD in sub-Saharan Africans.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Lagos State University Teaching Hospital. Written informed consent for participation was not required for this study in accordance with the Nigerian national legislation and the Nigerian institutional requirements. The Mayo Clinic IRB Committee approved this international collaboration.

AUTHOR CONTRIBUTIONS

LM contributed to analysis and interpretation of the data, drafting of the article, and generation/collection of images. OO and SO contributed to conception and design, collection, analysis, and interpretation of the data, and critical revision of the article. BB performed the functional experiments and contributed to the analysis and interpretation of the data, drafting and critical revision of the article, and generation of images. JL, AB, and RW contributed to experiments, analysis and interpretation of the data, and critical revision of the article. RH and AS contributed to analysis and interpretation of data and critical revision of the article. FF and WS contributed to conception and design, analysis and interpretation of the data, drafting and critical revision of the article, and generation of images. OR contributed to conception and design, analysis and interpretation of the data, and drafting and critical revision of the article. ZW contributed to conception and design, collection, analysis, and interpretation of the data, drafting and critical revision of the article, and generation/collection of images. All authors approved the final article.

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REFERENCES

1. Worldometer. *Nigeria Population (LIVE)* (2019).
2. Central Intelligence Agency. *The World Factbook.* Africa, Nigeria (2019).
3. Oluwole OG, Kuivaniemi H, Carr JA, Ross OA, Olaogun MOB, Bardien S, et al. Parkinson's disease in Nigeria: a review of published studies and recommendations for future research. *Parkinsonism Relat Disord.* (2019) 62:36–43. doi: 10.1016/j.parkreldis.2018.12.004
4. Ojo OO, Abubakar SA, Iwuozo EU, Nwazor EO, Ekenze OS, Farombi TH, et al. The Nigeria Parkinson disease registry: process, profile, and prospects of a collaborative project. *Mov Disord.* (2020) 35:1315–22. doi: 10.1002/mds.28123
5. Yoon WT, Comparison of dystonia between Parkinson's disease and atypical parkinsonism: the clinical usefulness of dystonia distribution and characteristics in the differential diagnosis of parkinsonism. *Neurol Neurochir Pol.* (2018) 52:48–53. doi: 10.1016/j.pjnns.2017.11.004
6. Toffoli M, Vieira SRL, Schapira AHV. Genetic causes of PD: a pathway to disease modification. *Neuropharmacology.* (2020) 170:108022. doi: 10.1016/j.neuropharm.2020.108022
7. Book A, Guella I, Candido T, Brice A, Hattori N, Jeon B, et al. A meta-analysis of α-synuclein multiplication in familial Parkinsonism. *Front Neurol.* (2018) 9:1021. doi: 10.3389/fneur.2018.01021
8. Truban D, Hou X, Caulfield TR, Fiesel FC, Springer W. PINK1, Parkin, and mitochondrial quality control: what can we learn about Parkinson's disease pathobiology? *J Parkinsons Dis.* (2017) 7:13–29. doi: 10.3233/JPD-160989
9. Okubadejo N, Britton A, Crews C, Akinyemi R, Hardy J, Singleton A, et al. Analysis of Nigerians with apparently sporadic Parkinson disease for mutations in LRRK2, PRKN and ATXN3. *PLoS One.* (2008) 3:e3421. doi: 10.1371/journal.pone.0003421
10. Okubadejo NU, Rizig M, Ojo OO, Jonvik H, Oshinaike O, Brown E, et al. Leucine rich repeat kinase 2 (LRRK2) GLY2019SER mutation is absent in a second cohort of Nigerian Africans with Parkinson disease. *PLoS One.* (2018) 13:e0207984. doi: 10.1371/journal.pone.0207984
11. Oluwole OG, Kuivaniemi H, Abrahams S, Haylett WL, Vorster AA, van Heerden CJ, et al. Targeted next-generation sequencing identifies novel variants in candidate genes for Parkinson's disease in Black South African and Nigerian patients. *BMC Med Genet.* (2020) 21:23. doi: 10.1186/s12881-020-0953-1
12. Yonova-Doing E, Atadzhanov M, Quadri M, Kelly P, Shawa N, Musonda ST, et al. Analysis of LRRK2, SNCA, Parkin, PINK1, and DJ-1 in Zambian patients with Parkinson's disease. *Parkinsonism Relat Disord.* (2012) 18:567–71. doi: 10.1016/j.parkreldis.2012.02.018
13. Cilia R, Sironi F, Akpalu A, Cham M, Sarfo FS, Brambilla T, et al. Screening LRRK2 gene mutations in patients with Parkinson's disease in Ghana. *J Neurol.* (2012) 259:569–70. doi: 10.1007/s00415-011-6210-y
14. Bardien S, Blanckenberg J, van der Merwe L, Farrer MJ, Ross OA. Patient-control association study of the Leucine-Rich repeat kinase 2 (LRRK2) gene in South African Parkinson's disease patients. *Mov Disord.* (2013) 28:2039–40. doi: 10.1002/mds.25637
15. Haylett WL, Keyser RJ, du Plessis MC, van der Merwe C, Blanckenberg J, Lombard D, et al. Mutations in the parkin gene are a minor cause of Parkinson's disease in the South African population. *Parkinsonism Relat Disord.* (2012) 18:89–92. doi: 10.1016/j.parkreldis.2011.09.022
16. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. *Neurology.* (2001) 57:1497–9. doi: 10.1212/wnl.57.8.1497
17. *Nucleotide*. National Center for Biotechnology Information, U.S. National Library of Medicine. Available online at: https://www.ncbi.nlm.nih.gov/ (accessed August 10, 2020).
18. Institute of Medical Genetics in Cardiff. *The Human Gene Mutation Database.* Available online at: http://www.hgmd.cf.ac.uk/ac/index.php (accessed August 10, 2020).
19. Genome Aggregation *Database, gnomAD*. Available online at: https://gnomad.broadinstitute.org/ (accessed August 10, 2020).
20. Watzlawik J, Hou X, Truban T, Ramnarine C, Barodia SK, Gendron TF, et al. Sensitive ELISA-based detection method for the mitophagy marker p-S65-Ub in human cells, autopsy brain, blood samples. *Autophagy.* (2020) 28:1–16. doi: 10.1080/15548627.2020.1834712
21. Yi W, MacDougall EJ, Tang MY, Krahn AI, Gan-Or Z, Trempe J-F, et al. The landscape of Parkin variants reveals pathogenic mechanisms and therapeutic targets in Parkinson's disease. *Hum Mol Genet.* (2019) 28:2811–25. doi: 10.1093/hmg/ddz080
22. Fiesel FC, Caulfield TR, Moussaud-Lamodière EL, Ogaki K, Dourado DF, Flores SC, et al. Structural and functional impact of Parkinson disease-associated mutations in the E3 ubiquitin ligase Parkin. *Hum Mutat.* (2015) 36:774–86. doi: 10.1002/humu.22808
23. Benamer HT, De Silva R. LRRK2 G2019S in the North African population: a review. *Eur Neurol.* (2010) 63:321–5. doi: 10.1159/000279653
24. Kilarski LL, Pearson JP, Newsway V, Majounie E, Knipe MD, Misbahuddin A, et al. Systematic review and UK-based study of PARK2 (parkin), PINK1, PARK7 (DJ-1) and LRRK2 in early-onset

Parkinson's disease. *Mov Disord.* (2012) 27:1522–9. doi: 10.1002/mds.25132
25. Ando M, Fiesel FC, Hudec R, Caulfield TR, Ogaki K, Górka-Skoczylas P, et al. The PINK1 p. I368N mutation affects protein stability and ubiquitin kinase activity. *Mol Neurodegener.* (2017) 12:32. doi: 10.1186/s13024-017-0174-z
26. Kasten M, Hartmann C, Hampf J, Schaake S, Westenberger A, Vollstedt EJ, et al. Genotype-phenotype relations for the Parkinson's disease genes Parkin, PINK1, DJ1: MDSGene systematic review. *Mov Disord.* (2018) 33:730–41. doi: 10.1002/mds.27352
27. Barc K, Kuzma-Kozakiewicz M. Positron emission tomography neuroimaging in neurodegenerative diseases: Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Neurol Neurochir Pol.* (2019) 53:99–112. doi: 10.5603/PJNNS.a2019.0013

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BRIEF RESEARCH REPORT
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APOE and *MAPT* Are Associated With Dementia in Neuropathologically Confirmed Parkinson's Disease

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Introduction: Cognitive decline and dementia are common and debilitating non-motor phenotypic features of Parkinson's disease with a variable severity and time of onset. Common genetic variation of the Apolipoprotein E (*APOE*) and micro-tubule associated protein tau (*MAPT*) loci have been linked to cognitive decline and dementia in Parkinson's disease, although studies have yielded mixed results. To further elucidate the influence of *APOE* and *MAPT* variability on dementia in Parkinson's disease, we genotyped postmortem brain tissue samples of clinically and pathologically well-characterized Parkinson's donors and performed a survival analysis of time to dementia.

Methods: We included a total of 152 neuropathologically confirmed Parkinson's disease donors with or without clinical dementia during life. We genotyped known risk variants tagging the *APOE* ε4 allele and *MAPT* H1/H2 inversion haplotype. Cox proportional hazards regression analyses adjusted for age at onset, sex and genetic principal components were performed to assess the association between the genetic variants and time from motor onset to onset of dementia.

Results: We found that both the *APOE* ε4 allele (HR 1.82, 95 % CI 1.16–2.83, $p = 0.009$) and *MAPT* H1-haplotype (HR 1.71, 95 % CI 1.06–2.78, $p = 0.03$) were associated with earlier development of dementia in patients with Parkinson's disease.

Conclusion: Our results provide further support for the importance of *APOE* ε4 and *MAPT* H1-haplotype in the etiology of Parkinson's disease dementia, with potential future relevance for risk stratification and patient selection for clinical trials of therapies targeting cognitive decline in Parkinson's disease.

Keywords: parkinson's disease, dementia, neuropathology, genetics, association study, APOE, MAPT

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INTRODUCTION

Parkinson's disease (PD) is a heterogenous disorder in terms of clinical presentation and rate of progression. Dementia is one of the most debilitating non-motor manifestations of the disease, with broad implications for both patients and caregivers (1–3). Longitudinal studies have shown that most patients ultimately develop Parkinson's disease dementia (PDD) if they survive long

enough, although the time of onset is highly variable (4, 5). Cognitive disability is not only a feature of advanced disease, as 36% of patients meet criteria for mild cognitive impairment already at clinical diagnosis (6) and 17% of patients develop dementia within five years from disease onset (7). Identification of biomarkers, including common genetic variants predicting early cognitive decline and dementia, could provide important insights into the biological and molecular underpinnings of PDD, benefit recruitment to clinical trials and identify potential targets for novel therapeutics.

Genome-wide association studies (GWAS) have identified genetic susceptibility loci for sporadic PD, with the latest meta-analysis bringing the number up to 90 risk signals across 78 loci (8). Genetic variability may not only affect the risk of developing PD, but also influence the clinical course of the disease. Several genetic loci have been hypothesized as risk factors for dementia in sporadic PD, among them *APOE* and *MAPT,* showing partly conflicting results in previously published reports (9).

Coding variation in *APOE* on chromosome 19 gives rise to three common alleles: ε2, ε3, and ε4. The *APOE* ε4 allele is a strong and well-established genetic risk factor for Alzheimer's disease (AD) (10), and the top GWAS signal in dementia with Lewy bodies (DLB) (11). While *APOE* does not seem to alter the risk for PD in itself according to GWAS results, the ε4 allele has been studied as a potential risk factor for cognitive decline and development of dementia in PD patients, with several larger studies reporting a significant association (12, 13).

An inversion polymorphism on chromosome 17q21, containing *MAPT* and several other genes, gives rise to the H1 and H2 haplotypes in European populations (14). Single-nucleotide polymorphisms (SNPs) tagging the H1-haplotype have consistently been among the most significant association signals in GWAS of PD-risk (8, 15, 16). The *MAPT* gene encodes the tau protein that is found to aggregate in neurofibrillary tangles (NFT), a core neuropathological feature of AD, but also found in varying degrees in PD and PDD patients upon autopsy (17, 18). Interestingly, the *MAPT* H1-haplotype has also been reported to be associated with an accelerated rate of cognitive decline and earlier development of dementia in PD patients (7, 19, 20), yet larger studies have not been able to replicate this finding (12, 21).

Discrepant results across previous genetic association studies of cognitive outcomes in PD could potentially arise from differences in methodology, in particular with respect to inclusion criteria, duration of follow-up and outcome measures used to assess cognitive decline. A study based on brain bank samples can take advantage of gold standard diagnostics and clinical data that cover the patients' entire lifespan. In this study, we investigated the association of SNPs in the *APOE* and *MAPT* loci with time to dementia by retrospective survival analysis in neuropathologically defined PD brain donors.

METHODS

Subjects

All subjects were neuropathologically confirmed patients with PD or PDD from the Netherlands Brain Bank (NBB, www.brainbank.nl). All brains available from the NBB from 1989 to 2017 (n = 3,853) were considered for study inclusion according to the selection criteria. Written, informed consent for the use of clinical information and tissue samples for research purpose, was collected from the donors or their next of kin.

Standardized brain autopsies and neuropathological examinations were performed by experienced neuropathologists (AR and WB). Neuropathological assessment of Lewy Body (LB)-related α-synuclein pathology was done according to BrainNet Europe guidelines (22) and assessment of AD neuropathologic change was done according to National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines (23).

Clinical information was extracted from the medical records provided by the NBB. The diagnosis of PD was based on the combination of the clinical syndrome of PD [UK Parkinson's Disease Society Brain Bank criteria (24)], and moderate to severe loss of neurons in the substantia nigra in association with Lewy pathology in at least the brainstem with or without limbic and cortical brain regions (25). When dementia had been diagnosed during life, donors fulfilling these criteria were classified as PDD. A diagnosis of dementia was made during life by a neurologist or geriatrician, or retrospectively based on neuropsychological test results showing disturbances in at least two core cognitive domains (26) or Mini-Mental State Examination (MMSE) score <20. Distinction between DLB and PDD was made based on the 1-year rule, where dementia presenting before or within 1 year of parkinsonism onset was diagnosed as DLB, and not included in this study (27). Cases diagnosed as having both PD and AD were also excluded from the study.

Genotyping

DNA was extracted from brain tissue. Genotyping was carried out on the Infinium® NeuroChip Consortium Array (Illumina, San Diego, CA USA) (28). Quality control was carried out in PLINK version 1.9 (29). Samples passing standard quality control, including filtering of variants and individuals based on call rate (< 0.95), Hardy-Weinberg equillibrium ($p < 0.000001$), relatedness (pi-hat > 0.125), excess heterozygosity (> 4SD from mean), sex-check and ancestry assessed by principal component plots, were imputed using the Michigan Imputation Server (30). We selected rs1800547 to discriminate between the *MAPT* H1 and H2 haplotypes, and used rs429358 and rs7412 to define the *APOE* ε2, ε3, and ε4 alleles as previously described (31, 32).

The NeuroChip array was also used to screen for known pathogenic mutations in relevant Mendelian PD genes. Covering the majority of definitely and probably pathogenic variants in the autosomal dominant genes *SNCA*, *LRRK2*, and *VPS35*, we identified no mutation carriers (**Supplementary Table 1**).

Statistical Analysis

All statistical analyses were carried out in R (version 4.0.2; http://www.r-project.org). Differences in baseline demographics and clinical variables between patients with PD and PDD were assessed using *t*-tests for continuous variables and chi-square tests for categorical variables. Ordinal variables (neuropathological scores) were compared using the Wilcoxon Rank Sum Test, while associations between neuropathology

TABLE 1 | Clinical characteristics of cases with Parkinson's disease non-demented (PDnD) and Parkinson's disease dementia (PDD).

	PDnD $N = 71$	PDD $N = 81$	*p*
Sex, male (%)	43 (60.6)	57 (70.4)	0.271
Age at disease onset, mean (SD)	61.3 (13.0)	64.2 (9.5)	0.117
Age at dementia onset, mean (SD)	-	73.7 (7.0)	-
Disease duration, mean (SD)	15.5 (7.7)	13.6 (6.7)	0.102
Motor dementia interval, mean (SD)	-	9.4 (5.8)	-
Dementia duration, mean (SD)	-	4.1 (2.8)	-
Age at death, mean (SD)	77.0 (9.3)	77.8 (6.5)	0.515

SD: standard deviation. P value from t-tests for continuous variables and chi-square tests for categorical variables (sex).

TABLE 2 | Risk variant frequencies and results from Cox proportional hazards regression models with age at onset, sex, and genetic principal components as covariates.

Variant	Frequency	HR	95% CI for HR	*p*
APOE ε4	PDnD: 0.11 PDD: 0.14	1.82	1.16–2.83	0.009*
MAPT H1/H1	PDnD: 0.68 PDD: 0.77	1.71	1.06–2.78	0.03*

APOE, Apoliporotein E; HR, hazard ratio; CI, confidence interval; MAPT, microtubule-associated protein tau.
**P value from the Wald test.*

and genotypes were measured by odds ratios using ordinal logistic regression adjusting for age at death and sex. For the survival analysis we used the R package "survival." Cox proportional hazards regression models were employed to assess the relationship between genotype and dementia onset. The event variable was presence of dementia. As time variable we used disease duration at dementia onset for PDD and disease duration at death for PD. Separate analyses were carried out for each risk locus, with sex, age at motor symptom onset and the first five genetic principal components as covariates. We estimated hazard ratio (HR) and the 95% confidence interval (CI). P values for each covariate were obtained from the Wald test. The results were visualized as Cox regression-adjusted curves using the R package "survminer." A combined plotting and testing approach was employed to check the proportional hazards assumptions. A $p < 0.05$ was used as significance threshold in this study.

RESULTS

One hundred sixty five donors (PD $n = 79$ and PDD $n = 86$) were identified. A total of 13 cases were excluded for missing clinical, neuropathological or genotype data, or failing quality control. A total of 152 cases (PD $n = 71$ and PDD $n = 81$) meeting clinical and neuropathological criteria were included in the final analysis. The demographic and clinical characteristics are displayed in **Table 1**. There were no significant differences in sex distribution, age at disease onset, disease duration or age at death between PD and PDD patients.

Braak α-synuclein stage ($p = 0.01$), Thal amyloid-β (Aβ) phase ($p = 0.001$), Braak NFT stage ($p = 0.003$) and CERAD neuritic plaque score ($p < 0.001$) were all higher in PDD compared to PD patients (**Figure 1** and **Supplementary Table 2**). Applying the NIA-AA criteria, intermediate or high AD co-pathology was present in 7% (5 of 67) of PD patients and 14% (11 of 80) of PDD patients. *APOE* ε4 was significantly associated with Thal Aβ phase (OR 4.85, $p < 0.001$) and CERAD neuritic plaque score (OR 4.97, $p < 0.001$), but not Braak NFT or Braak α-synuclein stage (**Supplementary Table 3**). The *MAPT* H1-haplotype was not significantly associated with any of the neuropathological scores.

In the Cox proportional hazards model the *APOE* ε4 allele was significantly associated with a shorter time between PD onset and diagnosis of PDD (HR per ε4 allele 1.82, 95 % CI 1.16–2.83, $p = 0.009$, **Table 2** and **Figure 2A**). When Thal Aβ phase or CERAD neuritic plaque score were added as covariates, the association with time to dementia was no longer significant ($p = 0.23$ and $p = 0.11$, respectively). The *MAPT* H1-haplotype was also significantly associated with a shorter time to dementia (HR per H1 haplotype 1.71, 95% CI 1.06–2.78, $p = 0.03$, **Table 2** and **Figure 2B**). Later age at onset was significantly associated with shorter time to dementia in both models (HR 1.09, 95% CI 1.06–1.12, $p < 0.001$).

DISCUSSION

In this study we explored the genetic effects of *MAPT* and *APOE* on onset of dementia in PD in a neuropathologically characterized cohort. With the advantages of definite diagnosis and clinical data from the patients' entire lifespan, we found that even in a small sample, both the *APOE* ε4 allele and the *MAPT* H1-haplotype were significantly associated with an accelerated onset of dementia in PD patients.

Several studies have examined the effects of *APOE* ε4 on cognitive decline and dementia in PD. Many of these have had cross-sectional design, and while some have demonstrated an association with *APOE* ε4 and lower cognitive performance (21), others have failed to do so (33). Consistent with our results, a previous study of PD patients demonstrated earlier development of dementia among *APOE* ε4-carriers (HR 1.90, 95% CI 1.05–3.44) (34). In line with our data, two recent meta-analyses reported an increased risk of dementia in PD patients who carried the *APOE* ε4 allele, although regional differences in effect size were noted (35, 36). Longitudinal studies have found associations with *APOE* ε4 and a more rapid cognitive decline measured on both screening instruments for global cognition (37, 38) and battery-style assessment of mental status (12, 39). In a recent GWAS on PD progression using longitudinal data from three large cohorts, the top hit for cognitive progression was rs429358 tagging *APOE* ε4 (40). In contrast, variants in the *APOE*-gene were not associated with cognitive decline or dementia at 3.5, 5, or 10 year follow-up in the CamPaIGN study, a UK incident cohort of PD patients (7, 20), or with shorter time to dementia

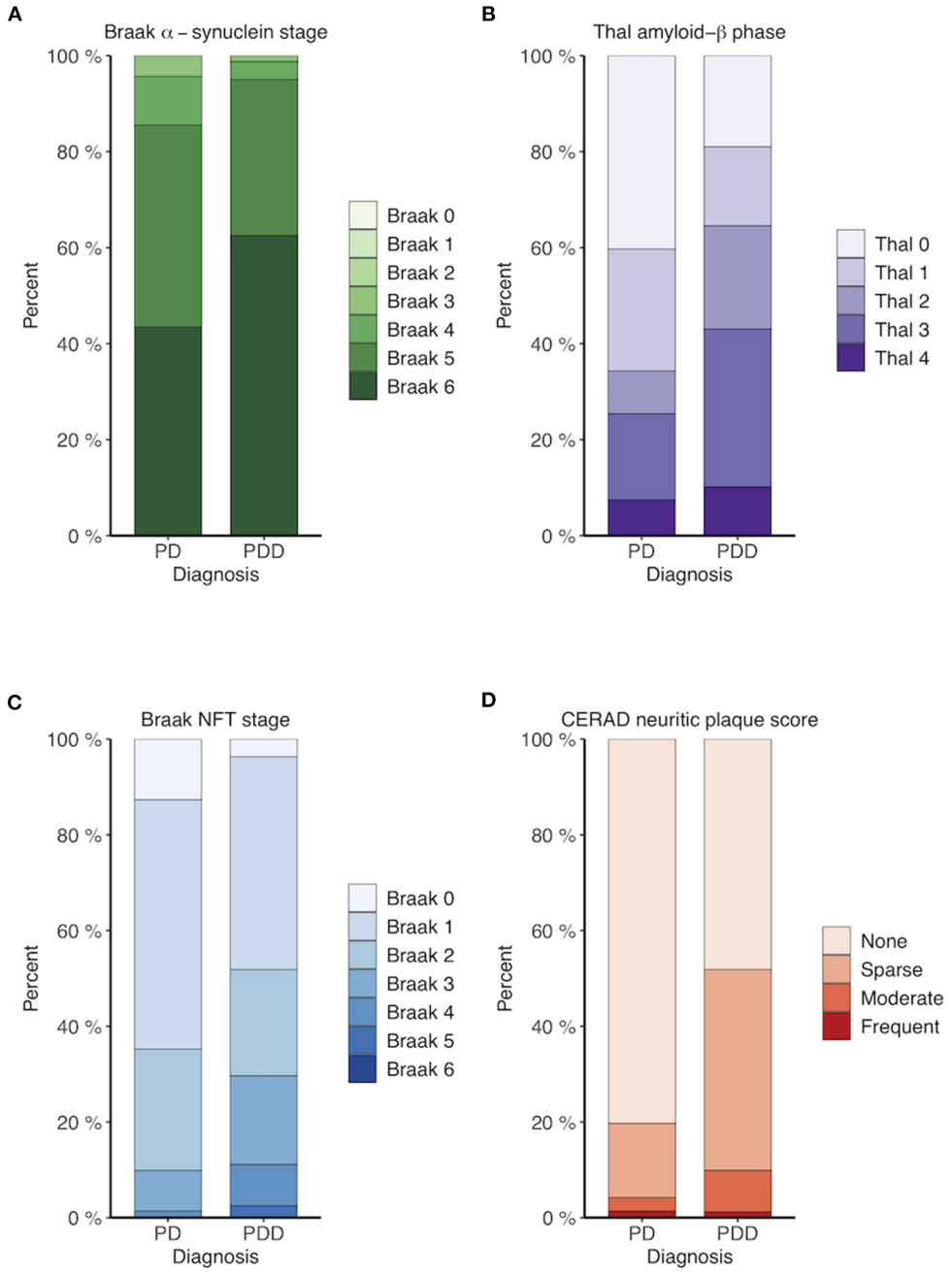

FIGURE 1 | Neuropathological scores for PD and PDD patients. **(A)** Braak α-synuclein stage. **(B)** Thal amyloid-β phase. **(C)** Braak neurofibrillary tangle (NFT) stage. **(D)** CERAD neuritic plaque score. PDD patients display more advanced LB, Aβ, and tau pathology compared to PD patients.

in another longitudinal study (41). While longitudinal designs represent a gold standard for tracking disease progression, they may be hampered by small sample size, short follow-up time and loss to follow-up. Taken together, the weight of evidence favors an effect of *APOE* on cognitive decline and dementia in PD, further supported by our results.

We also found a significant association between *MAPT* H1 and time to dementia in PD. This locus is less established than *APOE* in the previous literature on genetic risk factors of cognitive progression. The CamPaIGN study was the first to report an association between the *MAPT* H1/H1 genotype and cognitive decline in PD (19). The results were confirmed in the subsequent 5- and 10-year follow-up studies, supporting the *MAPT* H1/H1 genotype as predictive of dementia (7, 20). The association between *MAPT* genotype and PDD has later been replicated (42), while other studies have failed to do so

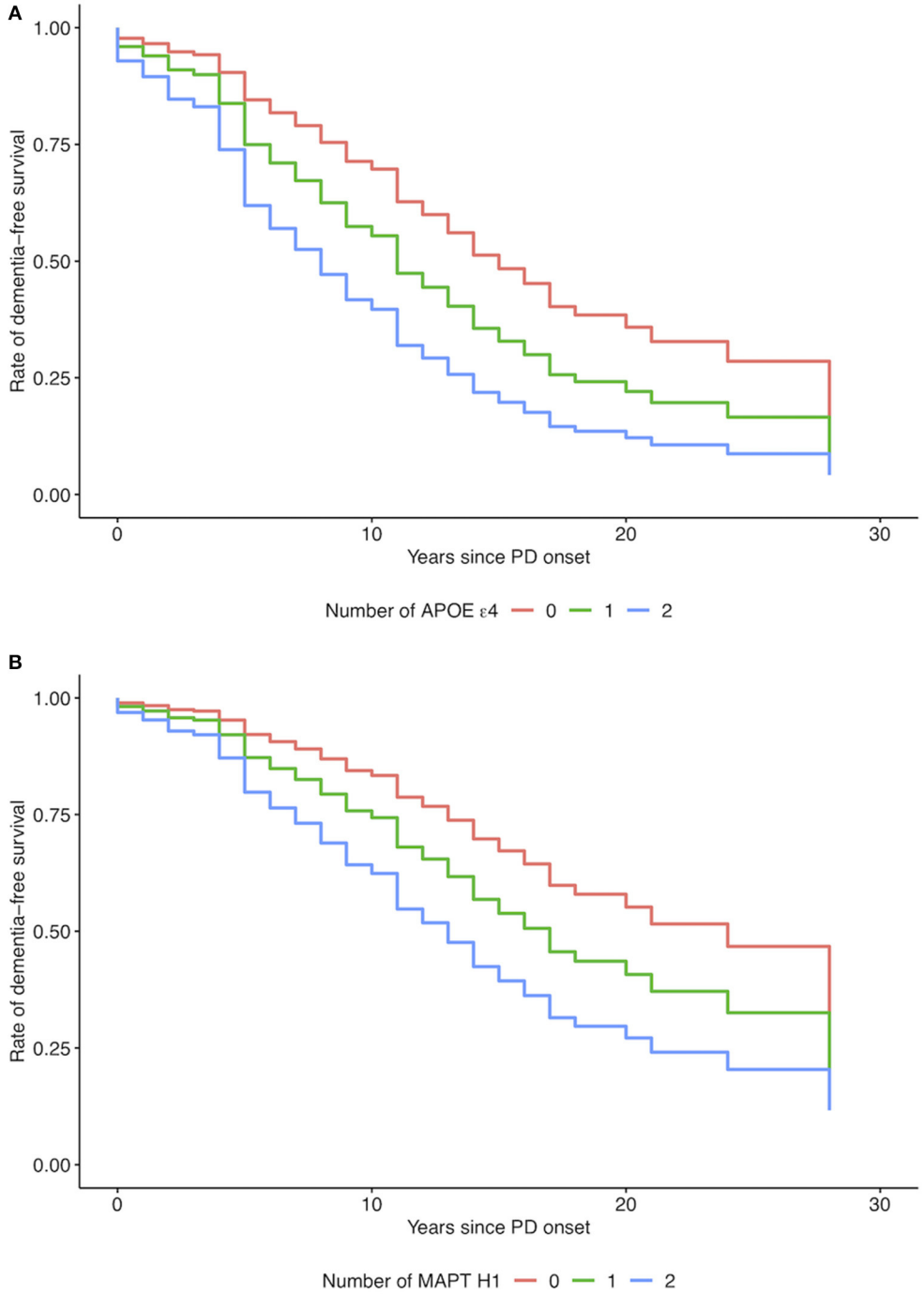

FIGURE 2 | Adjusted survival curves for Cox proportional hazards model. **(A)** Apolipoprotein E (*APOE*) ε4 (0 = negative, 1 = ε4 heterozygous, 2 = ε4 homozygous), and **(B)** Microtubule-associated protein tau (*MAPT*) H1-haplotype (0 = negative, 1 = heterozygous, 2 = homozygous).

(12, 21, 38). Contrary to our results, no association between *MAPT* H1/H1 genotype and dementia onset was found in a previous survival analysis of 298 PD patients where 59 progressed to dementia (34). A prospective investigation of 212 patients noted associations between *MAPT* H1 and specific cognitive outcome measures, but not with the overall rate of cognitive decline (12). The authors of this study hypothesized that the significant signal reported in the CamPaIGN study could represent an effect specific to early dementia development, as the CamPaIGN patients were included at diagnosis and assessed for progression to PDD at 3 years. Our data do not support this explanation of previously discrepant results, as the mean disease

duration at dementia onset in the PDD group was 9–10 years in our study.

The underlying mechanisms linking *APOE* and *MAPT* variants to dementia are unclear, however neuropathological studies suggests that protein aggregation is pivotal in this association. In our study *APOE* ε4 was significantly associated with both Thal Aβ phases and CERAD neuritic plaque scores, supporting that *APOE* ε4 exerts its genetic risk on dementia primarily through Aβ neuropathology. The *MAPT* H1 haplotype was not associated with any neuropathological scores in our study. Concomitant AD pathology (Aβ plaques and NFT) is found in variable amounts upon autopsy in PD and PDD brains, and is more prevalent in PDD compared to PD (17, 43, 44). This is indeed true for our cases, as neuropathological examination revealed significantly more advanced Thal Aβ phases, Braak NFT stages and CERAD neuritic plaque scores in PDD compared to PD samples.

Several lines of evidence support the role of cortical LB pathology as the major pathological driver of dementia in PD (17, 45), and in our study PDD donors had significantly more advanced Braak α-synuclein stages than PD donors. While it seems likely that *APOE* ε4 mediates dementia through an Aβ-dependent pathway, previous studies have also reported an effect of *APOE* ε4 on cognitive outcome and severity of cortical LB pathology in patients with low concomitant AD-pathology (46, 47). Corroborating these findings, two recent experimental studies have shown evidence that *APOE* ε4 may promote LB pathology independent of Aβ pathology (48, 49). In our results, however, the association with dementia was dependent on Aβ, as the signal was no longer significant when adjusting for Thal Aβ phase or CERAD neuritic plaque score.

While the presence of tau pathology has been correlated with reduced time to dementia (50), some evidence also supports that the *MAPT* H1-haplotype may influence the cortical LB burden (51), suggesting *MAPT* also may promote dementia in more than one way. This idea was not supported by our data, but we note that the size of our study provided limited statistical power to disentangle potentially complex correlations between genotype and various neuropathologies. We also acknowledge that although the H1 inversion haplotype on chromosome 17 is commonly named after *MAPT*, it contains a number of other genes, and the mechanism driving the association signal for PD risk has yet to be unequivocally established. Recent evidence suggest that rather than *MAPT*, the disease-relevant gene could be the neighboring *KANSL1*, which is involved in autophagy regulation (52).

The clinical diagnosis of PD can be challenging, with a diagnostic accuracy of 80.6% when pathological examination is used as the gold standard (53). The strength of this study lies in the neuropathological confirmation of diagnosis and the retrospective overview of the clinical disease course from the patients' entire lifespan. Some limitations of our study should be noted. First, clinical information was obtained by retrospective review of medical records posing a risk for information bias, in particular regarding approximation of timing of events. However, the timing of motor symptom onset and dementia onset observed in this study harmonize well with previous reports (17, 54). Second, we acknowledge that lack of extensive neuropsychological evaluation is a limitation. In theory, death and dementia may be competing events and potentially bias the estimated effect of genotypes on dementia development. *APOE* ε4 has been associated with decreased longevity, but we observed similar age at death in PD and PDD, and any theoretical bias from this effect would skew results in the opposite direction of our findings (55). Further corroboration of the genetic associations reported here is warranted, preferably in longitudinal cohorts. Third, given the limited sample size and statistical power of our study, we narrowly selected only two candidate loci among several previously reported as associated with cognition in PD. A broader perspective on the genetic architecture of PDD would have to consider the contribution from loci such as *SNCA*, *GBA*, *COMT* and potentially others (9), and ideally also the possibility of synergistic interactions between these.

In conclusion, our study adds to the growing evidence supporting the role for not only *APOE* ε4 but also the *MAPT* H1 haplotype in development of dementia in PD. Detecting significant associations in a small, but well-characterized neuropathological sample, we anticipate that larger genetic association studies of neuropathological phenotypes will be a fruitful strategy to further disentangle molecular mechanisms in neurodegenerative disorders. Ultimately, a better understanding of genotype-phenotype correlations may facilitate precision medicine in PD, improving risk prediction and patient stratification for novel targeted therapies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Committees for Medical and Health Research Ethics, Norway. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-AT performed statistical analyses and drafted the manuscript. HG and JR contributed clinical and neuropathological data. SH contributed to genotyping. MT contributed to study design and organized the study. WB contributed clinical and neuropathological data, contributed to study design and organized the study. LP designed and organized the study and contributed to genotyping, data analyses and drafting of the manuscript. All authors took part in critical revision of the manuscript and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Winter Y, von Campenhausen S, Arend M, Longo K, Boetzel K, Eggert K, et al. Health-related quality of life and its determinants in Parkinson's disease: results of an Italian cohort study. *Parkinsonism Relat Disord*. (2011) 17:265–9. doi: 10.1016/j.parkreldis.2011.01.003
2. Vossius C, Larsen JP, Janvin C, Aarsland D. The economic impact of cognitive impairment in Parkinson's disease. *Mov Disord*. (2011) 26:1541–4. doi: 10.1002/mds.23661
3. Fletcher P, Leake A, Marion MH. Patients with Parkinson's disease dementia stay in the hospital twice as long as those without dementia. *Mov Disord*. (2011) 26:919. doi: 10.1002/mds.23573
4. Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov Disord*. (2008) 23:837–44. doi: 10.1002/mds.21956
5. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch Neurol*. (2003) 60:387–92. doi: 10.1001/archneur.60.3.387
6. Foltynie T, Brayne CE, Robbins TW, Barker RA. The cognitive ability of an incident cohort of Parkinson's patients in the UK. The CamPaIGN study. *Brain*. (2004) 127:550–60. doi: 10.1093/brain/awh067
7. Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. *Brain*. (2009) 132:2958–69. doi: 10.1093/brain/awp245
8. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol*. (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
9. Fagan ES, Pihlstrom L. Genetic risk factors for cognitive decline in Parkinson's disease: a review of the literature. *Eur J Neurol*. (2017) 24:561–20. doi: 10.1111/ene.13258
10. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. (1997) 278:1349–56. doi: 10.1001/jama.278.16.1349
11. Guerreiro R, Ross OA, Kun-Rodrigues C, Hernandez DG, Orme T, Eicher JD, et al. Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study. *Lancet Neurol*. (2018) 17:64–74. doi: 10.1016/S1474-4422(17)30400-3
12. Morley JF, Xie SX, Hurtig HI, Stern MB, Colcher A, Horn S, et al. Genetic influences on cognitive decline in Parkinson's disease. *Mov Disord*. (2012) 27:512–8. doi: 10.1002/mds.24946
13. Pankratz N, Byder L, Halter C, Rudolph A, Shults CW, Conneally PM, et al. Presence of an APOE4 allele results in significantly earlier onset of Parkinson's disease and a higher risk with dementia. *Mov Disord*. (2006) 21:45–9. doi: 10.1002/mds.20663
14. Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, et al. A common inversion under selection in Europeans. *Nat Genet*. (2005) 37:129–37. doi: 10.1038/ng1508
15. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*. (2014) 46:989–93. doi: 10.1038/ng.3043
16. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet*. (2009) 41:1308–12. doi: 10.1038/ng.487
17. Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, et al. Neuropathologic substrates of Parkinson disease dementia. *Ann Neurol*. (2012) 72:587–98. doi: 10.1002/ana.23659
18. Geut H, Hepp DH, Foncke E, Berendse HW, Rozemuller JM, Huitinga I, et al. Neuropathological correlates of parkinsonian disorders in a large Dutch autopsy series. *Acta Neuropathol Commun*. (2020) 8:39. doi: 10.1186/s40478-020-00914-9
19. Goris A, Williams-Gray CH, Clark GR, Foltynie T, Lewis SJ, Brown J, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Ann Neurol*. (2007) 62:145–53. doi: 10.1002/ana.21192
20. Williams-Gray CH, Mason SL, Evans JR, Foltynie T, Brayne C, Robbins TW, et al. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *J Neurol Neurosurg Psychiatry*. (2013) 84:1258–64. doi: 10.1136/jnnp-2013-305277
21. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Hurtig HI, Van Deerlin VM, et al. APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease. *JAMA Neurol*. (2014) 71:1405–12. doi: 10.1001/jamaneurol.2014.1455
22. Alafuzoff I, Ince PG, Arzberger T, Al-Sarraj S, Bell J, Bodi I, et al. Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. *Acta Neuropathol*. (2009) 117:635–52. doi: 10.1007/s00401-009-0523-2
23. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. (2012) 123:1–11. doi: 10.1007/s00401-011-0910-3
24. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. (1992) 55:181–4. doi: 10.1136/jnnp.55.3.181
25. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol*. (2009) 8:1150–7. doi: 10.1016/S1474-4422(09)70238-8
26. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord*. (2007) 22:1689–707; quiz 837. doi: 10.1002/mds.21507
27. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology*. (2017) 89:88–100. doi: 10.1212/WNL.0000000000004058
28. Blauwendraat C, Faghri F, Pihlstrom L, Geiger JT, Elbaz A, Lesage S, et al. NeuroChip, an updated version of the NeuroX genotyping platform to rapidly screen for variants associated with neurological diseases. *Neurobiol Aging*. (2017) 57:247 e9–13. doi: 10.1016/j.neurobiolaging.2017.05.009

29. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. (2015) 4:7. doi: 10.1186/s13742-015-0047-8
30. Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. *Nat Genet*. (2016) 48:1284–7. doi: 10.1038/ng.3656
31. Mata IF, Yearout D, Alvarez V, Coto E, de Mena L, Ribacoba R, et al. Replication of MAPT and SNCA, but not PARK16–18, as susceptibility genes for Parkinson's disease. *Mov Disord*. (2011) 26:819–23. doi: 10.1002/mds.23642
32. Williams-Gray CH, Goris A, Saiki M, Foltynie T, Compston DA, Sawcer SJ, et al. Apolipoprotein E genotype as a risk factor for susceptibility to and dementia in Parkinson's disease. *J Neurol*. (2009) 256:493–8. doi: 10.1007/s00415-009-0119-8
33. Federoff M, Jimenez-Rolando B, Nalls MA, Singleton AB. A large study reveals no association between APOE and Parkinson's disease. *Neurobiol Dis*. (2012) 46:389–92. doi: 10.1016/j.nbd.2012.02.002
34. Huertas I, Jesus S, Garcia-Gomez FJ, Lojo JA, Bernal-Bernal I, Bonilla-Toribio M, et al. Genetic factors influencing frontostriatal dysfunction and the development of dementia in Parkinson's disease. *PLoS ONE*. (2017) 12:e0175560. doi: 10.1371/journal.pone.0175560
35. Pang S, Li J, Zhang Y, Chen J. Meta-analysis of the relationship between the APOE gene and the onset of parkinson's disease dementia. *Parkinsons Dis*. (2018) 2018:9497147. doi: 10.1155/2018/9497147
36. Sun R, Yang S, Zheng B, Liu J, Ma X. Apolipoprotein E polymorphisms and parkinson disease with or without dementia: a meta-analysis including 6453 participants. *J Geriatr Psychiatry Neurol*. (2019) 32:3–15. doi: 10.1177/0891988718813675
37. Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. *Lancet Neurology*. (2017) 16:66–75. doi: 10.1016/S1474-4422(16)30328-3
38. Paul KC, Rausch R, Creek MM, Sinsheimer JS, Bronstein JM, Bordelon Y, et al. APOE, MAPT, and COMT and parkinson's disease susceptibility and cognitive symptom progression. *J Parkinsons Dis*. (2016) 6:349–59. doi: 10.3233/JPD-150762
39. Tropea TF, Xie SX, Rick J, Chahine LM, Dahodwala N, Doshi J, et al. APOE, thought disorder, and SPARE-AD predict cognitive decline in established Parkinson's disease. *Mov Disord*. (2018) 33:289–97. doi: 10.1002/mds.27204
40. Tan MMX, Lawton MA, Jabbari E, Reynolds RH, Iwaki H, Blauwendraat C, et al. Genome-wide association studies of cognitive and motor progression in parkinson's disease. *Mov Disord*. (2020). doi: 10.1002/mds.28342
41. Phongpreecha T, Cholerton B, Mata IF, Zabetian CP, Poston KL, Aghaeepour N, et al. Multivariate prediction of dementia in Parkinson's disease. *NPJ Parkinsons Dis*. (2020) 6:20. doi: 10.1038/s41531-020-00121-2
42. Seto-Salvia N, Clarimon J, Pagonabarraga J, Pascual-Sedano B, Campolongo A, Combarros O, et al. Dementia risk in Parkinson disease: disentangling the role of MAPT haplotypes. *Arch Neurol*. (2011) 68:359–64. doi: 10.1001/archneurol.2011.17
43. Compta Y, Parkkinen L, O'Sullivan SS, Vandrovcova J, Holton JL, Collins C, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? *Brain*. (2011) 134:1493–505. doi: 10.1093/brain/awr031
44. Sabbagh MN, Adler CH, Lahti TJ, Connor DJ, Vedders L, Peterson LK, et al. Parkinson disease with dementia: comparing patients with and without Alzheimer pathology. *Alzheimer Dis Assoc Disord*. (2009) 23:295–7. doi: 10.1097/WAD.0b013e31819c5ef4
45. Kempster PA, O'Sullivan SS, Holton JL, Revesz T, Lees AJ. Relationships between age and late progression of Parkinson's disease: a clinico-pathological study. *Brain*. (2010) 133:1755–62. doi: 10.1093/brain/awq059
46. Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, et al. APOE epsilon4 increases risk for dementia in pure synucleinopathies. *JAMA Neurol*. (2013) 70:223–8. doi: 10.1001/jamaneurol.2013.600
47. Dickson DW, Heckman MG, Murray ME, Soto AI, Walton RL, Diehl NN, et al. APOE epsilon4 is associated with severity of Lewy body pathology independent of Alzheimer pathology. *Neurology*. (2018) 91:e1182–95. doi: 10.1212/WNL.0000000000006212
48. Zhao N, Attrebi ON, Ren Y, Qiao W, Sonustun B, Martens YA, et al. APOE4 exacerbates alpha-synuclein pathology and related toxicity independent of amyloid. *Sci Transl Med*. (2020) 12:eaay1809. doi: 10.1126/scitranslmed.aay1809
49. Davis AA, Inman CE, Wargel ZM, Dube U, Freeberg BM, Galluppi A, et al. APOE genotype regulates pathology and disease progression in synucleinopathy. *Sci Transl Med*. (2020) 12:eaay3069. doi: 10.1126/scitranslmed.aay3069
50. Irwin DJ, Grossman M, Weintraub D, Hurtig HI, Duda JE, Xie SX, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol*. (2017) 16:55–65. doi: 10.1016/S1474-4422(16)30291-5
51. Robakis D, Cortes E, Clark LN, Vonsattel JP, Virmani T, Alcalay RN, et al. The effect of MAPT haplotype on neocortical Lewy body pathology in Parkinson disease. *J Neural Transm*. (2016) 123:583–8. doi: 10.1007/s00702-016-1552-3
52. Soutar MPM, Melandri D, Annuario E, Welsh NJ, D'Sa K, Guelfi S, et al. *Regulation of Mitophagy by the NSL Complex Underlies Genetic Risk for Parkinson's Disease at Chr16q11.2 and on the MAPT H1 Allele*. BioRxiv (2020). Available online at: https://www.biorxiv.org/content/10.1101/2020.01.06.896241v1.full (accessed December 27, 2020). doi: 10.1101/2020.01.06.896241
53. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: a systematic review and meta-analysis. *Neurology*. (2016) 86:566–76. doi: 10.1212/WNL.0000000000002350
54. Prokopenko I, Miyakawa G, Zheng B, Heikkinen J, Petrova Quayle D, Udeh-Momoh C, et al. Alzheimer's disease pathology explains association between dementia with Lewy bodies and APOE-epsilon4/TOMM40 long poly-T repeat allele variants. *Alzheimers Dement*. (2019) 5:814–24. doi: 10.1016/j.trci.2019.08.005
55. Blauwendraat C, Heilbron K, Vallerga CL, Bandres-Ciga S, von Coelln R, Pihlstrom L, et al. Parkinson's disease age at onset genome-wide association study: defining heritability, genetic loci, and alpha-synuclein mechanisms. *Mov Disord*. (2019) 34:866–75. doi: 10.1002/mds.27659

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Association of *SNCA* Parkinson's Disease Risk Polymorphisms With Disease Progression in Newly Diagnosed Patients

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Objectives: To evaluate the impact of *SNCA* polymorphisms originally identified as risk factors for Parkinson's disease (PD) on the clinical presentation and progression of the disease in a large cohort of population-based patients with incident PD.

Methods: Four hundred thirty-three patients and 417 controls from three longitudinal cohorts were included in the study. Disease progression was recorded annually for up to 9 years using the Unified Parkinson's Disease Rating Scale (UPDRS) or Mini-Mental State Examination. Genotypes for five variants within the *SNCA* locus (rs2870004, rs356182, rs5019538, rs356219, and rs763443) were determined. We studied the association between each variant and disease progression using linear mixed-effects regression models.

Results: The clinical profile of the patients with PD at the point of diagnosis was highly uniform between genotype groups. The rs356219-GG genotype was associated with a higher UPDRS II score than A-allele carriers ($\beta = 1.52$; 95% confidence interval 0.10–2.95; $p = 0.036$), but no differences were observed in the rate of progression of the UPDRS II scores. rs356219-GG was also associated with a faster annual change in Mini-Mental State Examination score compared with A-carriers ($\beta = 0.03$; 95% confidence interval 0.00–0.06; $p = 0.043$).

Conclusions: We show that the known PD-risk variant rs356219 has a minor effect on modifying disease progression, whereas no differences were associated with rs2870004, rs356182, rs5019538, and rs763443. These findings suggest that *SNCA* variants associated with PD risk may not be major driving factors to the clinical heterogeneity observed for PD.

Keywords: *SNCA*, Parkinson's disease, disease progression, genetic association, cognitive impairment

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the core motor symptoms, bradykinesia, resting tremor, rigidity, and postural instability, though often accompanied by a wide spectrum of additional motor and non-motor signs (1). The severity and rate of progression of clinical symptoms in PD are highly variable between patients. Some patients experience mild motor decline and non-motor symptoms, whereas some experience fast deterioration in motor symptoms and prominent non-motor symptoms. These differences are in part predicted by sex, age at diagnosis, motor phenotype, and disease severity (2). Similarly, the timing and rate of cognitive decline vary widely among individuals with PD (3), and certain measures, including older age or differences in motor phenotype at diagnosis, predict a more rapid rate of cognitive decline in subgroups of patients (4). The observed heterogeneity can pose prognostic difficulties in a clinical setting, compromising both the planning of appropriate patient management and clinical trial design.

Although heterogeneity in PD is widely recognized, the biological factors modulating the progression remain largely unknown. Association studies have shown that common genetic variance contributes to the risk of developing idiopathic PD (5, 6), and some of these same variants may modify the progression of clinical symptoms (7, 8). The *SNCA* gene encodes α-synuclein, the main protein component of Lewy bodies, which are the pathological hallmark of sporadic PD (9), and genetic variants in the *SNCA* region repeatedly have the strongest association with PD risk in genome-wide association studies (GWASs) (5, 10–12). To date, data on the impact of these *SNCA* polymorphisms on PD progression are scarce, and further investigation in longitudinal studies of patients with PD is needed to refine the link between the genetic variance in *SNCA* and disease course.

Here, we explored the effects of five *SNCA* single nucleotide polymorphisms (SNPs), rs2870004, rs356182, rs5019538, rs356219, and rs763443, on the presentation of PD at the time of diagnosis and the progression of the motor, functional, and cognitive impairment over up to 9 years of regular follow-up, in three deeply phenotyped, longitudinal PD cohorts from Northern Europe.

METHODS

Study Participants

Three longitudinal cohorts were included in the study: the Norwegian ParkWest study (13), the Parkinsonism Incidence in North-East Scotland (PINE) study (14), and the Swedish New Parkinson Patient in Umeå (NYPUM) study (15). These cohorts provide on-going prospective follow-up of population-based incidence studies of all newly diagnosed PD patients identified in specific geographic regions, initiated between 2002 and 2009. Diagnosis of PD was made according to UK Brain Bank criteria by a neurologist specialized in movement disorders at the baseline visit with continued reassessment at follow-up visits. Participant recruitment and follow-up are summarized in **Supplementary Figure 1**. Briefly, 605 patients were enrolled: 212 in ParkWest, 211 in PINE, and 182 in NYPUM. Of these, 70 have had a diagnosis other than PD during follow-up, 7 did not consent to follow-up, 57 did not consent to genotyping, and 38 have no DNA sample available, or DNA could not be genotyped. Five hundred twenty-three control subjects were recruited from the same areas: 201 in ParkWest, 266 in PINE, and 56 in the NYPUM study. Of these, 70 have no DNA sample available, or DNA could not be genotyped, 30 did not consent to genotyping, and 6 were diagnosed with PD during follow-up. The remaining 433 PD patients and 417 controls consented to regular follow-up and were eligible for this study. At the time of the study, data from clinical visits for a period of up to 9 years were available (**Supplementary Figure 1**).

Respective ethical committees approved studies: The Western Norway Regional Committee for Medical and Health Research Ethics, the Multi-Centre Research Ethics Committee for Scotland, and the Regional Ethics Review Board in Umeå. All participants signed written informed consent.

Clinical Assessment

The clinical assessments have been described in detail, and the same procedures were followed for each cohort (13–15). At baseline, general medical and neurological examinations and semi-structured interviews were performed for all participants to establish medical, drug, and family history (first-degree relative with PD, self-reported). No cases of familial PD were recorded. Patients with PD were assessed at baseline and annual follow-up visits using Hoehn and Yahr staging (16), the Unified Parkinson's Disease Rating Scale (UPDRS) II (activities of daily living) and part III (motor examination) (17), and the Mini-Mental State Examination (MMSE) (18) (in ParkWest, MMSE was evaluated at baseline, the first annual visit and every second year after that), and controls were assessed at baseline and follow-up visits using the MMSE. Home visits were offered to those unable or unwilling to come to the clinic to minimize attrition bias.

Based on subscores of UPDRS III (motor examination), we derived measures of tremor (sum of items 20 and 21), rigidity (sum of item 22), bradykinesia (sum of items 23, 24, 25, 26, and 31), and axial impairment (sum of items 27, 28, 29, and 30). We calculated levodopa-equivalent doses (LEDs) in accordance with published recommendations (19).

Genotyping of *SNCA* Variants

We selected five *SNCA* polymorphisms (rs2870004, rs356182, rs5019538, rs356219, and rs763443) identified as contributing to a person's risk of developing PD in the largest genome-wide association studies (5, 20) and the largest dedicated genetic study of *SNCA* (6) to date.

Genomic DNA was extracted from peripheral blood using standard methods. Allelic discrimination analysis was performed using predesigned TaqMan SNP genotyping assay (Thermo Fisher Scientific) for rs2870004 (Assay ID: C__26455957_20), rs356182 (C___3208989_10), rs356219 (C___1020193_10), and rs763443 (C___1902284_10) and a custom assay for rs5019538 (Thermo Fisher Scientific). The amplification reactions were performed using the ABI PRISM 7300 Real-Time PCR System

(Applied Biosystems) with SDS v1.4 software. The call rates were >99% for each SNP, and the concordance rate was 98%.

Statistical Methods

All between-group comparisons were performed using IBM SPSS Statistics version 26.0 (Armonk, NY). The regression analysis was done in R version 4.0.2. No differences were observed between the unadjusted and adjusted analyses unless otherwise stated. Two-tailed p-values < 0.05 were considered significant, and correction for multiple testing was not performed in this exploratory analysis. As there is insufficient evidence regarding the best genetic model to analyze the effect of *SNCA* SNPs on disease progression, we took an exploratory approach and included both the recessive and dominant genetic models in the analysis plan.

Baseline Analysis

Continuous data were summarized using descriptive statistics, whereas categorical data were reported as counts and percentages. Between-group differences in demographic variables were assessed for significance using the Mann–Whitney U tests and χ^2 tests, as appropriate. Logistic regression (categorical outcome) or linear regression (continuous outcome) was used to test the association between *SNCA* genotypes and PD risk or clinical outcomes at baseline, without and with adjustment for age at baseline and sex. The results of multivariable analyses were presented as odds ratios (ORs) with 95% confidence intervals (CIs) and p-values.

Longitudinal Analysis

We investigated the association between each of the *SNCA* genotypes and disease progression using three different linear mixed models. The outcome variables for the three models were repeated measurements of UPDRS part II, UPDRS part III, or MMSE total score. MMSE total scores were transformed using log (30 – MMSE + 1) to achieve normality. Time in the study (as a continuous variable) and the *SNCA* genotype (as a binary categorical variable) were included as fixed effects. Patient IDs were included as random intercepts. The interaction between time and the genotype was included as a fixed effect to assess how the *SNCA* genotype influenced disease progression. The analyses were performed without adjustment and with adjustment for the following variables as fixed effects: study cohort, sex, age at baseline, and duration of motor symptoms at baseline. For MMSE, years of education were also included as a fixed effect. For UPDRS II and III, the effect sizes were similar after additional adjustment for LED at each visit (data not shown). Each model had a first-order autoregressive covariance structure. The plot of predictive margins was created using the command margins in Stata 16.00.

RESULTS

Baseline Characterization of Study Population

Of the total 850 participants eligible for the study, 433 were patients with PD, and 417 were control subjects (**Table 1**). The mean age at baseline for PD patients was 69.9 ± 9.6 years, with the proportion of males 60.7%. At the baseline examination, the patients and controls differed with regard to the level of family history of PD ($p < 0.001$), the years of education ($p = 0.005$), and the MMSE score ($p < 0.001$) but not the distribution of sex or age.

TABLE 1 | Baseline demographic and clinical characteristics of patients and controls included in study.

Variable[a]	NC	PD	*p* value
Total, *N*	417	433	
Male, *N* (%)	242 (58.0)	263 (60.7)	0.42
Age at baseline, years	69.6 (±10.2)	69.9 (±9.6)	0.68
Age at first motor symptoms, years		67.9 (±9.6)	
Positive family history, *N* (%)	25 (6.9)	56 (13.0)	**0.005**
Education, years	12.5 (±3.0)	11.2 (±3.6)	**<0.001**
UPDRS II		9.2 (±4.8)	
UPDRS III		24.7 (±11.4)	
Hoehn and Yahr		2.1 (±0.7)	
MMSE, median (IQR)	29.0 (2.0)	29.0 (3.0)	**<0.001**

NC, normal control; PD, Parkinson's disease; N, count; UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental State Examination, IQR, interquartile range. Values presented as mean (± standard deviation) unless stated otherwise.
Significant p-values ($p < 0.05$) indicated in bold. P-values calculated using χ^2 test, Mann–Whitney U-test, as appropriate.
[a]Missing data for family history, 1 PD and 56 NC; education, 20 NC; UPDRS II, 4 PD; MMSE, 76 PD and 7 NC.

SNCA Variants and Risk of Parkinson's Disease

The distributions of the five *SNCA* SNP genotypes and the minor allele frequencies in PD patients and controls are summarized in **Supplementary Table 1**. No deviations from Hardy–Weinberg equilibrium were observed for the allele frequencies in patients and controls. Logistic regression analysis was performed to determine if genotype status was associated with a higher incidence of PD, using either the recessive or dominant model (**Table 2**). In unadjusted analysis, rs356182-G allele carrier status was significantly associated with increased risk of PD compared with noncarriers (OR = 1.33; 95% CI 1.01–1.75; $p = 0.046$). This remained significant after adjustment for age at baseline and sex (OR = 1.32; 95% CI 1.00–1.75; $p = 0.049$). No other significant associations were identified between genotype status and risk of PD.

SNCA Variants and Baseline Parkinson's Disease Profile

Analysis of the association of *SNCA* genotypes and the demographic characteristics of the patients with PD showed no significant differences between groups, except for a higher mean number of years of education for the carriers of rs2870004-TT genotype as compared with rs2870004 A-allele carriers (13.2 ± 4.4 vs. 11.1 ± 3.5 years; $p = 0.021$, **Supplementary Table 2**). At the time of PD diagnosis, the rs356219-GG genotype was associated with higher UPDRS II scores ($p = 0.017$) (**Supplementary Table 2**). No differences were shown between baseline clinical presentation of PD and *SNCA* genotypes for the other SNPs investigated (**Supplementary Tables 2, 3**).

TABLE 2 | Comparison of genotypes of each *SNCA* variant between PD patients and controls.

SNP	Genotype	PD, *N* (%)	NC, *N* (%)	OR	(95% CI)	*p*-value
rs2870004						
Recessive	AA + AT	411 (95.1)	397 (95.7)	1.00		
	TT	21 (4.9)	18 (4.3)	1.13	(0.59–2.15)	0.71
Dominant	AA	275 (63.7)	258 (62.2)	1.00		
	AT + TT	157 (36.3)	157 (37.8)	0.94	(0.71–1.24)	0.66
rs356182						
Recessive	AA + AG	363 (83.8)	361 (87.2)	1.00		
	GG	70 (16.2)	53 (12.8)	1.31	(0.89–1.93)	0.17
Dominant	AA	151 (34.9)	172 (41.5)	1.00		
	AG + GG	282 (65.1)	242 (58.5)	1.32	(1.00–1.75)	**0.049**
rs5019538						
Recessive	AA + AG	382 (88.4)	371 (89.4)	1.00		
	GG	50 (11.6)	44 (10.6)	1.14	(0.72–1.70)	0.64
Dominant	AA	193 (44.7)	199 (48.0)	1.00		
	AG + GG	239 (55.3)	216 (52.0)	1.14	(0.87–1.50)	0.34
rs356219						
Recessive	AA + AG	354 (81.8)	355 (85.3)	1.00		
	GG	79 (18.2)	61 (14.7)	1.30	(0.90–1.87)	0.16
Dominant	AA	141 (32.6)	155 (37.3)	1.00		
	AG + GG	292 (67.4)	261 (62.7)	1.23	(0.92–1.63)	0.16
rs763443						
Recessive	CC + CT	331 (76.6)	317 (76.2)	1.00		
	TT	101 (23.4)	99 (23.8)	0.98	(0.71–1.34)	0.89
Dominant	CC	122 (28.2)	105 (25.2)	1.00		
	CT + TT	310 (71.8)	311 (74.8)	0.86	(0.63–1.16)	0.32

SNP, single nucleotide polymorphism; PD, Parkinson's disease; N, count; NC, normal control; OR, odds ratio; CI, confidence intervals.
Significant p-values indicated in bold. P-values calculated using logistic regression with adjustment.

Effect of *SNCA* Genotypes on Motor and Functional Impairment

Linear mixed-effects regression analysis with adjustment for age, sex, study cohort, and duration of motor symptoms at baseline revealed that there were no significant differences between any of the *SNCA* genotypes and the rate of annual changes in UPDRS II or III scores measured for up to 9 years (**Table 3**; **Supplementary Table 4**). Further adjustment for time-varying LED did not change the significance of the results (data not shown). However, the linear mixed-effects regression analysis reproduced the association between the rs356219 genotype and UPDRS II scores at baseline, with the carriers of rs356219-GG genotype having a 1.52-point higher UPDRS II score during all 9 years of the study in comparison with the carriers of A-allele in adjusted analysis ($\beta = 1.52$; 95% CI 0.10–2.95; $p = 0.036$) (**Table 3**, **Figure 1A**).

rs356219 Is Associated With Faster Cognitive Decline

The rs356219 genotype was associated with a difference in the rate of annual change in MMSE score (**Table 3**, **Figure 1B**), with carriers of rs356219-GG predicted to experience a faster decrease in MMSE scores over the 9 years of follow-up compared with an A-allele carrier ($\beta = 0.03$; 95% CI 0.00–0.06; $p = 0.043$) after the adjustment for study cohort, sex, age at baseline, duration of motor symptoms at baseline, and years of education. For MMSE, the estimated coefficients cannot be directly interpreted in terms of the annual change in performance, as the data were transformed before analysis. The adjusted model predicts that *SNCA* rs356219-GG carriers would experience on average a fall from 28.9 to 25.1 (95% CI 23.9–26.1) points during 9 years, whereas the MMSE score of A-allele carriers would fall from 29.0 to 26.6 (95% CI 26.2–27.0; $p = 0.043$). Analysis of the association of rs356219 with the rate of change in MMSE score in the control group showed no association between rs356219-GG status and the annual change in MMSE (data not shown). We did not observe any significant effects of the other *SNCA* genotypes on cognitive impairment measured using MMSE (**Table 3**; **Supplementary Table 4**).

DISCUSSION

In this study, we explored the effect of five *SNCA* polymorphisms linked to PD risk on the progression of the disease. Based on the prospective assessment of three population-based incident cohorts of patients with PD, we show an association between rs356219 and the rate of cognitive decline measured from the time of PD diagnosis. The predicted size of the effect of rs356219 on the annual change in cognitive impairment was small, and further, the four other PD risk SNPs investigated had no effect on longitudinal measures of disease severity. Together, these data suggest that although common variants in *SNCA* are important risk factors for PD, these SNPs play a minor role in modifying the progression of PD.

Patients with the rs356219-GG genotype experienced a faster rate of cognitive decline measured by MMSE than A-allele carriers over the 9 years of follow-up. No differences between genotype groups and the annual change in MMSE score were observed for the control subjects over the same follow-up period, indicating that this effect is disease-specific. Similar to our findings, Luo et al. (21) found an association between the rate of cognitive impairment and rs356219. However, in their study of patients with PD from China, carriers of the G-allele had a decreased risk of cognitive decline, indicating that the G allele might have a protective role in this population (21). In an analysis of European patients with PD, Goris et al. reported no association of rs356219 with the annual change in MMSE (22). Notably, this study only followed participants for the first 3.5 years from diagnosis, and based on the predictions from our population, a longer follow-up period would be required to observe the effects of rs356219 on changes in MMSE. In keeping with our findings at the time of PD diagnosis, no difference was observed between rs356219 and mild cognitive impairment in newly diagnosed patients (23). Further, in patients in the later stages of PD (average disease duration at examination 8.8 years), carriers of the rs356219-G allele were at higher risk of cognitive impairment (24). However, a large study analyzing a broad battery of cognitive tests found no association with this SNP in PD patients with a mean of 6.6 years disease duration (25).

Our models predicted that patients with the rs356219-GG genotype would experience on average a one and a half-point larger decrease in MMSE score compared with rs356219-A

TABLE 3 | Association between annual change in clinical assessments of PD and *SNCA* polymorphisms assuming a recessive model.

	rs2870004[a] AA + AT vs. TT		rs356182[a] AA + AG vs. GG		rs5019538[a] AA + AG vs. GG		rs356219[a] AA + AG vs. GG		rs763443[a] CC + CT vs. TT	
	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
UPDRS II[b]										
Main effect	−0.32 (−2.87; 2.23)	0.81	1.02 (−0.48; 2.52)	0.18	0.82 (−0.91; 2.54)	0.35	1.52 (0.10; 2.95)	**0.036**	−0.50 (−1.80; 0.79)	0.44
Interaction with time	0.13 (−0.30; 0.55)	0.56	0.09 (−0.18; 0.36)	0.52	−0.08 (−0.38; 0.23)	0.63	0.04 (−0.22; 0.29)	0.79	−0.01 (−0.24; 0.21)	0.92
UPDRS III[b]										
Main effect	0.27 (−4.82; 5.36)	0.92	0.86 (−2.13; 3.86)	0.57	−0.47 (−3.91; 2.97)	0.79	1.51 (−1.34; 4.36)	0.30	−0.11 (−2.70; 2.47)	0.93
Interaction with time	−0.24 (−1.03; 0.54)	0.55	0.17 (−0.33; 0.66)	0.51	−0.14 (−0.70; 0.41)	0.61	0.08 (−0.39; 0.55)	0.74	−0.29 (−0.70; 0.12)	0.17
MMSE[c]										
Main effect	0.03 (−0.29; 0.35)	0.86	0.06 (−0.12; 0.25)	0.50	0.01 (−0.21; 0.22)	0.95	0.03 (−0.14; 0.21)	0.70	−0.12 (−0.27; 0.04)	0.15
Interaction with time	−0.01 (−0.05; 0.04)	0.80	0.02 (−0.01; 0.05)	0.13	0.00 (−0.03; 0.03)	0.90	0.03 (0.00; 0.06)	**0.043**	0.00 (−0.03; 0.02)	0.94

UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental State Examination; CI, confidence intervals.
Significant p-values ($p < 0.05$) indicated in bold. Main effect indicates effect of carrier status on intercept, and interaction with time indicates effect of carrier status on slope (change in value per year) of model.
[a]*Genotypes grouped according to a recessive genetic model and association with change in clinical assessments assessed using linear mixed models. Reference group is given first.*
[b]*Adjusted for study cohort, sex, age at baseline, duration of motor symptoms at baseline.*
[c]*Adjusted for study cohort, sex, age at baseline, duration of motor symptoms at baseline, and years of education at baseline. MMSE score transformed before analysis.*

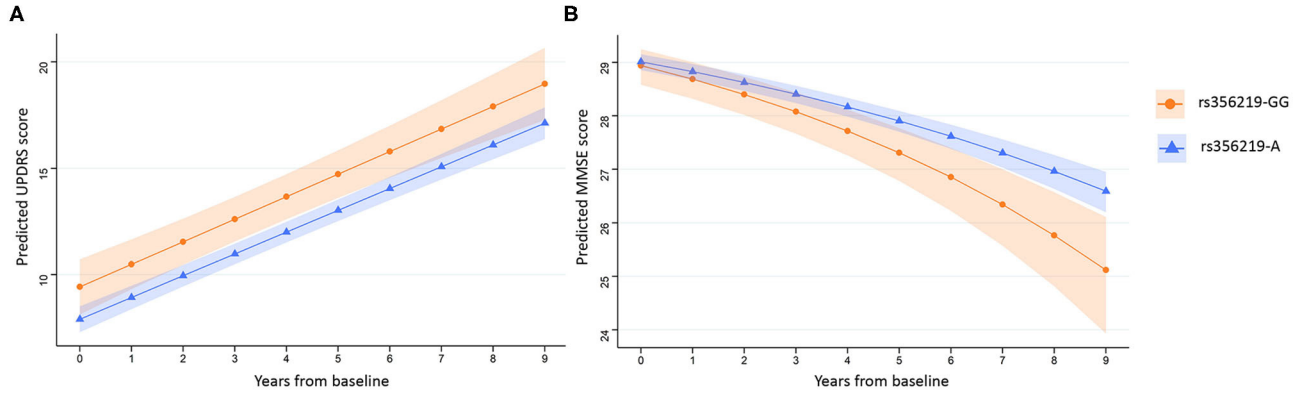

FIGURE 1 | Prediction of UPDRS II and MMSE scores over time. Average predicted UPDRS II **(A)** and MMSE **(B)** scores with confidence bands for first 9 years after diagnosis of PD for rs356219-GG allele carriers (orange, circles) and rs356219-A allele carriers (blue, triangles). UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental State Examination.

carriers after 9 years. This small difference suggests that the rs356219 genotype alone is not a strong predictor of cognitive decline in individuals with PD; however, subtle changes of cognitive function may prove to be clinically meaningful in combination with other risk factors. Recently, the rs356219 *SNCA* variant has been suggested to interact in a synergic manner with *GBA* variants to alter the disease course. In a longitudinal study of newly diagnosed patients with PD, rs356219-GG was associated with faster progression to Hoehn and Yahr stage 3 in *GBA*-associated PD but had no detectable effect in noncarriers of a *GBA* variant (26). This indicates that a synergistic interaction between different genetic risk variants could amplify their effect on disease outcomes.

In this study, we did not observe any significant associations between each of the five *SNCA* SNPs and the development of motor or functional impairment. Few studies have analyzed the effect of *SNCA* SNPs on the annual change in UPDRS scores (27, 28), and previously, only the rs356182-GG genotype has been linked to the rate of motor progression, with GG carriers exhibiting a slower rate of change in UPDRS III scores (28). A notable difference to our study is that the patients were not followed from the time of diagnosis but were first examined after a median disease duration of 7 years, and it is possible that the effect of this SNP on modifying motor impairment is more prominent in the later stages of PD. This highlights one of the many difficulties in modeling the relationship between measures of motor impairment and genetic variants, as, in addition to disease duration, the results can also be impacted by differences in the number and frequency of study visits and the length of follow-up. In our study, subjects were followed annually from the time of diagnosis. Although our findings support that common *SNCA* SNPs do not contribute to variability in the rate of motor impairment, it will be important to follow up these findings in the later phases of the disease.

Each of the *SNCA* variants included in our study has been previously linked to the risk of PD (5, 6). In our study population, we observed an association between rs356182 and disease risk under the dominant model. This variant appeared as the top hit with the strongest association with PD risk in consecutive GWASs (5, 10, 11). Two of the SNPs included in our study, rs2870004 and rs5019538, were only recently identified as risk variants for PD in the largest GWASs performed to date (5) or a comprehensive *SNCA* locus study (6) and have not previously been studied in the context of PD phenotype. In this study, we present the first assessment of the disease-modifying effect of these SNPs and find that they do not have a major impact on the presentation or progression of PD. This is in keeping with recent work showing that a genetic risk score based on 31 SNPs associated with the risk of PD was not associated with changes in clinical progression (8).

The present study has notable strengths. All cohorts included in our work are population-based and recruited incident cases representative of the general PD population, as opposed to general research studies, which are generally unrepresentative of the population age distribution of PD (29). Every center used the same standardized diagnostic criteria for PD and clinical outcomes, and patients were all recruited early in the disease and followed prospectively with more than 3,000 study visits. The rate of attrition for reasons other than death was very low, and potential selection bias was minimized by introducing remote visits for those unable to attend clinic visits. The study also has limitations. Firstly, we were only able to include 433 patients with PD and 417 controls, limiting the power of the study to detect small effects, although notably, our study is the largest to date to study the effects of these *SNCA* polymorphisms on disease progression. Furthermore, we acknowledge that our exploratory approach, including five *SNCA* SNPs and two genetic models, increases the risk of false positives. Therefore, the significance of our findings should be interpreted with caution, and this work should be validated. Further, we did not address the potential confounding effect of death on the association with disease outcomes in carriers of these *SNCA* SNPs.

In summary, we report the comprehensive analysis of five *SNCA* PD risk SNPs and their association with long-term disease progression in the largest study to date of patients with PD followed from diagnosis. We find that rs356219 is linked with subtle differences in PD clinical measures, whereas no differences are associated with rs2870004, rs356182, rs5019538, and rs763443, suggesting that these genetic variants do not play a large role in modifying disease progression. This illustrates that PD is a complex disease in which the mechanisms underlying the association of the *SNCA* GWAS signals with PD risk may not be driving factors to the large clinical heterogeneity observed throughout the disease.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the relevant ethical committees: The Western Norway Regional Committee for Medical and Health Research Ethics, the Multi-Centre Research Ethics Committee for Scotland and, the Regional Ethics Review Board in Umeå. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM-G, AS, CP, and JL: design of the study. AS, CP, and JM-G: writing the manuscript. LF, CC, GA, and AM: clinical data collection/organization of research project. CP and AS: molecular assays. AS and AU: statistical data analysis. AU, JL, LF, CC, GA, and AM: critical review of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.620585/full#supplementary-material

REFERENCES

1. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. *J Neurochem.* (2016) 139 (Suppl. 1):318–24. doi: 10.1111/jnc.13691
2. Lawton M, Ben-Shlomo Y, May MT, Baig F, Barber TR, Klein JC, et al. Developing and validating Parkinson's disease subtypes and their motor and cognitive progression. *J Neurol Neurosurg Psychiatry.* (2018) 89:1279–87. doi: 10.1136/jnnp-2018-318337
3. Aarsland D, Creese B, Politis M, Chaudhuri KR, Ffytche DH, Weintraub D, et al. Cognitive decline in Parkinson disease. *Nat Rev Neurol.* (2017) 13:217–31. doi: 10.1038/nrneurol.2017.27
4. Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. *Brain.* (2009) 132 (Pt 11):2958–69. doi: 10.1093/brain/awp245
5. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
6. Pihlstrøm L, Blauwendraat C, Cappelletti C, Berge-Seidl V, Langmyhr M, Henriksen SP, et al. A comprehensive analysis of SNCA-related genetic risk in sporadic parkinson disease. *Ann Neurol.* (2018) 84:117–29. doi: 10.1002/ana.25274
7. Iwaki H, Blauwendraat C, Leonard HL, Kim JJ, Liu G, Maple-Grodem J, et al. Genomewide association study of Parkinson's disease clinical biomarkers in 12 longitudinal patients' cohorts. *Mov Disord.* (2019) 34:1839–50. doi: 10.1002/mds.27845
8. Iwaki H, Blauwendraat C, Leonard HL, Liu G, Maple-Grodem J, Corvol JC, et al. Genetic risk of Parkinson disease and progression: an analysis of 13 longitudinal cohorts. *Neurol Genet.* (2019) 5:e348. doi: 10.1212/NXG.0000000000000348
9. Fields CR, Bengoa-Vergniory N, Wade-Martins R. Targeting alpha-synuclein as a therapy for Parkinson's disease. *Front Mol Neurosci.* (2019) 12:299. doi: 10.3389/fnmol.2019.00299
10. Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet.* (2017) 49:1511–6. doi: 10.1038/ng.3955
11. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* (2014) 46:989–93. doi: 10.1038/ng.3043
12. Foo JN, Tan LC, Irwan ID, Au W-L, Low HQ, Prakash K-M, et al. Genome-wide association study of Parkinson's disease in East Asians. *Hum Mol Genet.* (2016) 26:226–32. doi: 10.1093/hmg/ddw379
13. Alves G, Müller B, Herlofson K, HogenEsch I, Telstad W, Aarsland D, et al. Incidence of Parkinson's disease in Norway: the Norwegian ParkWest study. *J Neurol Neurosurg Psychiatry.* (2009) 80:851–7. doi: 10.1136/jnnp.2008.168211
14. Caslake R, Taylor K, Scott N, Gordon J, Harris C, Wilde K, et al. Age-, gender-, and socioeconomic status-specific incidence of Parkinson's disease and parkinsonism in northeast Scotland: the PINE study. *Parkinsonism Relat Disord.* (2013) 19:515–21. doi: 10.1016/j.parkreldis.2013.01.014
15. Linder J, Stenlund H, Forsgren L. Incidence of Parkinson's disease and parkinsonism in northern Sweden: a population-based study. *Mov Disord.* (2010) 25:341–8. doi: 10.1002/mds.22987
16. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology.* (1967) 17:427–42. doi: 10.1212/WNL.17.5.427
17. Fahn S. Unified Parkinson's disease rating scale. *Recent Dev Parkinsons Dis.* (1987) 2:153–163.
18. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* (1975) 12:189–98. doi: 10.1016/0022-3956(75)90026-6
19. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord.* (2010) 25:2649–53. doi: 10.1002/mds.23429
20. International Parkinson Disease Genomics Consortium, Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin U-M, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet. (2011) 377:641–9. doi: 10.1016/S0140-6736(10)62345-8
21. Luo N, Li Y, Niu M, Zhou L, Yao M, Zhu L, et al. Variants in the SNCA locus are associated with the progression of Parkinson's disease. *Front Aging Neurosci.* (2019) 11:110. doi: 10.3389/fnagi.2019.00110
22. Goris A, Williams-Gray CH, Clark GR, Foltynie T, Lewis SJ, Brown J, et al. Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Ann Neurol.* (2007) 62:145–53. doi: 10.1002/ana.21192
23. Yarnall AJ, Breen DP, Duncan GW, Khoo TK, Coleman SY, Firbank MJ, et al. Characterizing mild cognitive impairment in incident Parkinson disease: the ICICLE-PD study. *Neurology.* (2014) 82:308–16. doi: 10.1212/WNL.0000000000000066
24. Campelo CLC, Cagni FC, de Siqueira Figueredo D, Oliveira LG Jr, Silva-Neto AB, et al. Variants in SNCA gene are associated with Parkinson's disease risk and cognitive symptoms in a Brazilian sample. *Front Aging Neurosci.* (2017) 9:198. doi: 10.3389/fnagi.2017.00198
25. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Hurtig HI, Van Deerlin VM, et al. APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease. *JAMA Neurol.* (2014) 71:1405–12. doi: 10.1001/jamaneurol.2014.1455
26. Stoker TB, Camacho M, Winder-Rhodes S, Liu G, Scherzer CR, Foltynie T, et al. A common polymorphism in SNCA is associated with accelerated motor decline in GBA-Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (2020) 91:673–4. doi: 10.1136/jnnp-2019-322210
27. Davis AA, Andruska KM, Benitez BA, Racette BA, Perlmutter JS, Cruchaga C. Variants in GBA, SNCA, and MAPT influence Parkinson disease risk, age at onset, and progression. *Neurobiol Aging.* (2016) 37:209 e1–7. doi: 10.1016/j.neurobiolaging.2015.09.014
28. Cooper CA, Jain N, Gallagher MD, Weintraub D, Xie SX, Berlyand Y, et al. Common variant rs356182 near SNCA defines a Parkinson's disease endophenotype. *Ann Clin Transl Neurol.* (2017) 4:15–25. doi: 10.1002/acn3.371
29. Macleod AD, Henery R, Nwajiugo PC, Scott NW, Caslake R, Counsell CE. Age-related selection bias in Parkinson's disease research: are we recruiting the right participants? *Parkinsonism Relat Disord.* (2018) 55:128–33. doi: 10.1016/j.parkreldis.2018.05.027

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Cognitive Functioning of Glucocerebrosidase (*GBA*) Non-manifesting Carriers

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Mutations and variants in the glucocerebrosidase (*GBA*) gene are among the most common genetic risk factors for the development of Parkinson's disease (PD). Yet, penetrance is markedly reduced, and less is known about the burden of carrying a single mutation among those without diagnosed PD. Motor, cognitive, psychiatric, and olfactory functioning were assessed in 30 heterozygous *GBA* mutation carriers without PD (the majority of whom had mild *GBA* mutations) and 49 non-carriers without PD. Study focus was on domains affected in *GBA* mutation carriers with PD, as well as those previously shown to be abnormal in *GBA* mutation carriers without PD. *GBA* mutation carriers showed poorer performance on the Stroop interference measure of executive functioning when controlling for age. There were no group differences in verbal memory, Montreal Cognitive Assessment (MoCA), overall motor score, or presence of REM sleep behavior disorder or depression. Although total olfaction scores did not differ, *GBA* mutation carriers with hyposmia had lower global cognition scores than those without hyposmia. As anticipated by the low penetrance of *GBA* mutations, these findings suggest that pre-manifest non-motor or motor features of PD may not present in most *GBA* mutation carriers. However, there is support that there may be a subtle difference in executive functioning among some non-manifesting heterozygous *GBA* mutation carriers, and, combined with olfaction, this may warrant additional scrutiny as a potential biomarker for pre-manifest and pre-clinical *GBA* related PD.

Keywords: glucocerebrosidase, GBA, cognition, executive functioning, Parkinson's disease

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INTRODUCTION

GBA mutations are a common genetic risk factor for Parkinson disease (PD) and dementia with Lewy Bodies (DLB) (1–3). While harboring two copies of certain *GBA* mutations may lead to Gaucher disease, both mono- and biallelic *GBA* mutation carriers are at an increased risk of developing PD. *GBA* related PD (*GBA*-PD) may have earlier age of onset and more prominent non-motor features than idiopathic PD (4–6). This includes an increased risk of mild

cognitive impairment (7) and dementia (8–10). *GBA*-PD may exhibit a specific cognitive profile, with greater weakness in working memory/executive functioning (9), and visuospatial processing relative to idiopathic PD (9). Further, some studies have indicated more significant symptoms of depression, apathy, anxiety, and REM sleep behavior disorder (RBD) overall, or in a subset in *GBA*-PD (4, 11). Biallelic carriers may have significant olfactory disturbance (12), and monoallelic carriers experience more olfactory dysfunction relative to non-carriers (4), though the relationship between *GBA* status and olfaction has not been universally demonstrated (13).

Although earlier age at onset (14), and greater burden of both motor decline (15) and non-motor symptoms (16) are more pronounced among carriers of more "severe" GBA mutations, this phenotype is also reported among carriers of more "mild" mutations (16, 17). However, among carriers of mild GBA mutations, this prominent non-motor phenotype may manifest later in the disease course. A recent large scale, multi-center study suggests that individuals with *GBA*-PD, who are carriers of a mild *GBA* mutation (N409S), displayed a PD phenotype that was similar to non-mutation, sporadic PD, during the first 3 years of clinical disease (13).

Penetrance of *GBA* mutations for the development of PD is markedly reduced. A recent large scale investigation estimates that for monoallelic carriers, the risk of developing PD was 10% at age 60, 16% at age 70, and 19% by age 80 (18). Prior penetrance estimates assessing cohorts with known family history of PD report higher penetrance, reflecting either ascertainment bias or shared additional genetic factors (18). This overall risk further varies in relation to mutation severity (19). Severe mutations confer a 13.6-fold increased risk, while mild mutations confer a 2.2-fold increased risk for the development of PD (20, 21). Less is known about the burden of harboring a single *GBA* mutation outside of the context of PD. With emerging investigations of therapeutics targeting underlying disease process associated with *GBA* mutations (14), detailed characterization of *GBA* carriers is essential in order to determine early, pre-clinical markers of phenoconversion.

Recent studies have examined the prodromal course prior to PD onset, as well as other *GBA* associated conditions, such as DLB and RBD. However, findings vary in the extent of cognitive involvement in non-manifesting *GBA* mutation carriers (*GBA*-NMC), which may be attributable to mutation type and ascertainment. Studies investigating cohorts of mild or mild and severe mutation carriers ascertained in clinical settings and thus often consisting of relatives of individuals with PD, (15, 22, 23) have reported differences in global cognitive functioning, frequently assessed using the Montreal Cognitive Assessment (MoCA). In one study, *GBA*-NMC did not show worsening of MoCA score over a 6 year follow up period (23). We previously reported data from a community-based study in which carriers of mild mutations exhibited subtle decline in verbal memory (24).

In this study, we aim to extend prior work, assessing cognitive and other features among *GBA*-NMC relative to peers, in a sample comprised of first-degree relatives, spouses, and friends of PD patients from our outpatient clinic. This sample straddles the community based sample and a purely clinic based sample, and might be more representative of the cross-section of individuals in a New York sample who may seek counseling for *GBA* mutations.

MATERIALS AND METHODS

Participants

Participants were recruited as part of a larger ongoing study assessing the genetics of PD at Mount Sinai Beth Israel. Participants included in this analysis did not have PD. They participated because they had a first or second degree relative with PD, were spouses of individuals with known PD, or were community volunteers without family history of PD. Participants were evaluated by a movement disorders specialist neurologist, and a diagnostic checklist was completed that evaluated presence of clinical symptoms of a movement disorder. Participants with PD, cognitive impairment, or other major neurological diseases were excluded, as were those who also harbored a G2019S *LRRK2* mutation or Gaucher disease.

Inclusion as either a non-manifesting *GBA* carrier (NMC) or mutation negative control was based on the results of genotyping, and some spouses of PD patients harbored *GBA* mutations. *GBA* mutation/variant status was determined as previously described (12, 25). In brief, participants were screened for the eight most common *GBA* mutations (N409S, L483P, 84GG, IVS2+1, V433L, del55bp, D448H, and R535H) as well as E365K, T408M, and the G2019S *LRRK2* mutation. The Tag-It™ Mutation Detection Kit (Luminex Molecular Diagnostics, Toronto, ON, Canada) was used to perform genotyping according to the manufacturer's instructions. Using this system, the regions around the target genes were amplified by multiplex polymerase chain reaction (PCR). The regions were subjected to allele-specific primer extension, hybridized to specific Luminex® beads via Universal Tags, and sorted on a Luminex® 100 IS platform (Luminex Corporation, Austin, TX, USA). Genotyping was then completed using the Tag-It™Data Analysis Software (Luminex Molecular Diagnostics).

Participants provided written informed consent, and this study protocol was approved by the Mount Sinai Institutional Review Board.

Measures

Systematic neurological history and examination, including Unified Parkinson Disease Rating Scale-III (UPDRS-III), was completed by a neurologist. Non-motor symptoms were assessed using neuropsychological measures, administered by trained coordinators under the guidance of a clinical neuropsychologist. The following cognitive measures were used: Montreal Cognitive Assessment (MoCA), Hopkins Verbal Learning Test—Revised (HVLT-R), Stroop Test, Color Trails Test, Symbol Digit Modality Test, Digit Span, Letter Number Sequencing, Judgment of Line Orientation, FAS, and Animal Fluency.

Additional non-motor features were assessed using the Beck Depression Inventory—II (BDI-II), State-Trait Anxiety Inventory (STAI), REM Sleep Behavior Disorder Questionnaire

TABLE 1 | Univariate summary of demographics and outcome variables.

	Total ($N = 79$)	*GBA*-NMC ($n = 30$)	Non-carrier controls ($n = 49$)
Demographics			
Age, years, *mean (SD), range*	57.63 (12.53), 22–89	53.93 (14.41), 29–89	59.90 (12.58), 22–86
Median (IQR)	60.00 (48.00–66.00)	59.50 (38.75–63.25)	61.00 (52.50–68.50)
Education, *mean (SD)*	17.00 (2.00), 12–20	17.13 (1.78), 12–20	16.78 (6.23), 12–20
Gender, *n(%) Female*	23 (29.1%)	11 (36.7%)	12 (24.5%)
Family history of PD, *n(%)*	24 (30.4%)	17 (56.7%)	7(14.3%)
Primary analyses			
UPDRS-III, *mean (SD), range*	0.72 (1.29), 0.0–5.0	0.75 (1.19), 0.0–5	0.70 (1.36), 0.0–5.0
MoCA total score, *mean (SD), range*	27.72 (1.89), 21–30	27.60 (2.06), 23–30	27.78 (1.81), 21–30
HVLT-R Recall, *z* score, *mean (SD), range*	−0.31 (1.21), −3.22–1.22	−0.36 (0.85), −1.94 to 1.00	−0.27 (1.42), −3.22 to 1.22
Stroop Interference, *T*-score, *mean (SD), range*	49.00 (6.36), 37–70	49.60 (6.57), 37–60	48.68 (6.21), 37–70
BDI-II, *mean (SD), range* ≥ 14, *n(%)*	4.22 (5.71), 0–32, 6 (8.7%)	4.85 (5.17), 0–19, 3 (11.5%)	3.84 (6.05), 0–32, 3 (7.0%)
RBDSQ, *mean (SD), range* ≥ *5, n(%)*	1.60 (1.67), 0–6, 4 (5.8%)	2.00 (1.53), 0–6, 1 (4.0%)	1.36 (1.72), 0–6, 3 (6.9%)
UPSIT total correct„ *mean (SD), range*	33.06 (5.27), 18–40, 21 (30.9%)	32.89 (5.23), 18–39, 11 (40.7%)	33.17 (5.37), 18–40, 10 (24.4%)
Hyposmia, *n (%)*			

UPDRS-III, Unified Parkinson's Disease Rating Scale, motor section; MoCA, Montreal Cognitive Assessment; HVLT-R, Hopkins Verbal Learning Test—Revised; BDI-II, Beck Depression Inventory—II; STAI, State Trait Anxiety Inventory; REM Sleep Behavior Disorder Questionnaire; UPSIT, University of Pennsylvania Smell Identification Test.

(RBDQ), and University of Pennsylvania Smell Identification Test (UPSIT).

Analysis

As prior assessments have found differences between *GBA*-NMC and controls in motor functioning (15), both specific cognitive domains (24, 26) and overall cognition, depression, RBD, and olfaction (15), our primary hypothesis-driven analyses were on the effect of specific *GBA* status in these domains. In particular, primary cognitive analyses were performed on assessment of verbal memory (HVLT-R), executive function (Stroop), and MoCA. Primary motor comparisons were associated with continuous UPDRS-III scores. Depression (BDI-II) and RBD (RBDQ) were assessed using recommended cut scores of 14 and 5, respectively. As there are two major methods to assess olfaction, olfactory performance on the UPSIT was compared both continuously and dichotomously, using a cut-off score of 15 percentile adjusted for age and gender to define hyposmia (27).

Summaries of baseline demographics and outcome measures were presented and compared using parametric or nonparametric tests as appropriate (**Table 1**). Cognitive measures are reported in demographically adjusted standardized scores. For all primary outcomes, linear regression models were performed to assess the effect of mutation status on motor and non-motor functioning adjusting for age and sex when indicated by significant associations between outcome and demographics. Exploratory analyses evaluated additional cognitive domains (attention, working memory, processing speed, verbal learning, verbal fluency, and visuospatial functioning) and anxiety, as well as the relationships between olfaction and other domains. All analyses were conducted using SPSS 25 (SPSS INC., Chicago, IL, USA).

RESULTS

Thirty participants harbored a single *GBA* mutation, most with mild mutations (23 N409S, 1 R535H), two with a severe mutation (2 84GG), and four with other PD associated risk variants (2 T408M, 2 E362K), and 49 did not (controls). Among 30 *GBA*-NMC, 20 were recruited through a blood family member with PD, and 10 were spouses or friends. Seventeen *GBA*-NMC and 7 controls had a family history of PD in a first degree relative. Among the control group (non-*GBA*), all of whom were spouse (36) or friend (13) controls, 5 had family history of PD in first, and 2 had family history in first and in second degree relatives.

GBA-NMC (mean age ± SD: 53.93 ± 14.41 years, median 59.5, range 29–89) were not older than controls (59.90 ± 12.58, median 61.0, range 22–86). There were no group differences in sex (*GBA* mutation carriers 11 women; and controls 12 women) or years of education (*GBA* mutation carriers: mean ± SD 17.13 ± 1.78 years; controls: mean 16.78 ± 6.23 years). UPDRS-III, MoCA, HVLT-R Z-score, and Stroop interference *T*-score, and UPSIT scores did not differ between groups.

Table 1 shows results of linear regression models of hypothesized domains adjusting for demographics as appropriate. Stroop interference task was significantly associated with harboring a *GBA* mutation ($p < 0.05$), whereas UPDRS-III, HVLT-R, MoCA, UPSIT, presence, or significant symptoms of RBD or depression were not.

In the exploratory analyses, no difference was found between *GBA*-NMC and controls in the remaining cognitive measures listed above, or in anxiety, when correcting for multiple comparisons.

Forty percent of *GBA*-NMC were hyposmic, and 24% of controls were hyposmic. Among *GBA*-NMC, those with hyposmia had lower scores on the MoCA when controlling for age, ($p = 0.018$), with both hyposmia ($p = 0.046$) and age

($p = 0.019$) as significant predictors of MoCA score. Hyposmia was not significantly related to other primary outcome variables.

DISCUSSION

Findings from this study detail key associations between *GBA* mutation status and symptoms related to PD. *GBA* mutation carriers showed poorer performance on the inhibition component of the Stroop task. While there were no overall group differences in global cognitive functioning, assessed by the MoCA, *GBA* mutation carriers with hyposmia had lower global cognition scores than those without hyposmia. Additionally, *GBA* carriers were more likely to exhibit subtle motor signs.

Poorer performance in executive functioning tasks has been observed in *GBA* mutation carriers with and without parkinsonism (9, 28). We did not detect an association between *GBA* mutation status and global cognitive functioning, suggesting that overall there is limited, if any, deficit in most mild *GBA* mutation/variant carriers without PD. However, *GBA* mutation carriers did perform worse on the Stroop, an executive function task of response inhibition. Our study adds to the growing body of literature detailing a complex relationship between mutation status and performance on measures of global cognitive functioning (e.g., MoCA), albeit with a focus on mild mutation carriers. Most studies have reported worse performance on the MoCA among individuals with *GBA*-PD relative to idiopathic PD (1, 7), including a greater cognitive burden in severe mutation carriers (29). However, this may not occur early in disease as a recent large scale, multicenter study did not detect this difference among carriers, primarily of mild mutations (N409S), assessed early in the PD disease course (13). These prior studies suggest that among individuals with *GBA*-PD, disease duration, and mutation severity likely contribute to cognitive course.

In addition, *GBA* carriers without PD (*GBA*-NMC) have previously demonstrated greater cortical activation during a task of response inhibition (26), suggesting carriers may employ greater compensatory mechanisms in order to achieve similar performance, potentially revealing a mild burden of mutation status. Response inhibition, the ability to suppress a habitual or overlearned response, is a critical executive function, with cognitive and behavioral implications. Among *GBA*-NMC, such subtle cognitive changes may be indicative of an emerging disease process. Yet performance on this measure of response inhibition was within normal limits, reflecting mild, yet statistically significant difference between carriers and controls. Additionally, as relative weakness in this domain was mild, and as there were no differences between *GBA* mutation carriers and controls in other cognitive domains, this finding should be interpreted with caution.

Our finding of a subtle, isolated, relative cognitive weakness among *GBA*-NMC is in line with a prior study from our group that evaluated community dwelling older adults who were ascertained independent of mutation status. In that report, although global cognition was not worse, *GBA*-NMC did demonstrate greater, albeit mild, decline in verbal memory (24). Similar to the data reported herein, most participants had the mild *GBA* N409S mutation. These studies suggest that even among monoallelic carriers of mild mutations, relative cognitive vulnerabilities, which do not rise to the level of cognitive impairment, may be apparent. Our data do not disentangle the impact of mutation severity however, as the majority of the mutation carriers harbored mild mutations.

We did not detect an overall association between *GBA* mutation status and performance on a screening measure of global cognitive functioning (MoCA). This differs from results of a recent large, multicenter, study reporting worse performance on the MoCA among both *GBA*-NMC and *LRRK2*-NMC relative to control participants (22). Participants in this study were also predominantly mild mutation carriers. Our report also differs from a study of predominantly mid-life adult *GBA*-NMC comprised of both mild and severe mutation carriers, which found differences both at baseline and 2-year follow up on this measure (15). Of interest, 6-year follow up of this study, showed significant improvement in MoCA score among controls and improvement among carriers that did not reach the level of statistical significance (23). At follow up, while biallelic *GBA*-NMC performed significantly worse on the MoCA relative to controls, there was no longer a difference between monoallelic *GBA*-NMC and controls (23). Approximately half of the original *GBA*-NMC cohort was seen at 6 year follow up, raising the question of whether loss to follow up in that group was associated with worse cognition (23).

Further, our data support that while there was no difference between *GBA*-NMC and controls in overall olfaction scores, *GBA*-NMC carriers with hyposmia had poorer global cognitive functioning scores. This supports the longitudinal study showing olfaction as a potential preclinical marker, with reduced olfaction predicting worse cognition (23). While our relatively small sample size may have lacked the statistical power to detect an overall difference in MoCA score between carriers and controls, the relationship between olfaction and cognition in our sample may suggest variability in the impact of mutation status in our cohort of mild mutation carriers. Such a relationship emphasizes the need for continued, multi-modal assessment of this population in order to further our understanding of markers of disease burden, particularly among carriers of mild mutations.

Given that participants in this study were neurologically normal, there was limited variability in motor functioning scores, as neither *GBA*-NMC nor controls exhibited overt motor symptoms suggestive of PD. There were no group differences in continuous motor scores. As the UPDRS-III is a clinical tool, it may lack the sensitivity needed to determine if mild motor differences are present in our sample.

Our sample consisted predominantly of carriers of mild mutations (23/30 N409S, 1/30 R535H) or risk variants (2/30 T408M, 2/30 E362K). Penetrance of *GBA* mutations, particularly mild mutations and risk variants, is markedly reduced (21), and as such, most of the individuals in our sample are not expected to progress to PD. Other investigations which included a greater proportion of severe mutation carriers (15, 23) may yield evidence of greater disease burden that was not evident in our sample. Sensitivity analyses excluding the two carriers

of more severe mutations (84 GG) yielded similar findings. Mutation carriers continued to show poorer performance on the inhibition component of the Stroop task when controlling for age, at the trend level, and carriers with hyposmia had lower scores on the MoCA when controlling for age.

Through our sample, we identified both *GBA*-NMC and control participants with and without family members with PD. Some of our sample was ascertained through affected family members, and others were spouses of individuals with idiopathic PD. Similar to other studies in which non-manifesting carriers and controls were recruited through tertiary care clinics, our participants with first degree relatives with PD, likely share additional risk factors that were not directly accounted for in this study, and a larger proportion of individuals with such a family history were in the *GBA*-NMC cohort. To determine the degree to which included mutation negative, but positive family history controls influenced the analysis, sensitivity analyses were performed, in which individuals in the control group with a family history of PD were excluded. These yielded a similar pattern yet did not reach statistical significance. Mutation carriers continued to show poorer performance on the inhibition component of the Stroop task when controlling for age, although this finding no longer reached statistical significance. In excluding these individuals, it is possible this analysis was limited by the reduced sample size. However, additional unmeasured genetic and environmental factors seen in family members of affected individuals interact with GBA status, and contribute to the differences measured.

Additionally, as limited genetic testing was available for this study, we are unable to conclusively determine if presence of additional genetic factors that may be associated with PD impact our findings. As additional genetic factors may modify *GBA* associated PD risk (30), along with the small sample size, incomplete genetic testing among both carrier and control participants limits degree of certainty that we are measuring solely the effect of *GBA* status.

This study reports subtle differences between *GBA*-NMC and mutation negative controls on non-motor features. While statistically significant differences emerged, it is notable that across domains assessed in this study, these differences were reflective of mild vulnerabilities. Motor functioning was within normal limits, and cognitive findings were limited to one domain, and reflective of mild, relative weakness, rather than significant burden. While such differences may be suggestive of an emerging pathological process, the overall disease burden was low in this sample. Our study was limited in that we were not able to disentangle the effect of mutation severity, as the sample of severe and risk variant carriers was low. Further, additional longitudinal investigations are needed to determine if these mild differences represent prodromal disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mount Sinai Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RS-P and EM: study concept and design. EM, RS-P, and MZ: drafting of the manuscript. EM, RS-P, CW, and RO: Statistical analysis. RS-P and MZ: study supervision. RS-P: obtained funding. All authors: acquisition, analysis, or interpretation of data, critical revision of the manuscript for important intellectual content, administrative, technical, or material support.

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REFERENCES

1. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* (2009) 361:1651–61. doi: 10.1056/NEJMoa0901281
2. Clark LN, Kartsaklis LA, Wolf Gilbert R, Dorado B, Ross BM, Kisselev S, et al. Association of glucocerebrosidase mutations with dementia with lewy bodies. *Arch Neurol.* (2009) 66:578–83. doi: 10.1001/archneurol.2009.54
3. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA Neurol.* (2013) 70:727–35. doi: 10.1001/jamaneurol.2013.1925
4. Brockmann K, Srulijes K, Hauser AK, Schulte C, Csoti I, Gasser T, et al. GBA-associated PD presents with nonmotor characteristics. *Neurology.* (2011) 77:276–80. doi: 10.1212/WNL.0b013e318225ab77
5. Nichols WC, Pankratz N, Marek DK, Pauciulo MW, Elsaesser VE, Halter CA, et al. Mutations in GBA are associated with familial Parkinson disease susceptibility and age at onset. *Neurology.* (2009) 72:310–6. doi: 10.1212/01.wnl.0000327823.81237.d1
6. Aflaki E, Westbroek W, Sidransky E. The complicated relationship between gaucher disease and Parkinsonism: insights from a rare disease. *Neuron.* (2017) 93:737–46. doi: 10.1016/j.neuron.2017.01.018
7. Alcalay RN, Caccappolo E, Mejia-Santana H, Tang M, Rosado L, Orbe Reilly M, et al. Cognitive performance of GBA mutation carriers with early-onset PD: the CORE-PD study. *Neurology.* (2012) 78:1434–40. doi: 10.1212/WNL.0b013e318253d54b
8. Crosiers D, Verstraeten A, Wauters E, Engelborghs S, Peeters K, Mattheijssens M, et al. Mutations in glucocerebrosidase are a major genetic risk factor for Parkinson's disease and increase susceptibility to dementia in a Flanders-Belgian cohort. *Neurosci Lett.* (2016) 629:160–4. doi: 10.1016/j.neulet.2016.07.008
9. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, Van Deerlin VM, et al. GBA Variants are associated with a distinct pattern of cognitive deficits in Parkinson's disease. *Mov Disord.* (2016) 31:95–102. doi: 10.1002/mds.26359

10. Seto-Salvia N, Pagonabarraga J, Houlden H, Pascual-Sedano B, Dols-Icardo O, Tucci A, et al. Glucocerebrosidase mutations confer a greater risk of dementia during Parkinson's disease course. *Mov Disord.* (2012) 27:393–9. doi: 10.1002/mds.24045
11. Swan M, Doan N, Ortega RA, Barrett M, Nichols W, Ozelius L, et al. Neuropsychiatric characteristics of GBA-associated Parkinson disease. *J Neurol Sci.* (2016) 370:63–9. doi: 10.1016/j.jns.2016.08.059
12. Saunders-Pullman R, Hagenah J, Dhawan V, Stanley K, Pastores G, Sathe S, et al. Gaucher disease ascertained through a Parkinson's center: imaging and clinical characterization. *Mov Disord.* (2010) 25:1364–72. doi: 10.1002/mds.23046
13. Simuni T, Brumm MC, Uribe L, Caspell-Garcia C, Coffey CS, Siderowf A, et al. Clinical and dopamine transporter imaging characteristics of leucine rich repeat kinase 2 (LRRK2) and glucosylceramidase beta (GBA) Parkinson's disease participants in the Parkinson's progression markers initiative: a cross-sectional study. *Mov Disord.* (2020) 35:833–44. doi: 10.1002/mds.27989
14. Sardi SP, Simuni T. New Era in disease modification in Parkinson's disease: review of genetically targeted therapeutics. *Parkinsonism Relat Disord.* (2019) 59:32–8. doi: 10.1016/j.parkreldis.2018.10.025
15. Beavan M, McNeill A, Proukakis C, Hughes DA, Mehta A, Schapira AH. Evolution of prodromal clinical markers of Parkinson disease in a GBA mutation-positive cohort. *JAMA Neurol.* (2015) 72:201–8. doi: 10.1001/jamaneurol.2014.2950
16. Cilia R, Tunesi S, Marotta G, Cereda E, Siri C, Tesei S, et al. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann Neurol.* (2016) 80:662–73. doi: 10.1002/ana.24777
17. Gan-Or Z, Liong C, Alcalay RN. GBA-associated Parkinson's disease and other synucleinopathies. *Curr Neurol Neurosci Rep.* (2018) 18:44. doi: 10.1007/s11910-018-0860-4
18. Balestrino R, Tunesi S, Tesei S, Lopiano L, Zecchinelli AL, Goldwurm S. Penetrance of glucocerebrosidase (GBA) mutations in Parkinson's disease: a Kin Cohort Study. *Mov Disord.* (2020) 35:2111–4. doi: 10.1002/mds.28200
19. Gan-Or Z, Giladi N, Orr-Urtreger A. Differential phenotype in Parkinson's disease patients with severe versus mild GBA mutations. *Brain.* (2009) 132(Pt 10):e125. doi: 10.1093/brain/awp161
20. Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology.* (2015) 84:880–7. doi: 10.1212/WNL.0000000000001315
21. Gan-Or Z, Giladi N, Rozovski U, Shifrin C, Rosner S, Gurevich T, et al. Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset. *Neurology.* (2008) 70:2277–83. doi: 10.1212/01.wnl.0000304039.11891.29
22. Simuni T, Uribe L, Cho HR, Caspell-Garcia C, Coffey CS, Siderowf A, et al. Clinical and dopamine transporter imaging characteristics of non-manifest LRRK2 and GBA mutation carriers in the Parkinson's Progression Markers Initiative (PPMI): a cross-sectional study. *Lancet Neurol.* (2020) 19:71–80. doi: 10.1016/S1474-4422(19)30319-9
23. Avenali M, Toffoli M, Mullin S, McNeil A, Hughes DA, Mehta A, et al. Evolution of prodromal parkinsonian features in a cohort of *GBA* mutation-positive individuals: a 6-year longitudinal study. *J Neurol Neurosurg Psychiatry*. (2019) 90:1091–7. doi: 10.1136/jnnp-2019-320394
24. Moran EE, Wang C, Katz M, Ozelius L, Schwartz A, Pavlovic J, et al. Cognitive and motor functioning in elderly glucocerebrosidase mutation carriers. *Neurobiol Aging.* (2017) 58:239.e1–e7. doi: 10.1016/j.neurobiolaging.2017.06.010
25. Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med.* (2006) 354:424–5. doi: 10.1056/NEJMc055509
26. Bregman N, Thaler A, Mirelman A, Helmich RC, Gurevich T, Orr-Urtreger A, et al. A cognitive fMRI study in non-manifesting LRRK2 and GBA carriers. *Brain Struct Funct.* (2016) 222:1207–18. doi: 10.1007/s00429-016-1271-4
27. Postuma RB, Aarsland D, Barone P, Burn DJ, Hawkes CH, Oertel W, et al. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Mov Disord.* (2012) 27:617–26. doi: 10.1002/mds.24996
28. Kono S, Ouchi Y, Terada T, Ida H, Suzuki M, Miyajima H. Functional brain imaging in glucocerebrosidase mutation carriers with and without parkinsonism. *Mov Disord.* (2010) 25:1823–9. doi: 10.1002/mds.23213
29. Liu G, Boot B, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, et al. Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann Neurol.* (2016) 80:674–85. doi: 10.1002/ana.24781
30. Blauwendraat C, Reed X, Krohn L, Heilbron K, Bandres-Ciga S, Tan M, et al. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. *Brain.* (2020) 143:234–48. doi: 10.1093/brain/awz350

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REVIEW
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A More Homogeneous Phenotype in Parkinson's Disease Related to R1441G Mutation in the LRRK2 Gene

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Parkinson's disease (PD) is characterized by a great clinical heterogeneity. Nevertheless, the biological drivers of this heterogeneity have not been completely elucidated and are likely to be complex, arising from interactions between genetic, epigenetic, and environmental factors. Despite this heterogeneity, the clinical patterns of monogenic forms of PD have usually maintained a good clinical correlation with each mutation once a sufficient number of patients have been studied. Mutations in LRRK2 are the most commonly known genetic cause of autosomal dominant PD known to date. Furthermore, recent genome-wide association studies have revealed variations in LRRK2 as significant risk factors also for the development of sporadic PD. The LRRK2-R1441G mutation is especially frequent in the population of Basque ascent based on a possible founder effect, being responsible for almost 50% of cases of familial PD in our region, with a high penetrance. Curiously, Lewy bodies, considered the neuropathological hallmark of PD, are absent in a significant subset of LRRK2-PD cases. Indeed, these cases appear to be associated with a less aggressive primarily pure motor phenotype. The aim of our research is to examine the clinical phenotype of R1441G-PD patients, more homogeneous when we compare it with sporadic PD patients or with patients carrying other LRRK2 mutations, and reflect on the value of the observed correlation in the genetic forms of PD. The clinical heterogeneity of PD leads us to think that there may be as many different diseases as the number of people affected. Undoubtedly, genetics constitutes a relevant key player, as it may significantly influence the phenotype, with differences according to the mutation within the same gene, and not only in familial PD but also in sporadic forms. Thus, extending our knowledge regarding genetic forms of PD implies an expansion of knowledge regarding sporadic forms, and this may be relevant due to the future therapeutic implications of all forms of PD.

Keywords: R1441G, LRRK2, phenotype, Parkinson's disease, progression

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease. The worldwide incidence estimates range from 5 to more than 35 new cases per 100,000 individuals yearly (1). This relatively wide range is probably due to differences in study methodologies or in the demographic characteristics of the populations studied. The overall prevalence in the general population is estimated at 0.3% but rises sharply with age to more than 3% in those >80 years of age (2). PD is clinically defined by the presence of bradykinesia and at least one additional cardinal motor feature (rigidity or rest tremor), as well as additional supporting and exclusionary criteria (3). In addition, the disease is associated with a wide spectrum of non-motor symptoms (NMS). These include cognitive impairment, disorders of sleep–wake cycle regulation, autonomic dysfunction, and mood disorders, as well as sensory symptoms (most prominently hyposmia and pain). Some of these NMS can predate the onset of classic motor symptoms by years or even decades (4). PD is mainly characterized by a selective slow and progressive degeneration of dopaminergic neurons in the substantia nigra. Nevertheless, non-dopaminergic neurons are also known to degenerate in PD. In fact, the pathological process also affects even neurons outside the central nervous system (CNS), such as those in the mesenteric system or olfactory bulb. In addition to neuronal loss, this disorder is pathologically characterized by the presence of abnormal deposition of α-synuclein (αsyn) in the cytoplasm of certain neurons (Lewy bodies) in several different brain regions. However, Lewy bodies are not specific to the diagnosis of PD and PD can be diagnosed even in the absence of Lewy body pathology (5).

Despite intensive research, the etiopathogenesis of PD remains largely unknown, considering currently that there is no single cause and that there are multiple factors that play a role in its development, especially genetic, environmental, and also epigenetic factors. The genetic forms of PD justify only around 10–15% of cases of PD and are derived from mutations in genes that are involved in different cellular processes. The functional characterization of these genes has enabled the scientific community to understand a series of basic cellular mechanisms that intervene also in the sporadic form of PD (sPD), although its hierarchy in the latter is still unknown. Among these cellular mechanisms are those related to defects in folding, aggregation, and phosphorylation of αsyn, abnormalities in intracellular vesicular trafficking, neuroinflammation, and oxidative stress derived from mitochondrial dysfunction (6). All these proposed pathways might be involved, are non-mutually exclusive, and all are susceptible to be pharmacologically manipulated to try to intervene early in the etiopathogenesis of the disease.

The most frequent forms of familial PD transmitted with dominant inheritance are the forms linked to mutations in leucine-rich repeat kinase 2 (LRRK2) gene. LRRK2 is a large, multidomain protein containing two catalytic domains: a Ras of complex proteins (Roc) G-domain and a kinase domain. Multiple variants of this gene have been described, yet only 8 have been proved to be pathogenic (N1437H, R1441 G/H/C, Y1699C, I2012T, G2019S, and I2020T) (7). Mutations have been identified in both catalytic domains and in several of its multiple putative regulatory domains (8, 9). Moreover, recent genome-wide association studies (GWAS) have revealed variations in LRRK2 as significant risk factors also for the development of sPD (10, 11). Although definite molecular consequences of mutations in LRRK2 have not been fully elucidated, different studies indicate that mutations occurring in the LRRK2 gene are associated with an increase in kinase activity (12). Thus, the fact that these mutations result in hyperactivation of the LRRK2 kinase has broadened disease-modifying treatment's horizon and, indeed, LRRK2 kinase inhibitors are being developed and tested, suggesting that subjects with LRRK2 mutations may be one of the first precision medicine cohorts for PD.

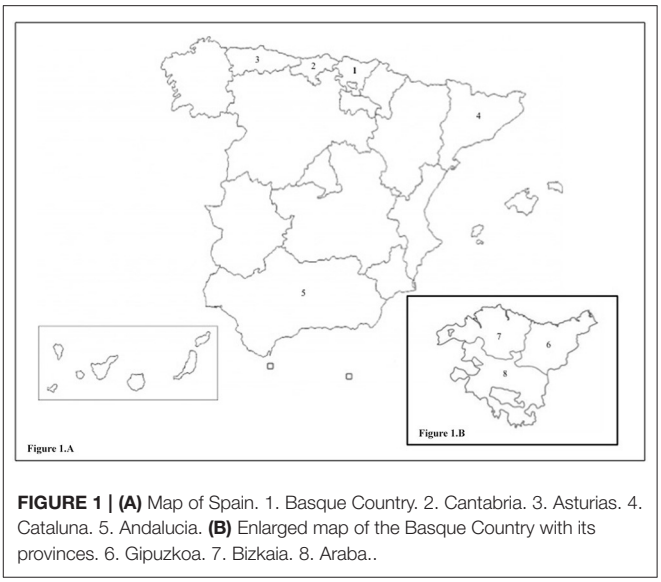

FIGURE 1 | (A) Map of Spain. 1. Basque Country. 2. Cantabria. 3. Asturias. 4. Cataluna. 5. Andalucia. **(B)** Enlarged map of the Basque Country with its provinces. 6. Gipuzkoa. 7. Bizkaia. 8. Araba..

The R1441G mutation is especially frequent in the Basque population, especially in the region of Gipuzkoa, based on a possible founder effect due to the presence of a common haplotype (13). The prevalence of this mutation is 46% in familial forms of PD and 2.5% in sporadic forms in patients of Basque origin in Gipuzkoa (14). The R1441G mutation has also been identified in Bizkaia (15) and in other regions near the Basque Country with a prevalence of 2.2% in Asturias (16), 1% in Cantabria (17), and 0.7% in Cataluña (18) (**Figures 1A,B**). It has been posited that a "north-south" gradient may exist, since R1441G-PD is significantly less common as we descend in the peninsula, with just isolated cases in Andalucía, which also share the same haplotype. In contrast, this mutation is very uncommon in other European populations, including other regions of Spain, North and South America, and Japan (14, 19–22). Lifetime penetrance of R1441G mutations increases with age, with figures of 12.8% at 65 years, from 50.2% at 70 years, reaching 83.4% at 80 years of age (23).

The main objective of this work is to summarize the results of the most relevant publications related to the clinical phenotype of patients with R1441G-PD. In addition, updated data from the

registry of our patient cohort, based on our collaboration in the Parkinson's Progression Markers Initiative (PPMI) and on our daily clinical practice, will be provided.

PUBLISHED CLINICAL PHENOTYPE OF THE R1441G-PD PATIENTS

Motor Symptoms

The motor aspects of R1441G-PD seem to be very similar to sPD. The first publications reported an age of onset around 60 years and frequently an asymmetric tremor-dominant phenotype (24, 25). The clinical course was slowly progressive, with an excellent response to dopaminergic treatment. Most of the patients developed motor fluctuations and dyskinesias over the years while dementia was uncommon even 15 years after the onset of the disease (25). In subsequent studies, it was found that asymmetric resting tremor predominated as onset symptom (60%) and that classic motor complications (fluctuations in 63.5%, dyskinesias in 56.5%) appeared at the same time as in sPD (26). Moreover, it was observed that the presence of dystonia throughout the disease stood out in 22.6% of cases predominantly in the lower limbs. In most reported cases, it was a tonic extension of the great toe. In some cases, it was a debut symptom, a characteristic sign and very homogeneous in certain families.

Cognitive Dysfunction

Cognitive impairment in PD associated with LRRK2 mutations is the most studied NMS, and existing evidence suggests that dementia is uncommon in these patients even one or two decades after the onset of the disease. Initial studies of cognitive dysfunction in patients with LRRK2-PD were based on clinical observations and suggested that dementia might occur less frequently in LRRK2-PD carrying R1441G and G2019S mutations than in sPD (25, 27). Kasten et al. evaluated the presence of dementia in ∼65% of patients with LRRK2-PD, finding a prevalence of 11% in these patients. These figures are lower than those observed in sPD (28), where around 25–30% of patients develop dementia (29, 30). Subsequent studies found similar cognitive abnormalities in G2019S-PD and sPD patients, being executive function the most frequently impaired domain (31–33). Our group performed a study to examine the cognitive status of R1441G-PD and compared this to that of sPD. A comprehensive cognitive assessment was performed using an extensive neuropsychological battery in order to evaluate the different cognitive domains. No differences were found in neuropsychological performance of R1441G-PD and sPD patients (34). The prevalence of mild cognitive impairment (PD-MCI) was 30% in both groups, with no differences in the number and type of domains impaired. Executive function, memory, and attention were the most frequently affected domains. Although the difference was not statistically significant, the prevalence of dementia was higher in the sPD group (27 vs. 13%). These results were in line with prior studies, which suggested that impairment of executive function and attention was frequent in LRRK2-PD patients, whereas dementia was not so common (35). Somme et al. examined cognition and psychiatric symptoms in 27 patients with LRRK2-PD (12 G2019S and 15 R1441G) and 27 patients with sPD. LRRK2-PD patients exhibited less frequently subjective cognitive complaints and mild cognitive impairment or dementia (36). More studies are needed, but what has been published to date suggests that cognitive impairment is less frequent in LRRK2-PD, more specifically in PD associated with the R1441G mutation.

A study of the prodromal phase of PD with asymptomatic, non-manifesting carriers (NMC) of LRRK2 mutations has recently been conducted. G2019S carriers scored higher in motor scores and had lower radioligand uptake compared to non-carriers, but no differences in NMS scores were observed. In contrast, R1441G carriers scored higher in motor scores, had lower radio ligand uptake, and had higher scores in depression, dysautonomia, as well as REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) scores, but had better cognition scores than non-carriers (37).

Affective and Neuropsychiatric Symptoms

In the cohort of patients with G2019S-PD studied by Goldwurm et al. it was found that the majority of subjects (14/16) had affective and behavioral alterations on the NPI-12 scale, mainly depression, anxiety, irritability, and hallucinations (32). According to the previously mentioned review carried out in patients with genetic PD (28), the prevalence of depression was 30% in LRRK2-PD patients. In contrast, in other studies prevalence reached 50–65% (31, 32). Compared with sPD, different results have hitherto been reported. In some studies, no differences have been noted (33, 38–41), while in others fewer depressive symptoms in patients with G2019S-PD were observed (42). With regard to anxiety, Belarbi et al. described a percentage of anxiety (measured through the NPI) of 69%. Compared to sPD, no significant differences were observed (56%) (31). In this study, the authors also found a high frequency of apathy (56 vs. 35%), irritability (34 vs. 20%), sleep disturbances (65 vs. 39%), and hallucinations (26 vs. 6%) compared with patients without mutation, with significant differences in the case of depression and anxiety. Somme et al. reported a lower prevalence of hallucinations and apathy in LRRK2-PD patients (R1441G and G2019S) compared to patients with sPD (36). In contrast, Gaig et al. found no differences between G2019S-PD and sPD in terms of hallucinations, anxiety, and depression (40). A link between bipolar disorder and LRRK2 gene has also been suggested (33). Our group evaluated affective symptoms in R1441G-PD. Thirty patients with R1441G-PD were compared with 30 sPD. The mean scores in the depression and anxiety scales were similar in both groups. Depressive symptoms were detected in 31.8% of R1441G-PD and 25% of sPD patients, and anxiety symptoms were evident in 4.5 and 15%, respectively (34). No further studies have hitherto been conducted to assess the presence of depressive symptoms and anxiety specifically in R1441G-PD.

Hyposmia

Hyposmia is one of the most common and best-characterized NMS and is often one of the earliest prodromal features to manifest. Due to the fact that prior existent evidence suggested that odor identification appeared to be distinctly affected in LRRK2-PD patients (27, 43, 44), our group performed a study

to assess olfactory function in PD patients using the Brief Smell Identification Test (B-SIT) and compared carriers of the G2019S and R1441G mutations in LRRK2 with non-carriers (45). A total of 190 PD patients were assessed, consisting of 146 non-carriers and 44 carriers of a mutation in LRRK2, 39 of which were R1441G mutations and 5 were G2019S mutations. Olfactory dysfunction was distributed distinctly between groups. Out of 44 LRRK2 mutation carriers, only 16 (36%) exhibited hyposmia. In contrast, hyposmia was evident in 110 of the 146 non-carriers (75%). Despite the fact that similar results were found in both mutations in this study, the small sample of patients carrying the G2019S mutation hindered reaching a definitive conclusion regarding this mutation. Nevertheless, according to the results of the study, a normal olfactory test result in a patient with typical PD may increase the probability that the patient is a LRRK2 mutation carrier, specifically the R1441G mutation.

Sympathetic Dysfunction

The sympathetic function can be evaluated by cardiac scintigraphy, measuring the uptake of 123I-metaiodobenzylguanidine (MIBG). Our group evaluated a total of 90 patients by cardiac MIBG scintigraphy, including 27 carriers of LRRK2 mutations (23 with the R1441G mutation and 4 with the G2019S mutation) and 63 non-carriers (45). Sixty-six percent of LRRK2 mutation carriers had low early MIBG uptake, compared to 86% of non-carriers ($P = 0.048$). Similarly, the heart/mediastinum ratio in delayed MIBG images appeared to differ between these groups of patients with PD, even though these results did not reach statistical significance.

Sleep Disorders

Sleep disorders such as insomnia, excessive daytime sleepiness (EDS), and REM Sleep Behavior Disorder (RBD) are common in sPD. In fact, RBD, and possibly EDS, may antedate the onset of parkinsonism in sPD. Iranzo et al. assessed sleep in 18 LRRK2-PD (17 carrying G2019S and one R1441G mutations), 17 NMC (11 G2019S, three R1441G, three R1441C), 14 non-manifesting non-carriers (NMNC), and 19 unrelated sPD through a comprehensive interview conducted by sleep specialists, validated sleep scales and questionnaires, and video-polysomnography followed by multiple sleep latency test (MSLT). They observed that sleep complaints were frequent in LRRK2-PD and showed a pattern that when compared to sPD was characterized by more frequent sleep onset insomnia, similar EDS, and less prominent RBD. Thus, unlike in sPD, RBD and EDS seemed to be not suitable markers of the prodromal stage of LRRK2-PD (46). However, further studies are needed to asses sleep disorders specifically in R1441G-PD.

OUR SERIES OF R1441G-PD PATIENTS AND NON-MANIFESTING MUTATION CARRIERS

In the last 25 years, we have been closely monitoring familial forms of PD in the Movement Disorders Unit of Donostia University Hospital (San Sebastián, Gipuzkoa, Spain). A total of 251 LRRK2 mutation carriers have been followed up. Sixty-six of them carry the G2019S mutation (46 PD patients and 20 NMC). Regarding the R1441G mutation, we have followed up 100 R1441G-PD patients, as well as more than 200 of their asymptomatic relatives, of which 85 NMC have been registered. Both patients and their families have collaborated altruistically in multiple projects and consortia. In this article, we would like to show the current general characteristics of this cohort, and some data about its follow-up obtained in the context of the Parkinson's Progression Markers Initiative (PPMI) as well as our general daily practice.

TABLE 1 | Characteristics of the R1441G-PD patients in 2012 and updated date about the current cohort.

	2012 Ruiz-Martínez J.	2020 Vinagre-Aragón A.
Basque origin	94%	81%
PD family history	91%	80%
Homozygous	0	1%
Phenocopy	0	1%
Gender (male/female)	48.5%	51%
Mean age (years)	74.6	77.9
Mean age at onset (years)	61.8	62.7
Mean disease duration (years)	12.7	14.9
Levodopa response	100%	100%
Equivalent levodopa (mg/day)	875	767.1
Advanced therapies - Deep Brain Stimulation (DBS) - Continuous apomorphine infusion (CAI) - Continuous intrajejunal infusion of levodopa/carbidopa gel (CIILG)	1 DBS	5 DBS, 3 CAI, 1CIILG
H&Y	2.91	2.71
Motor phenotype		
- Tremor	60%	55.36%
- Rigid-akinetic syndrome	27.7%	27.69%
- Mixt	7.7%	3.11%
- Gait predominant	4.6%	13.84%
Neuropathological study	2 (no αsyn aggregates)	5 (no αsyn aggregates)

Characteristics of the R1441G Series of Patients

The general characteristics and the motor phenotype of R1441G-PD patients remain very similar to those described in 2012 by Ruiz-Martinez J. in his doctoral thesis (**Table 1**) (26). The 100 patients currently included in the database have the diagnosis of PD according to the Gelb and Brain Bank clinical criteria. Ninety-nine patients are heterozygous for the R1441G mutation, and one is homozygous. Ninety-three percent have positive family history, mostly first-order (80%). All these patients belong to a total of 49 families. We have been able to follow-up patients from three generations. Some of these families have up to 20 members suffering from PD. We have only had one case of phenocopy.

Eighty-one percent are of Basque origin based on the presence of at least their first two surnames with this root.

Regarding the distribution by sex, 51 are women and 49 are men. In this series, the current mean age of the patients ($n = 95$) is 77.95 years (40-96), the mean age of disease onset ($n = 90$) is 62.71 years (35-81), and the mean time of duration of disease ($n = 89$) is 14.9 years (4-33).

The clinical phenotype at disease onset was analyzed in 65 patients, and tremor (55.36%) predominates clearly over rigid-akinetic syndrome (27.69%) and gait disorder (13.84%). The current mean H&Y stage ($n = 0$) is 2.71, with patients predominating in stages 3 (45.71%) and 2 (24.2%). Regarding NMS, there are data available published in 2012 by Ruiz-Martinez J. in his Doctoral Thesis (26). The frequency of NMS was analyzed in 68 R1441G-PD patients and compared with 28 G2019S-PD patients. At the time of the evaluation, 35.9% had signs of cognitive impairment, 4.8% suffered from hallucinations, and 31.3% had depressive symptoms. Other NMS were observed with the following frequency: constipation 30.6%, orthostatic hypotension 11.3%, RBD 11.5%, and EDS 4.8%. No case associated with restless leg syndrome was reported. When comparing R1441G-PD and G2019S-PD, there were only significant differences in hallucinations (4.8 vs. 29.2%) and urinary symptoms (19.4 vs. 43.5%).

The equivalent dose of levodopa (L-dopa) is 767.1 mg/day. Regarding advanced therapies, 5 patients have undergone deep-brain stimulation (DBS), two associate continuous infusion of apomorphine, and one patient is on intrajejunal duodopa infusion. All the patients that underwent DBS evolved well. In all of them, the fluctuations and dyskinesia improved significantly, and none of them developed complications. In 3 of them, a 50% reduction in L-dopa was achieved. Freezing was the symptom that improved the least, as is to be expected in PD patients in general.

Within the subgroup of patients with an earlier age of onset, there is a patient who is also a carrier of a mutation that justifies cerebral calcifications observed in him and his relatives. Another patient associates a mutation in Parkin gene, and another suffers from Down syndrome. The Parkin/LRRK2 double-mutation case has a homozygous deletion in Parkin considered pathogenic, and the phenotype of a very tremulous early onset coincides with the classic phenotype of Parkin-associated PD.

In this historical series, 42 patients have already died at a mean age of 82.02 years (63–93). The age at disease onset in these cases was 65.79 years (4–80), and they died after a mean duration of disease of 16.02 years (5–27). It was possible to perform an autopsy in 5 of these patients. Curiously, Lewy bodies and Lewy neurites were absent in all of them in the neuropathological study (also in the double mutation case).

Non-manifesting Carriers of the R1441G Mutation Follow-Up

In a study carried out by our group on asymptomatic relatives with the R1441G mutation, there was evidence of dopaminergic nigrostriatal denervation in R1441G mutation carriers and it was associated with a decrease in the performance of complex motor tests. These tests could be early indicators of ongoing dopamine deficit in this group at risk for PD (47). It should be pointed out that to date, we still are in touch with 89 NMC (49 women and 40 men) with a mean age of 52.4 years (38-73).

PPMI Cohort

As previously mentioned, our hospital is one of the clinical sites in which the PPMI takes place with a total of 28 participants carrying the R1441G mutation involved (14 PD patients and 14 asymptomatic mutation carriers). Taking part in this study has enabled us to closely monitor these subjects during a 6-year follow-up period.

The mean age at onset of disease in R1441G-PD patients was 63 (46-80) years. The mean duration of disease when follow-up began was 9 (5–14) years. The PD phenotype at disease onset was tremulous in 70%, with first Parkinson's symptom being leg rest tremor in all these cases. Ninety percent had positive family history. As shown in **Figure 2**, motor progression was certainly slow, with the mean UPDRSIII at baseline of 14 ± 5 (5–26) and the mean UPDRSIII at year 6 of 16 ± 11 (4–44). Likewise, the mean HY at baseline was 1.79 and 2.00 at year 6 of follow-up. The mean score on the Schwab and England ADL scale at baseline was 92 ± 7 (80–100) and 84 ± 17 (60–100) at year 6. Patients remained cognitively stable during follow-up. The mean score on MOCA at baseline was 23 ± 3 (18-28) and 24 ± 3 (19-29) at year 6.

The mean age of NMC when follow-up began was 59 (51-65). In 40%, basal DaTSCAN was consistent with evidence of dopamine transporter deficit. In the context of PPMI, DaTSCANs were performed in NMC at 2, 4, and 6 years from the start of follow-up, but these data have yet not been published. To date, none of them have manifested symptoms or signs of the disease.

DISCUSSION

The first descriptions in 2004 of PD associated with the R1441G mutation on a small sample of patients (13, 24, 25) showed motor features very similar to those obtained in the doctoral thesis of Ruiz Martinez J., in 2012 (26) and in the current work, which includes a significantly larger sample. The clinical profile of PD associated with R1441G mutation is very similar to that usually described in sPD. Certain NMS are less common in R1441G-PD, and in addition, these PD patients appear to have also less cognitive impairment. In fact, the latter could be a possible clinical difference between R1441G-PD and sPD patients or patients carrying other mutations in the LRRK2 gene, but further studies with larger samples are needed. However, the fact that the same features are maintained after increasing the sample size and expanding the follow-up period supports the initial hypothesis that R1441G-PD is characterized by a homogeneous clinical phenotype.

A close follow-up of patients and NMC has allowed us to know more accurately the evolution of the disease in these cases and to analyze the role of different clinical markers for the detection of early signs of the disease. There is no doubt that

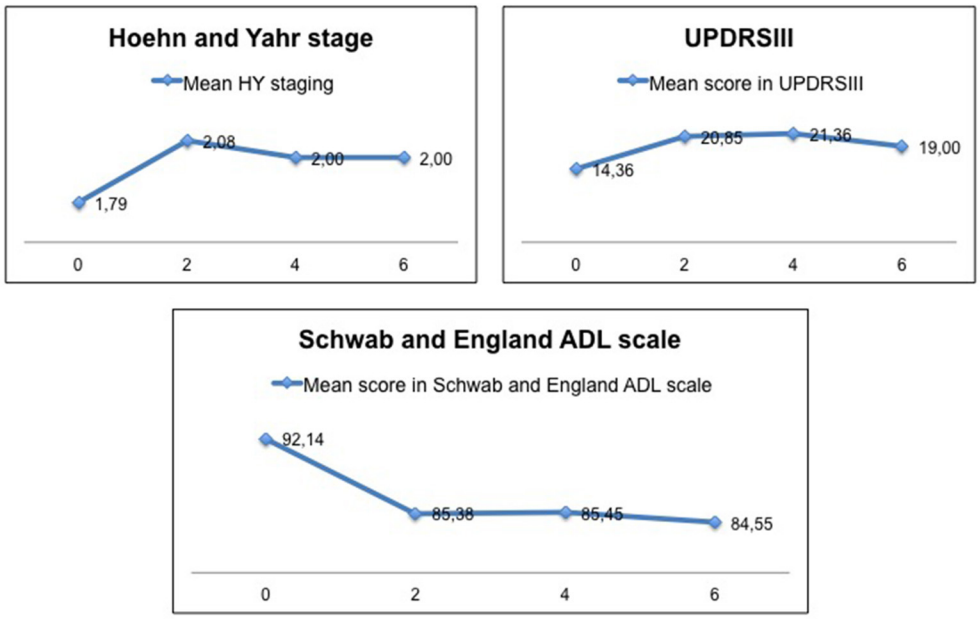

FIGURE 2 | Motor progression and its impact on activities of daily living in the subgroup of patients of our cohort participating in the PPMI.

the study and follow-up of NMC is an excellent opportunity for the detection of these markers. The relevance of certain NMS is known due to their early presence before the so-called motor phase of the disease, and therefore the study of these NMS in R1441G mutation carriers allows us to approximate the value of these symptoms as PD predictor markers. However, within NMS, olfactory and sympathetic dysfunction do not seem to be highly represented in R1441G-PD patients. Thus, neither seems to be a marker in NMC. Similarly, RBD and constipation appear to be also less represented in R1441G-PD patients according to previously published data. In contrast, as might be expected in a predominantly motor disease in R1441G-PD patients, the follow-up data of NMC and their correlation with the DaTSCAN (47) indicate that the study of early markers should be aimed at evaluating areas or systems that have shown loss of activity, and in this sense the nigrostriatal dopaminergic pathway stands out as it defines the motor profile of the illness. Thus, the DaTSCAN as well as other tests evaluating the nigrostriatal motor pathway may be more effective in preclinical stages as they evaluate areas where neuronal degeneration has been demonstrated.

In addition, long-term follow-up has enabled to obtain mortality data as well as the performance of neuropathological studies that help to improve knowledge about the etiopathogenesis of the disease. So far, 42 R1441G-PD patients have died. The mean age of death, after more than a mean of 16 years of duration of disease, reaches 82 years. This figure is very similar to the mean age of death expected in the Spanish population, which is around 83 years. This data also indicates that it is a disease with a less aggressive course.

In 5 of the deceased patients, informed consent to perform the neuropathological study was obtained. In these cases, the course of the disease was characterized by a lower frequency of NMS and all of them showed isolated nigral degeneration in the absence of Lewy pathology (48). Takanashi et al. reported the same findings (49). Some of these cases were included in the work published by Kalia et al. (50). They performed a clinical–pathological correlation study in a series of patients with LRRK2-PD and showed that the cases with less accumulation of αsyn corresponded to forms of PD with a pure motor phenotype and less presence of NMS. Unlike in R1441G-PD, G2019S, and I2020T-PD did show greater heterogeneity in the results of the hitherto performed neuropathological studies (5).

It should also be pointed out that one of the recently deceased patients carried also a Parkin mutation. In this specific case, the age of disease onset was early (44 years) and the phenotype was very tremulous coinciding with the classic phenotype of Parkin-associated PD. The course was less aggressive with few NMS. The patient died 22 years after the onset of symptoms due to a lymphoma, and αsyn aggregates were absent in the neuropathological study.

The absence of αsyn aggregates in ours and other descriptions of LRRK2-PD patients can be understood from different perspectives. The surmise reason of this difference may be the location of the mutation in the protein domain. G2019S and I2020T mutations are located in the kinase domain, whereas R1441G/H is located in the Roc domain. The kinase domain is known to be associated with the Rab family of proteins, and therefore, it seems to be more likely related to vesicular trafficking, autophagy, and/or lysosomal

dysfunction resulting in a cascade that induces Lewy body pathology (51). Nevertheless, R1441G/H mutation may more directly relate to the dopaminergic neuronal loss. Actually, many patients with R1441G/H pathologically showed isolated nigral degeneration in the absence of Lewy pathology (48, 49). The later histopathological features may constitute a marker of slower neuronal loss, which could justify the less aggressive clinical course observed in these patients. Patients carrying other LRRK2 mutations (G2019S and I2020T) have shown heterogeneity in the results of the performed neuropathological studies, which corresponds to a more variable clinical phenotype. This variability makes us consider that part of the pathophysiological mechanisms considered pivotal in the development of the disease up to now could not play such a central role.

Different physiopathogenic hypotheses have been proposed, based on abnormal aggregation and subsequent deposition of αsyn, with a toxic effect in the areas where it is deposited. In contrast, in R1441G-PD patients where these inclusions appear to be absent, the neuronal death mechanism has to be explained in another way. This plausible different physiopathogenic mechanism seems to affect the nigrostriatal pathway and determine a similar clinical motor phenotype. Nevertheless, it does not seem to affect other areas involved in the pathophysiology of NMS in sPD. Thus, the deposit of αsyn may constitute an epiphenomenon of the toxicity of other abnormal proteins involved in the pathogenesis of neurodegeneration in the context of aging and certain environmental factors.

It is extremely important to know in detail the specific phenotype of each type of PD in order to be able to study early-onset clinical markers and, based on this, to be able to develop disease-modifying therapies. The R1441G mutation has been consistently related to a motor phenotype similar to that seen in sPD over the years of follow-up. Without taking into account other potential neuroimaging or metabolic markers, the lower presence of NMS leads us to design the search for early markers of the disease focused on the nigrostriatal pathway. The homogeneity of these results correlates with a specific neuropathological pattern without αsyn aggregates as in other case descriptions of LRRK2-PD.

AUTHOR CONTRIBUTIONS

AV-A: literature review, data processing and analyses, results interpretation, language editing, review, and drafting of the first manuscript. JR-M: conception and article design, literature review, data processing and analyses, results interpretation, and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Twelves D, Perkins KS, Counsell C. Systematic review of incidence studies of Parkinson's disease. *Mov Disord.* (2003) 18:19–31. doi: 10.1002/mds.10305
2. Pringsheim T, Jette N, Frolkis A, Steeves TDL. The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Mov Disord.* (2014) 29:1583–90. doi: 10.1002/mds.25945
3. Postuma RB, Berg D, Stern M, Poewe W, Olanow WC, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord.* (2015) 30:1591–601. doi: 10.1002/mds.26424
4. Chaudhuri KR, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol.* (2009) 8:464–74. doi: 10.1016/S1474-4422(09)70068-7
5. Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with Parkinsonism – review of the literature. *Mov Disord.* (2017) 32:1504–23. doi: 10.1002/mds.27193
6. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson's disease. *Nat Rev Primers.* (2017) 23:17013. doi: 10.1038/nrdp.2017.13
7. Deng H, Wang P, Jankovic J. The genetics of Parkinson disease. *Ageing Res Rev.* (2018) 42:72–85. doi: 10.1016/j.arr.2017.12.007
8. Biosa A, Trancikova A, Civiero L, Glauser L, Bubacco L, Greggio E, et al. GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated LRRK2. *Hum Mol Genet.* (2013) 22:1140–56. doi: 10.1093/hmg/dds522
9. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's Disease-Associated Mutations in Leucine-Rich Repeat Kinase 2 Augment Kinase Activity. *Proc Natl Acad Sci USA*. (2005) 102:16842–7. doi: 10.1073/pnas.0507360102
10. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genetics.* (2009) 41:1303–7. doi: 10.1038/ng.485
11. Simón-sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet.* (2010) 41:1308–12. doi: 10.1038/ng.487
12. Purlyte E, Dhekne HS, Sarhan AR, Gomez R, Lis P, Wightman M, et al. Rab 29 activation of the Parkinson's disease-associated LRRK 2 *Kinase*. (2018) 37:1–18. doi: 10.15252/embj.201798099

13. Simón-Sánchez J, Martí-Massó JF, Sánchez-Mut JV, Paisán-Ruiz C, Martínez-Gil A, Ruiz-Martínez J, et al. Parkinson's disease due to the R1441G mutation in dardarin: a founder effect in the basques. *Mov Disord.* (2006) 21:1954–9. doi: 10.1002/mds.21114
14. Gorostidi A, Ruiz-Martinez J, López de Munain A, Alzualde A, Martí Massó JF. LRRK2 G2019S and R1441G mutations associated with Parkin-Son's disease are common in the basque country, but relative prevalence is determined by ethnicity. *Neurogenetics.* (2009) 10:157–9. doi: 10.1007/s10048-008-0162-0
15. González-Fernández MC, Lezcano E, Ross OA, Gómez-Esteban JC, Gómez-Busto F, Velasco F, et al. LRRK2-associated Parkinsonism is a major cause of disease in Northern Spain. *Parkinsonism Relat Disord.* (2007) 13:509–15. doi: 10.1016/j.parkreldis.2007.04.003
16. Mata IF, Ross OA, Kachergus J, Huerta C, Ribacoba R, Moris G, et al. LRRK2 mutations are a common cause of Parkinson's disease in Spain. *Eur J Neurol.* (2006) 13:391–4. doi: 10.1111/j.1468-1331.2006.01256.x
17. Infante J, Rodríguez E, Combarros O, Mateo I, Fontalba A, Pascual J, et al. LRRK2 G2019S is a common mutation in Spanish patients with late-onset Parkinson's disease. *Neurosci Lett.* (2006) 395:224–6. doi: 10.1016/j.neulet.2005.10.083
18. Gaig C, Ezquerra M, Marti MJ, Muñoz E, Valldeoriola F, Tolosa E. LRRK2 Mutations in Spanish patients with Parkinson disease: frequency, clinical features, and incomplete penetrance. *Arch Neurol.* (2006) 63:377–82. doi: 10.1001/archneur.63.3.377
19. Deng H, Le W, Guo Y, Hunter CB, Xie W, Huang M, et al. Genetic analysis of LRRK2 mutations in patients with Parkinson's disease. *J Neurol Sci.* (2006) 251:102–6. doi: 10.1016/j.jns.2006.09.017
20. Hatano T, Funayama M, Kubo SI, Mata IF, Oji Y, Mori A, et al. Identification of a Japanese family with LRRK2 p.R1441G-related Parkinson's disease. *Neurobiol Ageing.* (2014) 35:2656. doi: 10.1016/j.neurobiolaging.2014.05.025
21. Li Y, Ikeda A, Yoshino H, Oyama G, Kitani M, Daida K, et al. Clinical characterization of patients with leucine-rich repeat kinase 2 genetic variants in Japan. *J Hum Genet.* (2020) 65:771–81. doi: 10.1038/s10038-020-0772-4
22. Mata IF, Cosentino C, Marca V, Torres L, Mazzetti P, Ortega O, et al. LRRK2 mutations in patients with Parkinson's disease from Peru and Uruguay. *Parkinsonism Relat Disord.* (2009) 15:370–3. doi: 10.1016/j.parkreldis.2008.09.002
23. Ruiz-Martínez J, Gorostidi A, Ibañez B, Alzualde A, Otaegui D, Moreno F, et al. Penetrance in Parkinson's disease related to the LRRK2 R1441G mutation in the Basque Country (Spain). *Mov Disord.* (2010) 25:2340–5. doi: 10.1002/mds.23278
24. Paisán-Ruíz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* (2004) 44:595–600. doi: 10.1016/j.neuron.2004.10.023
25. Paisan-Ruiz C, Sàenz A, Lòpez de Munain A, Martì I, Martìnez Gil A, Martì-Massò JF, et al. Familial Parkinson's disease: clinical and genetic analysis of Four Basque families. *Ann Neurol.* (2005) 57:365–72. doi: 10.1002/ana.20391
26. Ruiz-Martinez J. *Enfermedad de Parkinson Por Mutación Vasca de La Dardarina (LRRK2): Estudio Epidemiológico, Clínico y Genético*. San Sebastián: University of the Basque Country - UPV/EHU Press (2012).
27. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* (2008) 7:583–90. doi: 10.1016/S1474-4422(08)70117-0
28. Kasten M, Kertelge L, Brüggemann N, van der Vegt J, Schmidt A, Tadic V, et al. Nonmotor symptoms in genetic Parkinson disease. *Arch Neurol.* (2010) 67:670–6. doi: 10.1001/archneurol.67.6.670
29. Aarsland D, Perry R, Brown A, Larsen JP, Ballard C. Neuropathology of dementia in Parkinson's disease: a prospective, community-based study. *Ann Neurol.* (2005) 58:773–6. doi: 10.1002/ana.20635
30. Hely MA, Reid WGJ, Adena MA, Halliday GM, Morris JGL. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov Disord.* (2008) 23:837–44. doi: 10.1002/mds.21956
31. Belarbi S, Hecham N, Lesage S, Kediha MI, Smail N, Benhassine T, et al. LRRK2 G2019S mutation in Parkinson's disease: a neuropsychological and neuropsychiatric study in a large algerian cohort. *Park Relat Disord.* (2010) 16:676–9. doi: 10.1016/j.parkreldis.2010.09.003
32. Goldwurm S, Zini M, Di Fonzo A, De Gaspari D, Siri C, Simons EJ, et al. LRRK2 G2019S mutation and Parkinson's disease: a clinical, neuropsychological and neuropsychiatric study in a large Italian Sample. *Park Relat Disord.* (2006) 12:410–9. doi: 10.1016/j.parkreldis.2006.04.001
33. Shanker V, Groves M, Heiman G, Palmese C, Saunders-Pullman R, Ozelius L, et al. Mood and cognition in leucine-rich repeat kinase 2 G2019S Parkinson's disease. *Mov Disord.* (2011) 26:1875. doi: 10.1002/mds.23746
34. Estanga A, Rodriguez-Oroz MC, Ruiz-Martinez J, Barandiaran M, Gorostidi A, Bergareche A, et al. Cognitive dysfunction in Parkinson's disease related to R1441G mutation in LRRK2. *Parkinsonism Relat Disord.* (2014) 20:10971100. doi: 10.1016/j.parkreldis.2014.07.005
35. Berg D, Schweitzer KJ, Leitner P, Zimprich A, Lichtner P, Belcredi P, et al. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease. *Brain.* (2005) 128:3000–11. doi: 10.1093/brain/awh666
36. Somme JH, Molano Salazar A, Gonzalez A, Tijero B, Berganzo K, Lezcano E, et al. Cognitive and behavioral symptoms in Parkinson's disease patients with the G2019S and R1441G mutations of the LRRK2 Gene. *Parkinsonism Relat Disord.* (2015) 21:494–9. doi: 10.1016/j.parkreldis.2015.02.019
37. Pont-Sunyer C, Tolosa E, Caspell-Garcia C, Coffey C, Alcalay RN, Chan P, et al. The Prodromal phase of leucine-rich repeat kinase 2–associated Parkinson disease: clinical and imaging studies. *Mov Disord.* (2017) 32:726–38. doi: 10.1002/mds.26964
38. Alcalay RN, Mirelman A, Saunders-Pullman R, Tang MX, Mejia-Santana H, Raymond D, et al. Parkinson disease phenotype in ashkenazi jews with and without LRRK2 G2019S mutations. *Mov Disord.* (2013) 28:1966–71. doi: 10.1002/mds.25647
39. Alcalay RN, Mejia-Santana H, Mirelman A, Saunders-Pullman R, Raymond D, Palmese C. et al. Neuropsychological performance in LRRK2 G2019S carriers with Parkinson's disease. *Park Relat Disord.* (2015) 21:106–10. doi: 10.1016/j.parkreldis.2014.09.033
40. Gaig C, Vilas D, Infante J, Sierra M, García-Gorostiaga I, Buongiorno M, et al. Nonmotor symptoms in LRRK2 G2019S associated Parkinson's disease. *PLoS ONE.* (2014) 9:e108982. doi: 10.1371/journal.pone.0108982
41. Pankratz N, Marder KS, Halter CA, Rudolph A, Shults CW, Nichols WC, et al. Clinical correlates of depressive symptoms in familial Parkinson's disease. *Mov Disord.* (2008) 23:2216–23. doi: 10.1002/mds.22285
42. Marras C, Schüle B, Munhoz RP, Rogaeva E, Langston JW, Kasten M, et al. Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers. *Neurology.* (2011) 77:325–33. doi: 10.1212/WNL.0b013e318227042d
43. Khan NL, Jain S, Lynch JM, Pavese N, Abou-Sleiman P, Holton JL, et al. Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. *Brain.* (2005) 128:2786–96. doi: 10.1093/brain/awh667
44. Markopoulou K, Larsen KW, Wszolek EK, Denson MA, Lang AE, Pfeiffer RF, et al. Olfactory dysfunction in familial Parkinsonism. *Neurology.* (1997) 49:1262–7. doi: 10.1212/wnl.49.5.1262
45. Ruiz-Martínez J, Gorostidi A, Goyenechea E, Alzualde A, Poza JJ, FRodríguez F, et al. Olfactory deficits and cardiac 123I-MIBG in Parkinson's disease related to the LRRK2 R1441G and G2019S mutations. *Move Disord.* (2011) 26:2026–31. doi: 10.1002/mds.23773
46. Pont-Sunyer C, Iranzo A, Gaig C, Fernández-Arcos A, Vilas D, Valldeoriola F, et al. Sleep disorders in Parkinsonian and Nonparkinsonian LRRK2 mutation carriers. *PLoS ONE.* (2015) 10:1–20. doi: 10.1371/journal.pone.0132368
47. Bergareche A, Rodríguez-Oroz MC, Estanga A, Gorostidi A, López de Munain A, Castillo-Triviño T, et al. DAT imaging and clinical biomarkers in relatives at genetic risk for LRRK2R1441G Parkinson's disease. *Mov Disord.* (2016) 31:335–43. doi: 10.1002/mds.26478
48. Marti-Masso JF, Ruiz-Martínez J, Bolaño MJ, Ruiz I, Gorostidi A, Moreno F, et al. Neuropathology of Parkinson's Disease with the R1441G Mutation in LRRK2. *Mov Disord.* (2009) 24:1998–2019. doi: 10.1002/mds.22677

49. Takanashi M, Funayama M, Matsuura E, Yoshino H, Li Y, Tsuyama S, et al. Isolated nigral degeneration without pathological protein aggregation in autopsied brains with LRRK2 p.R1441H homozygous and heterozygous mutations. *Acta Neuropathol Commun.* (2018) 6:105. doi: 10.1186/s40478-018-0617-y
50. Kalia LV, Lang AE, Hazrati LH, Fujioka S, Wszolek ZK, Dickson DW, et al. Clinical correlations with lewy body pathology in LRRK2-related Parkinson Disease. *JAMA Neurol.* (2015) 72:100–5. doi: 10.1001/jamaneurol.2014.2704
51. Alessi DR, Sammler E. LRRK2 kinase in Parkinson's disease. *Science.* (2018) 360:36–7. doi: 10.1126/science.aar5683

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Clinical Variability of *SYNJ1*-Associated Early-Onset Parkinsonism

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Autosomal recessive early-onset parkinsonism is clinically and genetically heterogeneous. Mutations of three genes, *PRKN*, *PINK1*, and *DJ-1* cause pure phenotypes usually characterized by levodopa-responsive Parkinson's disease. By contrast, mutations of other genes, including *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *SYNJ1*, *VPS13C*, and *PTRHD1,* cause rarer, more severe diseases with a poor response to levodopa, generally with additional atypical features. We performed data mining on a gene panel or whole-exome sequencing in 460 index cases with early-onset (≤ 40 years) Parkinson's disease, including 57 with autosomal recessive disease and 403 isolated cases. We identified two isolated cases carrying biallelic mutations of *SYNJ1* (double-heterozygous p.D791fs/p.Y232H and homozygous p. Y832C mutations) and two siblings with the recurrent homozygous p.R258Q mutation. All four variants were absent or rare in the Genome Aggregation Database, were predicted to be deleterious on *in silico* analysis and were found to be highly conserved between species. The patient with both the previously unknown p.D791fs and p.Y232H mutations presented with dystonia-parkinsonism accompanied by a frontal syndrome and oculomotor disturbances at the age of 39. In addition, two siblings from an Algerian consanguineous family carried the homozygous p.R258Q mutation and presented generalized tonic-clonic seizures during childhood, with severe intellectual disability, followed by progressive parkinsonism during their teens. By contrast, the isolated patient with the homozygous p. Y832C mutation, diagnosed at the age of 20, had typical parkinsonism, with no atypical symptoms and slow disease progression. Our findings expand the mutational spectrum and phenotypic profile of *SYNJ1*-related parkinsonism.

Keywords: Parkinson's disease, SYNJ1, autosomal recessive inheritance, early-onset parkinsonism, atypical Parkinson's disease

INTRODUCTION

Parkinson's disease (PD), the second most frequent neurodegenerative disorder after Alzheimer's disease, affects about 2% of people over the age of 60 years. PD affects dopaminergic neurons in the substantia nigra, causing characteristic motor signs, such as bradykinesia, rigidity with resting tremor and postural instability; it also affects other brain areas, causing non-motor signs, such as olfactory dysfunction, cognitive impairment, psychiatric symptoms and autonomic dysfunction (1). PD is monogenic and caused by rare, highly penetrant mutations in 10–15% of PD patients, but most PD cases are sporadic and probably due to a combination of environmental, genetic and epigenetic factors. The last 25 years have seen great progress toward understanding the genetic basis of this disease, with the identification of disease-causing genes. At least 23 loci and 13 genes clearly linked to inherited forms of parkinsonism have been identified, including 10 causing early-onset (EO) autosomal recessive (AR) forms (*PRKN*, *PINK1*, *DJ-1*, *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *SYNJ1*, *VPS13C*, and *PTRHD1*) [reviewed in Lunati et al. (2)]. AR EO PD is clinically and genetically heterogeneous: it is most frequently caused by *PRKN*, *PINK1* and *DJ-1* mutations, particularly in patients with a positive family history and/or consanguinity, with phenotypes resembling typical levodopa-responsive EO PD and slow disease progression. However, rare mutations of *ATP13A2, PLA2G6, FBXO7*, *DNAJC6*, *SYNJ1*, *VPS13C*, and *PTRHD1* cause more severe disease with additional neurological signs and symptoms, such as cognitive decline, dystonia, epilepsy, pyramidal features, and a less consistent response to levodopa [reviewed in Lunati et al. (2)].

Synaptojanin 1, encoded by *SYNJ1* on chromosome 21q22.11, was first identified in 1994 as a brain-specific 145 kDa protein highly conserved throughout evolution (3). It seems to be involved in synaptic vesicle endocytosis and recycling (4, 5). Biallelic mutations of *SYNJ1* are associated with two distinct phenotypes: EO PD (PARK20) and a severe neurodegenerative disorder with intractable seizures and tauopathies (6–20). Patients with *SYNJ1* mutations therefore display highly variable phenotypes.

We performed data mining on a gene panel or whole-exome sequencing in 460 index cases with EO PD. We identified biallelic *SYNJ1* variants in a consanguineous family and two isolated cases of PD.

MATERIALS AND METHODS

In total, 460 index cases with EO [$\leq$ 40 years, mean age at onset (AAO): 33.1 $\pm$ 6.9 years] parkinsonism without mutations of genes known to cause PD and related disorders were analyzed for the presence of biallelic coding (small insertions/deletions, missense, and stop-gain changes) or splice-site ($\pm$ 5 base pairs from the coding exons or synonymous variants predicted to create splice defects) variants of *SYNJ1*. Index cases were recruited through the French network for the study of Parkinson's disease genetics (PDG group) and diverse collaborations with Mediterranean countries. There were 296 male and 164 female patients; most were Caucasian (n = 397, 86.3%), and French (n = 314, 79%), the others were North African (n = 41, 8.9%), or of other origins (n = 22). A family history of PD, consistent with AR transmission, was reported in 12% of the index cases (n = 57), 403 of the index cases were isolated cases with suspected consanguinity (n = 16) or without consanguinity (n = 387). According to the clinical diagnostic criteria of the UK Parkinson Disease Society Brain Bank (21), most of the index cases (n = 431) had EO typical PD, the remaining 29 patients had EO parkinsonism with some atypical features (poor response to levodopa, pyramidal signs, oculomotor disturbances, or dementia). This study was approved by the appropriate institutional review boards, and written informed consent was obtained from all participants.

We investigated *SYNJ1* mutations, by performing data mining on a customized next-generation sequencing (NGS) targeted gene panel containing 9–70 PD-associated genes, depending on the incremental version used (**Supplementary Table 1**), or whole-exome sequencing data obtained as previously described (22, 23) from a large cohort of patients with EO PD.

Sanger sequencing was used to confirm variants and co-segregation analyses, where possible.

RESULTS

Genetic Findings

We identified a familial case (FPD-1458-9) with the recurrent homozygous missense mutation, p.R258Q (c.773G>A in exon 5) in *SYNJ1* (GenBank accession number for the longer 1,612 amino acid isoform: NM_003895.3) and two isolated cases—one consanguineous patient (SPD-174-10) carrying a homozygous missense mutation (c.2495A>G in exon 19, p.Y832C) and another patient (SPD-68-1) carrying two heterozygous mutations (a missense variant, c.694T>C in exon 5, p.Y232H and a truncating deletion, c.2371delG in exon 18, p.D791Ifs*4) (**Figure 1A**). No additional family members were available for determining parental phase for these last two mutations. The index case, FPD-1458-9, came from a consanguineous family consisting of two healthy parents, two affected and five unaffected siblings.

All four mutations were verified by Sanger sequencing. Co-segregation analyses performed in the FPD-1458 family revealed that the proband's affected sister harbored the same homozygous p.R258Q mutation, whereas both the unaffected parents and one of the five unaffected siblings for whom DNA was available were heterozygous for this mutation (**Figure 1A**).

No other rare homozygous or biallelic deleterious variants of PD-associated genes were identified on gene panel or whole exome analyses, for any of the three index cases.

Abbreviations: AAO, age at onset; AR, autosomal recessive; CADD, combined annotation-dependent depletion; EO, early-onset; GnomAD, Genome Aggregation Database; MMSE, Mini Mental State Examination; MRI, magnetic resonance imaging; NGS, next-generation sequencing; PD, Parkinson's disease; UPDRS-III, Unified Parkinson's Disease Rating Scale part III.

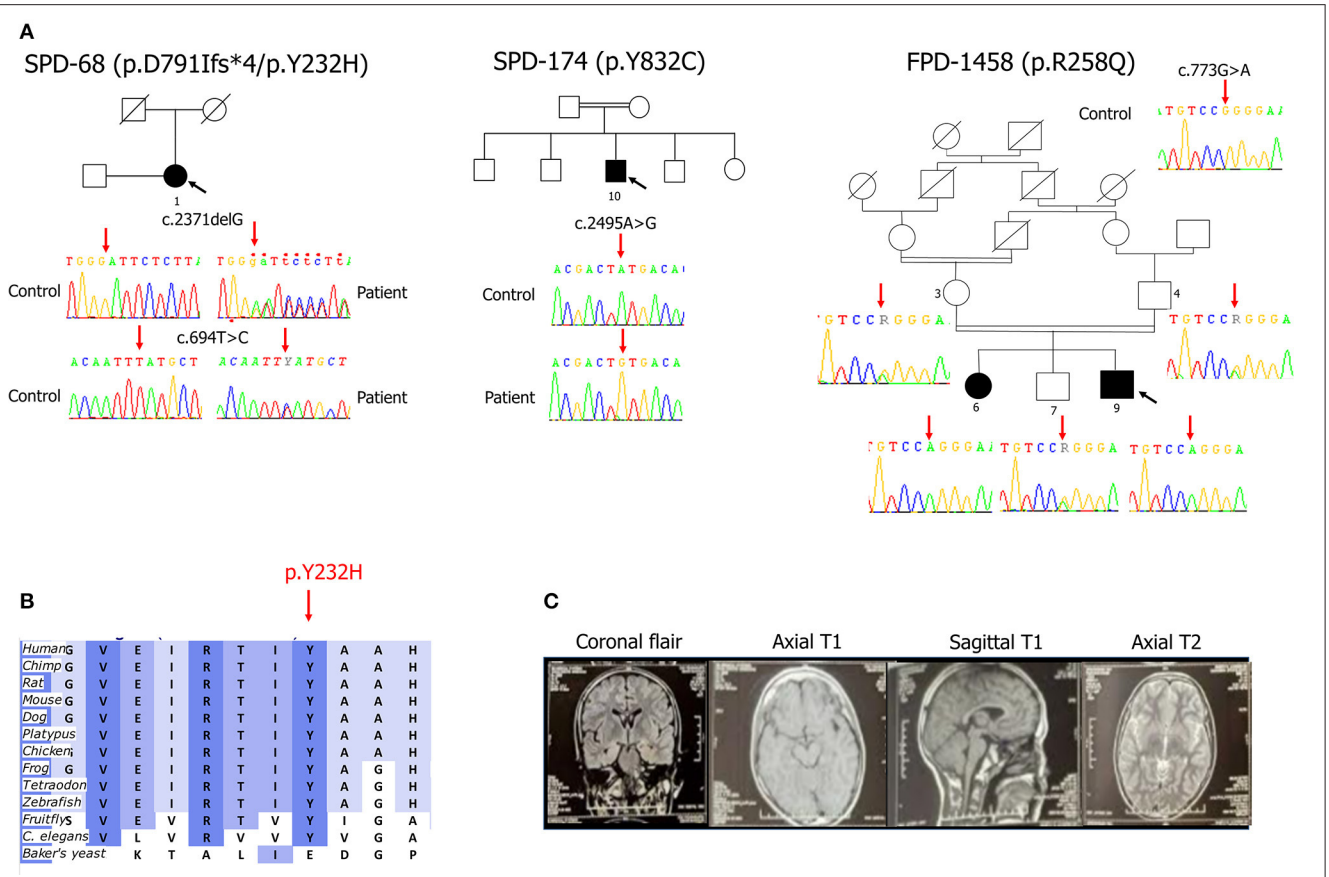

FIGURE 1 | (A) Pedigrees of the family and the two isolated cases with early-onset Parkinson's disease carrying *SYNJ1* mutations. Affected family members are represented by black circles (female) or squares (male). The arrow indicates the index cases. Double lines indicate consanguineous parents. The corresponding Sanger sequence electrophoregrams are shown. The p.R258Q mutation segregated with the phenotype in the FPD-1458 family: the p.R258Q genotypes are highlighted by a red arrow (heterozygous state for individuals 3, 4, and 7 and homozygous for the two affected siblings, 6 and 9). **(B)** Evolutionary conservation of the regions of the p.Y232 amino-acid sequences (download from Alamut® Visual software, https://www.interactive-biosoftware.com/alamut-visual/). **(C)** Brain magnetic resonance imaging (MRI) for patient FPD-1458-9 showing the normal appearance of the various slices.

Both the *SYNJ1* p.R258Q, and p.Y832C mutations were rare or absent from public databases and highly conserved between species (6, 7, 10). By contrast, neither of the new *SYNJ1* mutations, p.Y232H or p.D791Ifs*4, is present in any public database, including the Genome Aggregation Database (GnomAD). The missense variant p.Y232H is predicted to be pathogenic [SIFT: deleterious; Polyphen-2; probably damaging; MutationTaster: disease-causing; Align GVGD: Class C0 (GV: 122.78–GD: 0.00); combined annotation-dependent (CADD)_phred: 28] and has been shown to be conserved in orthologous sequences from *C. elegans* onwards (Alamut® Visual v.2.11 software, Interactive Biosoftware, Rouen, France) (**Figure 1B**). Both these mutations are located in functional domains: p.Y232H in the Sac1 domain and p.D791Ifs*4, in the 5′phosphatase domain (**Figure 2**).

Clinical Outcome

Case Report Family FPD-1458

The two affected siblings were born to healthy first-cousin parents originating from Algeria.

The proband, FPD-1458-9, was a 25-year-old man who had suffered episodes of generalized tonic-clonic seizures at the age of 2 years after a bout of fever. These episodes were treated with sodium valproate and carbamazepine. The patient's psychomotor development was normal and he started school at the age of 6 years. Three years later, he presented a cognitive decline, leading to an interruption of his schooling. At the age of 20 years, the patient presented dysarthria, which was followed, 1 year later, by weakness of the muscles of the left arm and dystonic postures that were more marked distally and a progressive slowing of lower limb movements limiting the distance that the patient could walk. A few months later, a distal resting tremor appeared in the left upper limb, subsequently extending to the contralateral upper limb. Initial treatment with dopamine agonists was not tolerated. The patient was therefore given low doses of levodopa (50 mg), leading to a partial improvement of his motor symptoms, but rapidly resulting in disabling levodopa-induced dyskinesia. There was no evidence of autonomic dysfunction, other than urinary urgency. Clinical examination at the age of 25 years revealed a poorly cooperative

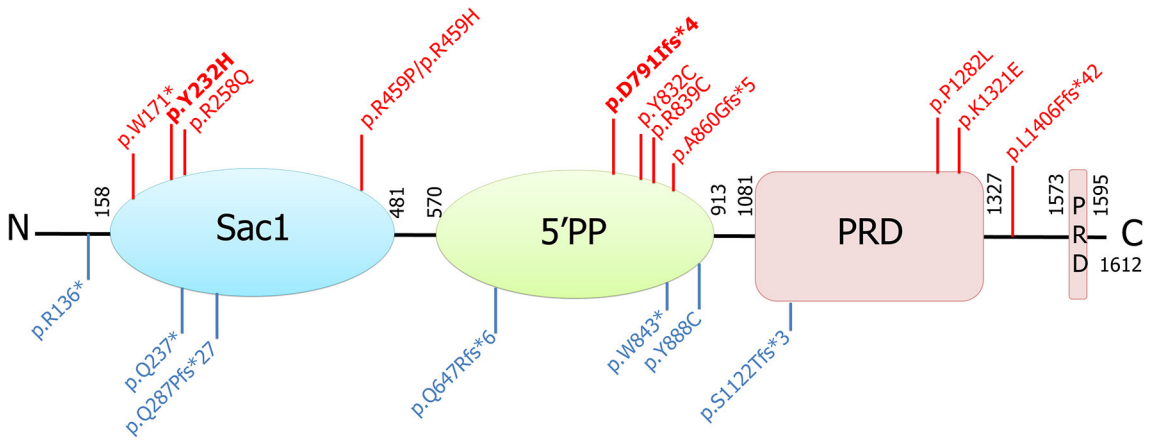

FIGURE 2 | Schematic representation of the longer isoform of the synaptojanin 1 protein, its functional domains, and all associated mutations identified to date. Those found in patients with parkinsonism are shown in red (in bold, those identified in this study), and those found in patients with intractable seizures and severe neurodegeneration are shown in blue. Sac1, suppressor of actin (Sac1-like inositol domain); 5′PP, inositol-5′-phosphatase domain; PRD, prolin-rich domain.

patient with parkinsonism combining an intermittent resting and postural tremor in both upper limbs, head tremor, bilateral but asymmetric plastic hypertonia with cogwheel rigidity on both sides, a global motor slowing and slowing of the gait, with a reduction of the swing of the arms predominantly on the left side, slight forward camptocormia, an inexhaustible nasopalpebral reflex, excessive drooling, but no oculomotor abnormalities or pyramidal signs. Off medication, the patient had a Unified Parkinson's disease rating scale part III (UPDRS-III) score of 35/108, and a Hoehn and Yahr stage of 2.5/5. The patient's cognitive disability made it impossible to perform a cognitive evaluation. Brain magnetic resonance imaging (MRI) results were normal (**Figure 1C**). An electroencephalogram (EEG) showed slow activity at 5 Hz/s, with no epileptic anomalies.

The patient's 31-year-old sister (FPD-1458-6) had treated generalized epilepsy, from the age of 14–20 years. Her psychomotor development was normal until the age of 10 years when she presented cognitive decline and was unable to continue her schooling. At the age of 26 years, she displayed progressive parkinsonism with dystonia, resting tremor and upper limb postural tremor predominantly on the left side, and progressive bradykinesia. Levodopa treatment, initiated at the age of 27 years, led to a significant improvement in clinical signs, but the patient developed early levodopa-induced dyskinesia, motor fluctuations, and end-of-dose dystonia. No autonomic dysfunction was observed. Neurological examination at the age of 29 years, on "off" medication, revealed a cooperating patient with parkinsonism characterized by resting tremor in the two upper limbs, predominantly on the left, plastic hypertonia, a loss of arm swing, slow gait and facial amimia, but no oculomotor abnormalities or pyramidal syndrome. The patient had a UPDRS-III motor score of 26/108 and a Hoehn and Yahr stage of 2/5. It was not possible to perform a neuropsychological evaluation.

Other family members, including both unaffected parents and the five other unaffected siblings, displayed no signs or symptoms of epilepsy or parkinsonism.

Isolated Case SPD-174-10

Incomplete clinical information was obtained for patient SPD-174-10, who was lost to follow-up. This 35-year-old man was born to consanguineous parents in Senegal. He presented an extrapyramidal akineto-rigid syndrome, at the age of 20. On neurological examination at the age of 35 years, a bilateral resting tremor was observed, with no other pyramidal signs or symptoms, cerebellar syndrome, apraxia, dystonia, or oculomotor disturbances. During the "on" stage, this patient had a Hoehn and Yahr stage of 5/5 after 16 years of disease progression. No specific treatment or response to treatment was recorded, but the patient developed urinary incontinence and erectile dysfunction. No neuropsychological evaluation or brain MRI scan was recorded at his last examination.

Isolated Case SPD-68-1

Patient SPD-68-1 was a 61-year-old French woman, with no family history of PD or known parental consanguinity. She developed micrography, slow gait and a chin tremor at the age of 39, rapidly followed by facial and cervical dystonia. Parkinsonism was diagnosed at the age of 43. Akinesia and rigidity responded to levodopa (100 mg, 3 times/day), but with the immediate development of motor fluctuations and disabling dyskinesias. One year later, the patient presented a bilateral, asymmetric, levodopa-responsive (50%) extrapyramidal syndrome (left > right): akinesia and rigidity but no resting tremor, motor fluctuations with diphasic (facial and lower limb dystonia) and peak-dose choreic dyskinesia (oromandibular, right upper limb), permanent posterior cervical dystonia, vertical gaze palsy, freezing of gait when "off" levodopa, but no postural instability, moderate dysarthria and hypophonia. A frontal syndrome was detected with perseverations and an applause sign [Mini-Mental State Examination (MMSE) score = 27/30]. Deep tendon reflexes were brisk with no Babinski sign. There was no cerebellar dysfunction or dysautonomia. Brain computed tomography (CT) scan findings were normal. Gait freezing became more frequent, with the development of postural

instability with falls 3 years after diagnosis, and a worsening of vertical gaze palsy and dysarthria. Levadopa sensitivity persisted (70% improvement following levodopa challenge, 9 years after diagnosis), but motor fluctuations and dyskinesias (diphasic and peak-dose) worsened. A moderate worsening of the frontal syndrome was observed, but instrumental functions were preserved [Mattis Dementia Rating Scale (MDRS) = 134/144 and MMSE score = 21/30, 9 years after diagnosis]. Eighteen years after diagnosis, this patient had severe dysarthria and mild dysphagia on medication.

DISCUSSION

SYNJ1-related diseases are heterogeneous in terms of their symptoms, ranging from EO typical PD (10) to EO complex parkinsonism (6–8, 10–16) (both designated PARK20), and severe neurodegeneration with intractable seizures (17–20) (**Table 1**).

We report here the molecular and associated clinical findings for two familial cases with the recurrent homozygous p.R258Q mutation and two apparently sporadic cases, each carrying, either double-heterozygous of new p.D791fs/p.Y232H variants or the known homozygous p. Y832C mutation.

Two independent groups initially reported the presence of the same *SYNJ1* p.R258Q missense mutation in the homozygous state in two consanguineous sib-pairs of Sicilian and Iranian origin (6, 7). A third family from Naples, was subsequently found to have the same recurrent *SYNJ1* mutation in two non-consanguineous siblings (8). Previous haplotype analyses in the two Italian families did not support the hypothesis of a common founder for the p.R258Q variant (8), instead suggesting a possible mutational hotspot. An additional non-consanguineous family of German origin was found to carry this mutation in the heterozygous state, together with a non-sense mutation at a *trans* location (**Table 1**). We report here the identification of a fourth consanguineous family of Algerian origin with the homozygous p.R258Q mutation. These families were characterized by EO atypical parkinsonism, with an onset in the third decade of life, with rapid progression through the initial stages and a stabilization of the disease at later stages (24). The principal clinical features of parkinsonian disease in these patients were a combination of tremor, dystonia, bradykinesia, and, a poor response to levodopa treatment in all but our case. Additional atypical signs, such as seizures, cognitive impairment, developmental delay, and oculomotor disturbances, were variable. Indeed, our siblings presented generalized tonic-clonic seizures, as seen in the Iranian siblings, whereas the Neapolitan carriers suffered from episodes of clonic seizures. Unlike five of the other six p.R258Q carriers, our patients presented no oculomotor disturbances. Finally, mild or severe cognitive decline was observed in the Sicilian, Neapolitan and Algerian families, but not in the Iranian siblings. In both the German siblings harboring the p.R258Q variant in the compound heterozygous state, the principal clinical trait was early epilepsy followed by generalized dopa-responsive dystonia in infancy (13).

We also identified an isolated patient of Senegalese origin, from a consanguineous family, who harbored the same homozygous pY832C variant as recently reported in two Chinese consanguineous siblings with PD (10). Very little clinical information for this patient was collected at a single follow-up assessment, but the same typical parkinsonism was observed, with no atypical signs/symptoms.

Finally, we identified a non-consanguineous isolated case with two new heterozygous *SYNJ1* variants, p.Y232H and p.D791Ifs*4, that may be pathogenic, based on the rarity of these variants and *in silico* analyses. We thus report a case extending the age at onset for *SYNJ1* mutations carriers (39 years), identify, for the first time, *SYNJ1* mutations in apparently isolated cases. However, the parental phase of these two variants is unknown, but the phenotype of this patient with two *SYNJ1* mutations resembles that of the other PARK20 mutation carriers, who had atypical parkinsonism with early-onset disease (at 20–31 years), a rapid development of dyskinesias on levodopa, predominantly axial symptoms with rapidly progressing gait impairment and falls, oculomotor disturbances and orofacial dystonia at onset (**Table 1**). Parkinsonism in our case was levodopa-responsive (> 50% response after acute challenge and presence of motor fluctuations), as also reported in a few previous cases (6, 13, 14) (**Table 1**), but the response was difficult to evaluate in the other six patients, due to severe dyskinesia and other adverse effects (6, 7, 12). Unlike patients from most PARK20 families with atypical EO parkinsonism, this patient displayed no seizures. However, susceptibility to seizures varies considerably, even within families (14, 15). Seizures also occur in some patients with mutations of *DNAJC6* (PARK19), encoding auxilin, which has been implicated in the uncoating of synaptic vesicles, potentially resulting in alterations to synaptic vesicle recycling (25). Like auxilin, synaptojanin 1 is involved in the post-endocytic recycling of synaptic vesicles, providing additional support for the link between synaptic endosomal trafficking and PD. In addition, a link between seizures and the accumulation of tau protein has been suggested, based on the brain autopsy of a single patient with intractable epilepsy and a homozygous *SYNJ1* truncating mutation showing tau-immunoreactive neurofibrillary degeneration in the substantia nigra (17).

Synaptojanin 1 is encoded by two open-reading frames (ORFs) of 170 and 145 kDa, encoding two major isoforms of 1,612 and 1,350 amino acids, respectively. The 145 kDa ORF is strongly expressed in the brain, and the corresponding protein localizes to presynaptic nerve terminals (26). Both isoforms contain two consecutive phosphatase domains: an N-terminal Sac1-like inositol domain and a central 5′-phosphatase domain followed by a C-terminal proline-rich domain (PRD). The longer 170 kDa isoform contains an additional PRD. Interestingly, a single *SYNJ1* mutation reported by Ben Romdhan et al. (14) is located in the C-terminal domain of the longer isoform.

In total 33 *SYNJ1* mutation carriers originating from 19 families and isolated cases (13 with the PARK20-*SYNJ1* phenotype and six with infancy treatment-resistant seizures

TABLE 1 | Clinical features of patients with biallelic mutations of the *SYNJ1* gene.

	Patient	*SYNJ1* mutations	Origin	Sex/AAE (y)	Transmission/ consanguinity	Age at onset of motor symptoms (y)	Phenotype	Seizures (age at onset)	Response to levodopa	Imaging data
Early-onset typical or atypical parkinsonism										
Our study	FPD-1458-9	p.R258Q (hom)	Algeria	M/25	AR/Yes	20	Parkinsonism, dysarthria, dystonia, drooling, postural instability, CI	Yes (2 y)	Partial with dyskinesia	Normal brain MRI
	FPD-1458-6	p.R258Q (hom)	Algeria	F/31	AR/Yes	26	Parkinsonism, dystonia, amimia, CI	Yes (14 y)	Good, with adverse effects (dyskinesia, motor fluctuations, dystonia)	NA
	SPD-174-10	p.Y832C (hom)	Senegal	M/35	Spo/Yes	20	Parkinsonism	No information	Unknown	NA
	SPD-68-1	p.Y232H/ p.D791Ifs*4[#] (double het)	France	F/61	Spo/No	39	Parkinsonism, facial and cervical dystonia, vertical gaze palsy, moderate dysarthria, hypophonia, postural instability, brisk deep tendon reflexes, MMSE 21/30	No	Good, with adverse effects (oromandibular and limb dystonias)	Normal brain CT scan
Krebs et al. (6)	Patient I	p.R258Q (hom)	Iran	M/29	AR/yes	20	Parkinsonism, eyelid apraxia and dysarthria at the age of 22 years, hypophonia, resting tremor, chin tremor, postural instability, no CI	Yes (3 y)	Not tolerated (severe dyskinesia)	Mild cortical atrophy, bilateral white matter hyperintensity
	Patient II	p.R258Q (hom)	Iran	F/39	AR/yes	Early twenties	Parkinsonism, right hand tremor, eyelid apraxia, severe jaw tremor, anarthria, requiring assistance to walk at the age of 32, bedbound at 37, no CI	Febrile convulsion in infancy	Not tolerated (severe dyskinesia)	Foramen magnum meningioma at the age of 37
Quadri et al. (7)	NAPO-16	p.R258Q (hom)	Italy (Sicily)	M/47	AR/yes	22	Parkinsonism, rest and action tremor, dystonia in both hands, postural instability, anarthria, severe dysarthria, eyelid apraxia and supranuclear vertical gaze palsy, dysphagia, CI	No	Not tolerated (oromandibular and limb dystonias, postural hypotension)	Diffuse cortex atrophy, hyperintensity of hippocampi, thinning midbrain quadrigeminal plate, nigrostriatal dopaminergic deficit, cortical hypometabolism
	NAPO-17	p.R258Q (hom)	Italy (Sicily)	F/31	AR/yes	28	Parkinsonism, rest and action tremor, dystonia in the hands and feet, dysarthria, dysphonia, mild dysphagia, postural instability, supranuclear vertical gaze palsy, brisk deep tendon reflexes, CI (MMSE 26/30)	No	Not tolerated (oromandibular and limb dystonias, postural hypotension)	Diffuse cortex atrophy, hyperintensity of hippocampi, thinning midbrain quadrigeminal plate, cortical hypometabolism

(Continued)

TABLE 1 | Continued

	Patient	*SYNJ1* mutations	Origin	Sex/AAE (y)	Transmission/ consanguinity	Age at onset of motor symptoms (y)	Phenotype	Seizures (age at onset)	Response to levodopa	Imaging data
Olgiati et al. (8)	NAPO-41	p.R258Q (hom)	Italy (Naples)	M/31	AR/No	28	Parkinsonism, hypomimia, oromandibular tremor, trunk dystonia, impaired speech, postural instability, mild supranuclear vertical gaze limitation, drooling, dysphagia at the age of 31, MMSE 28/30	One episode (uncertain)	Not treated	No gross abnormalities, normal brain MRI, nigrostriatal dopaminergic deficit, mild bilateral hypometabolism
	NAPO-42	p.R258Q (hom)	Italy (Naples)	F/27	AR/No	26	Parkinsonism, hypomimia, oromandibular tremor, impaired speech, brisk deep tendon reflexes, MMSE 25/30	One episode (16 y)	Not treated	No gross abnormalities, normal brain MRI, nigrostriatal dopaminergic deficit, mild bilateral hypometabolism
Kirola et al. (12)	H1_1	p.R459P (hom)	India/M	M/32	AR/yes	12	Parkinsonism, drooling, dystonia, dysarthria, dysphagia, hypophonia, intense constipation, falling backwards, postural instability, no dementia	No information	Not tolerated (dyskinesia and dystonia)	Hyperintensity in substantia nigra
	H1_2	p.R459P (hom)	India/F	F/22	AR/yes	18	Parkinsonism, drooling, dystonia, dysathria, dysphagia, hypophonia, falling backwards, constipation, no dementia	No information	Not tolerated (dyskinesia and dystonia)	NA
Rauschendorf et al. (13)	Patient 1	p.W171*/ p.R258Q (compound het)	Germany	M/21	AR/No	15	Generalized dystonia, Parkinsonism, severe action tremor of the tongue, head, and extremities, anarthria, CI	Yes (3–4 y)	Excellent (with L-dopa-induced dyskinesia)	Bilateral nigrostriatal dopaminergic deficit, bilateral caudate hypometabolism
	Patient 2	p.W171*/ p.R258Q (compound het)	Germany	M/32	AR/No	13	Generalized dystonia, Parkinsonism, action tremor of the upper extremities, chin and tongue	First days of life	Good (no dyskinesia)	Bilateral nigrostriatal dopaminergic deficit, bilateral caudate hypometabolism
Taghavi et al. (15)	F22P1	p.R839C (hom)	Iran	M/30	AR/Yes	24	Parkinsonism, chin tremor, dysarthria, longitudinally fissured tongue, postural instability	Yes (24 y)	Poor	NA
	F22P2	p.R839C (hom)	Iran	F/47	AR/Yes	27	Parkinsonism, postural instability	No	Poor	NA
Ben Romdhan et al. (14)	PD1	p.L1406Ffs*42/ p.K1321E (compound het)	Tunisia	M/23	AR/Yes	16	Parkinsonism, postural instability dystonia in the left arm, dysarthria, moderate CI (MMSE 20/30)	Yes (7 y)	Good (no dyskinesia)	Normal brain MRI
	PD2	p.L1406Ffs*42/ p.K1321E (compound het)	Tunisia	F/24	AR/Yes	21	Parkinsonism, postural instability, supranuclear vertical gaze palsy, moderate CI (MMSE 21/30)	No	Good (no motor complications)	Normal brain MRI
Hong et al. (16)	II1	p.A860Gfs*5/ p.P1282L (compound het)	China	F/35	AR/No	31	Parkinsonism, mild dysarthria, diplopia, dystonia, MMSE 28/30	No information	Poor	Mild cortical atrophy

(Continued)

TABLE 1 | Continued

	Patient	*SYNJ1* mutations	Origin	Sex/AAE (y)	Transmission/ consanguinity	Age at onset of motor symptoms (y)	Phenotype	Seizures (age at onset)	Response to levodopa	Imaging data
	II3	p.A860Gfs*5/ p.P1282L (compound het)	China	M/30	AR/No	28	Parkinsonism, diplopia, dystonia, MMSE 29/30	No information	Poor	Normal brain MRI
Xie et al. (10)	Patient 1	p.Y832C (hom)	China	F/52	AR/Yes	40	Parkinsonism, MMSE 30/30	No	Good	NA
	Patient 2	p.Y832C (hom)	China	M/54	AR/Yes	52	Parkinsonism	No	Not prescribed levodopa	NA
Kumar et al. (11)	114	p.R459H (hom)	India	No information	No information	34	Parkinsonism with poor information	No information	Unknown	NA
Early-onset treatment-resistant seizures and severe neurodegenerative decline										
Dyment et al. (17)	1	p.R136* (hom)	Pakistan	M/died at 6.5 years of age	Spo/Yes	NA	No parkinsonism, progressive neurodegenerative course, feeding intolerance at age of 1 year, and G tube dependence at the age of 2 years, hypotonia progressing to multiple contractures, no vocalization, cortical blindness	Yes (9 d)	Unknown	Brain MRI: mild cerebral atrophy at the age of 5 years
Hardies et al. (18)	Family A/1	p.Y888C (hom)	Morocco	F/7	AR/Yes	NA	No parkinsonism, progressive neurodegenerative course, feeding problems, hypotonia progressing to spastic tetraplegia, central visual impairment, severe intellectual disability	Yes (2.5 m)	Unknown	Normal brain MRI
	Family A/2	p.Y888C (hom)	Morocco	M/6	AR/Yes	NA	No parkinsonism, progressive neurodegenerative course, feeding problems, hypotonia progressing to spastic tetraplegia, central visual impairment, severe intellectual disability	Yes (6 m)	Unknown	Normal brain MRI
	Family B/1	p.W843* (hom)	Morocco	F/5	AR/Yes	NA	No parkinsonism, profound intellectual disability, progressive spastic tetraplegia, feeding problems with gastrostomy	Yes (1 d)	Unknown	Normal brain MRI
	Family B/2	p.W843* (hom)	Morocco	F/2.5	AR/Yes	NA	No parkinsonism, profound intellectual disability, progressive spastic tetraplegia, feeding problems with gastrostomy	Yes (1 d)	Unknown	Normal brain MRI
	Family C/1	Q647Rfs*6/ p.S1122Tfs*3 (compound het)	Caucasian	M/died at the age of 2.5 years	AR/No	NA	No parkinsonism, profound intellectual disability, tube fed, dystonia	Yes (12 d)	Unknown	Normal brain MRI at age 6

(Continued)

TABLE 1 | Continued

	Patient	*SYNJ1* mutations	Origin	Sex/AAE (y)	Transmission/ consanguinity	Age at onset of motor symptoms (y)	Phenotype	Seizures (age at onset)	Response to levodopa	Imaging data
	Family C/2	Q647Rfs*6/ p.S1122Tfs*3 (compound het)	Caucasian	M/died at the age of 8 years	AR/No	NA	No parkinsonism, profound intellectual disability, progressive spastic tetraplegia, feeding problems with gastrostomy	Yes (1 d)	Unknown	Brain MRI: thin corpus callosum and limited gliosis and atrophy of the periventricular white matter
Al Zaabi et al. (19)	Case 1	p.Q237*(hom)	Oman	F/2	Spo/Yes	NA	No parkinsonism, profound intellectual disability, no feeding difficulties, scoliosis, significant truncal and peripheral hypotonia, and persistent palmer and plantar reflexes	Yes (2 d)	Unknown	Normal brain MRI
	Case 2	p.Q237*(hom)	Oman	M/2	First cousin of case 1/Yes	NA	No parkinsonism, microcephaly at the age of 2 years, profound intellectual disability, feeding problems, dysphagia and palatal insufficiency, head lag and axial hypotonia with hyperreflexia and clonus	Yes	Unknown	Brain MRI: mild dilation of the ventricles and subarachnoid spaces
Samanta et al. (20)	Patient 1	p.Q287Pfs*27 (hom)	Saudi Arabia	F/2	Spo/Yes	NA	No parkinsonism, profound intellectual disability, profound hypotonia, feeding problems, severe cortical visual impairment, hypotonia progressing to spastic tetraplegia, brisk deep tendon reflexes, dystonia of upper extremities	Yes (2 d)	Unknown	Normal brain MRI

AAE, age at last examination; AR, autosomal recessive; CI, cognitive impairment; CT, computed tomography; d, day; F, female; het, heterozygote; hom, homozygote; M, male; MMSE, Mini-Mental State Examination; m, months; MRI, magnetic resonance imaging; NA, not available; Spo, sporadic; y, year.

[#]*unknown parental phase.*

and severe neurodegenerative decline) were identified (**Table 1**). These last ten patients presented in the neonatal period with intractable seizures, hypotonia, feeding difficulties, and severe developmental delay but no parkinsonian signs/symptoms. These patients harbored six loss-of-function mutations (p.R136*, p.W843*, Q647Rfs*6, p.S1122Tfs*3, p.Q237*, p.Q287Pfs*27, **Figure 2**) in the homozygous or compound heterozygous state, shown in some cases to reduce the levels of the mutant transcript, whereas the homozygous missense mutant p.Y888C, affecting an amino acid located in the 5′-phosphatase domain of the protein, was reported to affect both the Sac1 and 5′-phosphatase activity of synaptojanin 1 (18). However, other homozygous missense mutations, such as p.R839C and p.Y832C, also affecting amino acids located in the 5′-phosphatase domain of the protein, result in typical PD or EO atypical parkinsonism, indicating that clinical severity does not depend exclusively on the protein domain affected by the missense mutations (10, 15, this study). Conversely, it could be speculated that mutations leading to premature truncation of the protein in the homozygous or compound heterozygous state, in addition to the p.Y888C mutation, may lead to severe progressive neurodegeneration, whereas homozygous missense variants or compound heterozygous variants with a missense and a premature stop variant in the *SYNJ1* gene lead to milder phenotypes associated with parkinsonism and a higher susceptibility to seizures.

In conclusion, this study has extended the mutational and clinical spectrum of *SYNJ1* associated with EO typical or atypical parkinsonism and suggests that the screening of this gene should be considered in isolated cases and in patients with a later AO.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: www.ncbi.nlm.nih.gov/clinvar/ SCV001469064, SCV001469065.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by INSERM, CCPPRB du Groupe Hospitalier Pitié-Salpêtrière, Paris, France and by the appropriate institutional review boards. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SL conceived, designed and organized the study, wrote the first draft, reviewed, and critically revised the manuscript. J-CC and AB conceived the project, reviewed, and critically revised the manuscript. GM, CT, HB, MB, SK, MA, and AS contributed to the execution of the research project and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Halliday G, Lees A, Stern M. Milestones in Parkinson's disease–clinical and pathologic features. *Mov Disord.* (2011) 26:1015–21. doi: 10.1002/mds.23669
2. Lunati A, Lesage S, Brice A. The genetic landscape of Parkinson's disease. *Rev Neurol.* (2018) 174:628–43. doi: 10.1016/j.neurol.2018.08.004
3. Cremona O, Nimmakayalu M, Haffner C, Bray-Ward P, Ward DC, De Camilli P. Assignment of SYNJ1 to human chromosome 21q22.2 and Synj12 to the murine homologous region on chromosome 16C3-4 by *in situ* hybridization. *Cytogenet Cell Genet.* (2000) 88:89–90. doi: 10.1159/000015493
4. McPherson PS, Czernik AJ, Chilcote TJ, Onofri F, Benfenati F, Greengard P, et al. Interaction of Grb2 via its Src homology 3 domains with synaptic proteins including synapsin I. *Proc Natl Acad Sci USA.* (1994) 91:6486–90. doi: 10.1073/pnas.91.14.6486
5. McPherson PS, Takei K, Schmid SL, De Camilli P. p145, a major Grb2-binding protein in brain, is co-localized with dynamin in nerve terminals where it undergoes activity-dependent dephosphorylation. *J Biol Chem.* (1994) 269:30132–9. doi: 10.1016/S0021-9258(18)43787-8
6. Krebs CE, Karkheiran S, Powell JC, Cao M, Makarov V, Darvish H, et al. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. *Hum Mutat.* (2013) 34:1200–7. doi: 10.1002/humu.22372
7. Quadri M, Fang M, Picillo M, Olgiati S, Breedveld GJ, Graafland J, et al. Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset Parkinsonism. *Hum Mutat.* (2013) 34:1208–15. doi: 10.1002/humu.22373
8. Olgiati S, De Rosa A, Quadri M, Criscuolo C, Breedveld GJ, Picillo M, et al. PARK20 caused by SYNJ1 homozygous Arg258Gln mutation in a new Italian family. *Neurogenetics.* (2014) 15:183–8. doi: 10.1007/s10048-014-0406-0
9. Drouet V, Lesage S. Synaptojanin 1 mutation in Parkinson's disease brings further insight into the neuropathological mechanisms. *Biomed Res Int.* (2014) 2014:289728. doi: 10.1155/2014/289728
10. Xie F, Chen S, Cen ZD, Chen Y, Yang DH, Wang HT, et al. A novel homozygous SYNJ1 mutation in two siblings with typical Parkinson's disease. *Parkinsonism Relat Disord.* (2019) 69:134–7. doi: 10.1016/j.parkreldis.2019.11.001

11. Kumar S, Yadav N, Pandey S, Muthane UB, Govindappa ST, Abbas MM, et al. Novel and reported variants in Parkinson's disease genes confer high disease burden among Indians. *Parkinsonism Relat Disord.* (2020) 78:46–52. doi: 10.1016/j.parkreldis.2020.07.014
12. Kirola L, Behari M, Shishir C, Thelma BK. Identification of a novel homozygous mutation Arg459Pro in SYNJ1 gene of an Indian family with autosomal recessive juvenile Parkinsonism. *Parkinsonism Relat Disord.* (2016) 31:124–8. doi: 10.1016/j.parkreldis.2016.07.014
13. Rauschendorf MA, Jost M, Stock F, Zimmer A, Rösler B, Rijntjes M, et al. Novel compound heterozygous synaptojanin-1 mutation causes l-dopa-responsive dystonia-parkinsonism syndrome. *Mov Disord.* (2017) 32:478–80. doi: 10.1002/mds.26876
14. Ben Romdhan S, Sakka S, Farhat N, Triki S, Dammak M, Mhiri C. A Novel SYNJ1 mutation in a Tunisian Family with juvenile Parkinson's disease associated with epilepsy. *J Mol Neurosci.* (2018) 66:273–8. doi: 10.1007/s12031-018-1167-2
15. Taghavi S, Chaouni R, Tafakhori A, Azcona LJ, Firouzabadi SG, Omrani MD, et al. A clinical and molecular genetic study of 50 families with autosomal recessive parkinsonism revealed known and novel gene mutations. *Mol Neurobiol.* (2018) 55:3477–89. doi: 10.1007/s12035-017-0535-1
16. Hong D, Cong L, Zhong S, He Y, Xin L, Gao X, et al. Clonazepam improves the symptoms of two siblings with novel variants in the SYNJ1 gene. *Parkinsonism Relat Disord.* (2019) 62:221–5. doi: 10.1016/j.parkreldis.2018.11.020
17. Dyment DA, Smith AC, Humphreys P, Schwartzentruber J, Beaulieu CL, FORGE Canada Consortiumet al. Homozygous nonsense mutation in SYNJ1 associated with intractable epilepsy and tau pathology. *Neurobiol Aging.* (2015) 36:1222.e1–5. doi: 10.1016/j.neurobiolaging.2014.09.005
18. Hardies K, Cai Y, Jardel C, Jansen AC, Cao M, May P, et al. Loss of SYNJ1 dual phosphatase activity leads to early onset refractory seizures and progressive neurological decline. *Brain.* (2016) 139:2420–30. doi: 10.1093/brain/aww180
19. Al Zaabi N, Al Menhali N, Al-Jasmi F. SYNJ1 gene associated with neonatal onset of neurodegenerative disorder and intractable seizure. *Mol Genet Genomic Med.* (2018) 6:109–13. doi: 10.1002/mgg3.341
20. Samanta D, Arya K. Electroclinical findings of SYNJ1 epileptic encephalopathy. *J Pediatr Neurosci.* (2020) 15:29–33.
21. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry.* (1992) 55:181–4. doi: 10.1136/jnnp.55.3.181
22. Bouhouche A, Tesson C, Regragui W, Rahmani M, Drouet V, Tibar H, et al. Mutation analysis of consanguineous Moroccan patients with Parkinson's disease combining microarray and gene panel. *Front Neurol.* (2017) 8:567. doi: 10.3389/fneur.2017.00567
23. Lesage S, Drouet V, Majounie E, Deramecourt V, Jacoupy M, Nicolas A, et al. Loss of VPS13C function in autosomal recessive parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. *Am J Hum Genet.* (2016) 98:500–13. doi: 10.1016/j.ajhg.2016.01.014
24. Picillo M, Ranieri A, Orefice G, Bonifati V, Barone P. Clinical progression of SYNJ1-related early onset atypical parkinsonism: 3-year follow-up of the original Italian family. *J Neurol.* (2014) 261:823–4. doi: 10.1007/s00415-014-7270-6
25. Upreti C, Otero R, Partida C, Skinner F, Thakker R, Pacheco LF, et al. Altered neurotransmitter release, vesicle recycling and presynaptic structure in the pilocarpine model of temporal lobe epilepsy. *Brain.* (2012) 135:869–85. doi: 10.1093/brain/awr341
26. McPherson PS, Garcia EP, Slepnev VI, David C, Zhang X, Grabs D, et al. A presynaptic inositol-5-phosphatase. *Nature.* (1996) 379:353–7. doi: 10.1038/379353a0

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REVIEW
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ATP1A3-Related Disorders: An Ever-Expanding Clinical Spectrum

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The Na+/K+ ATPases are Sodium-Potassium exchanging pumps, with a heteromeric α-β-γ protein complex. The α3 isoform is required as a rescue pump, after repeated action potentials, with a distribution predominantly in neurons of the central nervous system. This isoform is encoded by the ATP1A3 gene. Pathogenic variants in this gene have been implicated in several phenotypes in the last decades. Carriers of pathogenic variants in this gene manifest neurological and non-neurological features in many combinations, usually with an acute onset and paroxysmal episodes triggered by fever or other factors. The first three syndromes described were: (1) rapid-onset dystonia parkinsonism; (2) alternating hemiplegia of childhood; and, (3) cerebellar ataxia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS syndrome). Since their original description, an expanding number of cases presenting with atypical and overlapping features have been reported. Because of this, ATP1A3-disorders are now beginning to be viewed as a phenotypic continuum representing discrete expressions along a broadly heterogeneous clinical spectrum.

Keywords: ATP1A3, sodium-potassium-exchanging ATPase, rapid-onset dystonia parkinsonism, Dyt12, alternating hemiplegia, CAPOS syndrome, ataxia

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INTRODUCTION

Considered rare, *ATP1A3*-related disorders have been capturing our attention in the last decade by virtue of cumulative cases reporting an expanding range of clinical and genetic variability. In the same manner, next-generation sequencing technologies have arisen fulfilling a major role in the understanding of the genotype-phenotype association of these newfangled syndromes. These have been discussed by the authors of this article in a recent editorial (1).

The Na^+/K^+ ATPase is a transmembrane ion-pump located at the cellular plasma membrane. This pump extrudes three Na^+ and import two K^+ into the cell for every adenosine triphosphate (ATP) split. Its main role is to regulate electrochemical gradients, and it is involved in the action potential propagation during neuronal depolarization.

The Na^+/K^+ ATPase is a heterotrimeric α-β-γ protein complex. Humans express four α isoforms (α1–4), encoded by the ATP1A 1-4 genes, respectively (2). The α3 isoform, encoded by *ATP1A3* located on chromosome 19q, is expressed almost exclusively in neurons (3). This isoform is specifically required as a rescue pump, after repeated action potentials, for rapid restoration of large transient increases in intracellular Na^+ concentration (4). Conditions associated with α3 deficiency are therefore likely aggravated by supra-threshold neuronal activity. The α3 isoform has been also suggested to support re-uptake of neurotransmitters (3, 5).

In the adult mouse brain Bøttger et al. found high expression of the Na^+/K^+ -ATPase1 α3 isoform in the striatum, globus pallidus, subthalamic nucleus, substantia nigra, thalamus, cerebellum, red nucleus, oculomotor nucleus, reticulo-tegmental nucleus of pons, and hippocampus, mainly in co-location with GABAergic neurons (6). In the retina, photoreceptor and all neuronal-type cells express Na^+/K^+ -ATPase1 α3 isoform (4). Within the cochlea, it is found in membranes of the spiral ganglion somata and organ of Corti, affecting the innervation pathways of inner hair cell synapses (7).

The main exception for nervous system-specific expression is the cardiac muscle (4).

Familial or most commonly *de novo* heterozygous pathogenic variants of *ATP1A3* are responsible of Na^+/K^+ ATPase dysfunction, due to α3 isoform defects. Not surprisingly carriers manifest a range of distinctive neurological syndromes, with some cases presenting atypical manifestations and others overlapping phenotypes.

The causative role of *ATP1A3* variants in the pathogenesis of several neurological disorders with a similar pattern of inheritance has been previously documented in several reviews (8–11). Here we have performed an up-to-date review of this topic, including several novel recently reported phenotypes in *ATP1A3* pathogenic variant carriers.

REVIEW

Classical ATP1A3-Related Syndromes

In their original descriptions, the three classic phenotypes related to *ATP1A3* pathogenic variants—rapid-onset dystonia parkinsonism (RDP), alternating hemiplegia of childhood (AHC), and cerebellar ataxia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS)—diverge in several clinical features with different pathogenic variants associated with each syndrome. However, in more recent years these pragmatic limits are less clear. In this section, we describe these syndromes in the chronological order of their association with *ATP1A3* pathogenic variants.

Rapid-Onset Dystonia-Parkinsonism

In 1993 Dobyns et al. reported a previously undescribed "rapid-onset dystonia-parkinsonism" (RDP) syndrome with an autosomal dominant inheritance pattern in a large family (12). The association of this syndrome with *ATP1A3* pathogenic variants was made by De Carvalho and colleagues in 2004. This was the first disorder that was found to be caused by variants in *ATP1A3* (13). Pathogenic variants presenting as RDP are evenly distributed throughout the *ATP1A3* gene (8). Nowadays p.Thr613Met is known to be the most common pathogenic variant in RDP (14).

RDP is an autosomal dominant disorder with variable penetrance, although some cases may appear sporadic due to *de novo* pathogenic variants, with an onset most commonly in the teens to twenties (15). Approximately half of the pathogenic variants occurred *de novo* (16).

Typically RDP debuts with an abrupt onset, and a limited progression over weeks. Usual manifestations include: bulbar symptoms (generally dysarthria and hypophonia with mild to moderate dysphagia), cranio-cervical dystonia, mild limb dystonia, and parkinsonism (mainly bradykinesia and postural instability) with no pill-rolling tremor, diurnal fluctuation, nor response to L-dopa.

It is habitually triggered by a physical or psychological stressor such as exercising, alcohol binges, minor head injuries, overheating, emotional stress, infections, or childbirth (8).

Before the onset of RDP, several patients report vague symptoms of dystonia associated with bradykinesia, typically lasting for hours to days (15). In the majority, this was mild and confined to the distal arm or leg. Generalized or truncal dystonia was never reported as a preceding symptom. Several cases were rarely followed by abrupt exacerbations, occurring 1–9 years after the initial onset. Seizures may rarely appear several years later (12, 15).

Initially RDP was considered a well-defined stereotypical phenotype associated to certain *ATP1A3* variants, with a nearly non-overlapping set of pathogenic variants associated with AHC or RDP. However, RDP phenotypical variability has been reported among non-related carriers of the same pathogenic variant, and even among individuals of the same family. More recently intermediate RDP-AHC phenotypes with a genotype-phenotype overlapping have also been reported (15, 17).

Psychiatric symptoms are common. Bipolar disorder, dysthymia, and agoraphobia have been reported (18). Across different families with distinct *ATP1A3* pathogenic variants, Brashear et al. found higher prevalence of mood disorders and psychosis in patients with RDP who had motor symptoms, compared to controls and non-motor manifesting carriers (19). Cognitive impairment, especially verbal learning and memory, non-contextual visual memory, processing speed, attention, and executive functioning appear to be part of RDP syndrome (20).

Brashear and colleagues proposed the minimal clinical criteria for RDP (15):

1. Abrupt onset of dystonia with features of parkinsonism over a few minutes to 30 days.
2. A clear rostro caudal (face > arm > leg) gradient of involvement.
3. Prominent bulbar findings.

In addition, other features suggestive of RDP include:

4. Minimal or no tremor at onset.
5. Occasional mild limb dystonia prior to the primary onset of RDP.
6. Common reports of triggers associated with the abrupt onset of symptoms.
7. Rare "second onsets" or abrupt worsening of symptoms later in life.
8. Stabilization of symptoms within a month.
9. Minimal improvement overall but with limited improvement in gait (seen in a few patients).

Investigations of a large cohort allowed Haq et al. to show that not all of the considered classical features of RDP are really characteristic, and that even characteristic features may be absent (16). Remarkably, rapid onset and bulbar predominance was

not universally present in pathogenic variant carriers. Non-rapid onset (over more than 30 days) was the clinical onset in about 20% of cases. Arms were the first body part affected (41%), followed by legs (21%), and face (2%). At longer follow-up, arms and voice were most severely affected. A strict rostro-caudal gradient of dystonia severity was present in only 7% of carriers, while parkinsonism was strongly correlated with dystonia (16).

Atypical signs have been reported in RDP, including prominent lower limb dystonia, late age of onset (>50 years of age) (18); dystonia without signs of parkinsonism, typical writer's cramp (12); pyramidal signs (p.Glu277Lys) (21); Myoclonus, ataxia, chorea (22, 23); and hyporeflexia (23).

For a comparison of the main clinical features of RDP with other classical syndromes see **Table 1**.

Brashear and colleagues reported in 2012 a novel phenotype in two unrelated children with onsets at age 9 months and 4 years, respectively. The former carrying the *ATP1A3* p.Arg756His and the later the p.Asp923Asn variant. Case 1's initial symptoms were three episodes of intermittent flaccidity preceded by illness with and without fever. Case 2 had a baseline history of hypotonia with superimposed spells of flaccidity and bulbar symptoms before sudden onset of dystonia of the limbs. Subsequently both patients developed bulbar symptoms including severe dysarthria and dysphagia, which is more characteristic of RDP (24).

Gradual onset of dystonia and parkinsonism has been reported (25). An insidious onset of asymmetrical parkinsonism evolving over a year, remaining stable for ∼3.5 years before an acute episode of bulbar signs with oromandibular dystonia and more severe parkinsonism in a 38 year-old p.Ile274Thr carrier was reported (26).

Decreased CSF levels of homovanillic acid have been inconsistently reported. Dopamine pre-synaptic SPECT imaging has been normal in all cases, thus supporting anatomo-pathological data of intact nigro-striatal neurons in RDP (27).

Alternating Hemiplegia of Childhood

Alternating hemiplegia of childhood (AHC) was first defined by Verret and Steele as a distinct syndrome in 1971, in a report that described eight patients with episodes of intermittent hemiplegia on alternating sides of the body, developmental delay, dystonia, and choreoathetosis beginning in infancy (28).

Onset usually occurs before the age of 6 months. In a cohort of 157, hemiplegic attacks were always present usually involving ipsilateral limbs, with face generally spared; 86.5% reported episodes of bilateral weakness without pyramidal signs; 88% with dystonic attacks involving one or more limbs, occurring alone or mixed with hemiplegic episodes, rarely involving the tongue; 49% with events of autonomic dysfunction; 53% with epilepsy; 72% developed chorea and/or dystonia; and 92% had developmental delay. Abnormal ocular movements, often monocular nystagmus, and hypotonia were common and tend to regress into adulthood (29).

Common triggers include stress, excitement, extreme heat or cold, water exposure, physical exertion, lighting changes, and foods (8).

AHC diagnostic criteria were proposed by Neville in 2007. Typical cases satisfied criteria 1, 2, 3, and 7 (30):

1. Onset of symptoms before 18 months of age.
2. Repeated attacks of hemiplegia involving either side of the body, at least in some episodes.
3. Episodes of bilateral hemiplegia or quadriplegia as generalization of a hemiplegic episode or bilateral from the beginning.
4. Other paroxysmal disturbances including tonic or dystonic crises, oculomotor abnormalities (e.g., strabismus or nystagmus), and autonomic phenomena occurring during hemiplegic episodes or in isolation.
5. Immediate disappearance of symptoms upon sleeping, which later may resume, usually 10–20 min after waking.
6. Evidence of developmental delay and neurological abnormalities including choreoathetosis, dystonia, or ataxia.
7. Not attributable to another disorder.

The incidence of AHC is about one in one million individuals (30).

In 2012 Heinzen and colleagues, and Rosewich and colleagues reported heterozygous variants in *ATP1A3* associated with AHC (31, 32).

De novo ATP1A3 pathogenic variants explain the majority of patients with AHC (32).

Variants presenting with AHC are clustered within certain regions of that gene. Typically, AHC occurs in carriers of variants in an amino acid position before 400 or above 800 (8).

The most frequent *ATP1A3*pathogenic variants causing AHC are p.Asp801Asn, p.Glu815Lys, and p.Gly947Args. The former variant accounting for up to 43% of all *ATP1A3*-related AHC cases (8).

In particular, p.Glu815Lys (16–35% of cases) is associated with a severe intellectual and motor disability, high prevalence of epilepsy with early onset of seizures and poor prognosis, whereas p.Asp801Asn (30–43% of cases) results in a moderate/mild form of the disease, and p.Gly947Arg (8–15% of cases) has a favorable prognosis (33).

Unlike *ATP1A3* variants that cause RDP, AHC-causing variants in this gene cause consistent reductions in ATPase activity without affecting the level of protein expression (31).

Cerebellar vermian atrophy has been observed (34). A brain magnetic resonance spectroscopy showed an increase time in choline and in lipids at the pons region, with normal NAA levels at the age of 40 and 44 years, similar to those findings observed in chronic inflammatory or hypo-myelinating CNS disorders (34).

Several atypical AHC cases have been reported, including benign familial nocturnal AHC, mild AHC, dystonia-predominant AHC, familial autosomal dominant pedigree, late-onset AHC, and AHC without quadriparesis (35).

A patient with p.Ser137Phe and AHC developed in her twenties episodes of loss of consciousness related to recurrent periods of asystole up to 5 s long, which required a peacemaker (36).

Gurrieri et al. found that 22 of 26 AHC patients with confirmed *ATP1A3* pathogenic variants shared a similar physical

TABLE 1 | Clinical characteristics of ATP1A3 classical syndromes.

	AHC	CAPOS	RDP
Age of onset	Before 18 months of age	Infancy-childhood (Frequently between 1 and 5 years)	After 18 months (often second to third decade)
Triggers	Fever, stress, excitement, extreme heat or cold, water exposure, physical exertion, lighting changes, and foods	Fever	Fever, running, alcohol binges, minor head injuries, overheating, emotional stress, infections, sleep deprivation, or childbirth
Onset	Acute onset	Acute-subacute ataxia	Abrupt onset over a few minutes to 30 days.
Typical manifestation	- Hemiplegia or quadriplegia - Tonic or dystonic crises - Oculomotor abnormalities - Cognitive impairment - autonomic phenomena	- Early onset cerebellar ataxia with a relapsing course - Areflexia - Pes-cavus - Optic atrophy - Sensorineural hearing loss.	- Bulbar and limb dystonia - Parkinsonism. - Rostro caudal gradient
Precede symptoms	Paroxysmal ocular manifestations, seizures, developmental delay	Fever-induced transient encephalopathy	Vague symptoms of dystonia in distal limbs
Course	Polyphasic (Relapsing-remitting)	Relapsing course of ataxia-encephalopathy (one to three episodes) with slow progression of other features	Rarely "secondary" exacerbations (2–3 episodes) occurring 1–9 years after the initial onset
Atypical manifestation	Benign familial nocturnal AHC; Mild AHC; Dystonia-predominant AHC, Familial dominant pedigree; Late-onset AHC; AHC without quadriparesis	Urinary urgency; Cardiac arrhythmia, left ventricular enlargement; Scoliosis; Cognitive dysfunction; Autistic traits; Bradykinesia; Myoclonus, Chorea, Tremor, Oral dyskinesias; Dystonia	Prominent lower limb dystonia; Late onset (>50 years of age); Gradual onset; Pure dystonia; Writer's cramp; Pyramidal signs; Myoclonus; Ataxia, Chorea; Hyporeflexia.

AHC, alternating hemiplegia of childhood; CAPOS, cerebellar ataxia, areflexia, pes-cavus, optic atrophy, sensorineural hearing loss; RDP, rapid onset dystonia parkinsonism.

phenotype consisting of generalized hypotonia, long face, thin and well-defined eyebrows, strabismus, widely spaced eyes, long palpebral fissures, downturned mouth, and slender habitus. Authors considered this phenotype sufficiently typical to delineate a recognizable phenotype (37).

CAPOS Syndrome

In 1996 Nicolaides, Appleton and Fryer described three members of a family affected by a likely dominantly inherited syndrome characterized by early onset cerebellar ataxia with a relapsing course, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss. They encompassed this association under the acronym of "CAPOS" (38). It was not until 2014 that Demos and colleagues found a heterozygous missense *ATP1A3* variant, p.Glu818Lys, in individuals from two independent families manifesting CAPOS syndrome (39).

Heimer and colleagues described in 2015 a phenotype highly resembling the patients described by Demos et al. except for the lack of pes cavus. They identified the same heterozygous p.Glu818Lys variant in the *ATP1A3* gene. They named this syndrome CAOS (Episodic Cerebellar Ataxia, Areflexia, Optic Atrophy, and Sensorineural Hearing Loss) (40) and remarked that pes cavus was found only in 3 of 10 patients described by Demos et al. Since the prevalence of pes cavus in the general population is ∼10% and because pes cavus was also absent in 7 patients in their cohort, it might be an incidental finding rather than a key feature of the disorder (40).

So far, more than 50 CAPOS or CAOS patients with *ATP1A3* p.Glu818Lys have been reported (41).

Roenn and colleagues characterized the functional defects of the CAPOS *ATP1A3* p.Glu818Lys using a combination of biochemical and electrophysiological measurements, which allowed demonstration of a reduced Na^+ affinity of the transport sites of the CAPOS mutant in internally as well as externally facing conformations. Consequently, the CAPOS mutant pump may fail to clear the neuron fast enough of the accumulated Na^+ in relation to action potentials, and this defect might be part of the pathophysiological mechanism (41).

CAPOS/CAOS frequently starts between 1 and 5 years of age, with a fever-induced, acute-onset cerebellar ataxia, accompanied by encephalopathic features, disturbed eye movements, hypotonia, areflexia, and mild weakness. Other less common episodic symptoms include paresis (hemi/para/tetra paresis), transient hearing and visual loss. Most patients have a complete recovery, although persistent ataxia is not rare. A relapsing course is characteristic (10, 42). Patients classically manifest two to three episodes before transitioning to a slowly progressive evolution (42).

Sensorineural hearing loss with a sudden-onset and progressive nature is a distinctive disabling feature of CAPOS syndrome (43). Han and colleagues reported 3 sporadic cases of auditory neuropathy spectrum disorder (ANSD) with an onset after language acquisition. Interestingly two were *ATP1A3* p.Glu818Lys carriers. The first proband did not manifest any features of CAPOS, while the second proband was compatible with a CAPOS syndrome (44).

Progressive loss of vision, with poor color discrimination and diminished brightness sensitivity and bilateral optic disc atrophy

indicative of optic neuropathy are expected findings (39), and very rarely may be absent (42).

Nystagmus and strabismus are also frequent characteristics in long term follow-up (42).

Areflexia is always present, with normal nerve conduction velocities (NCVs) reported (38, 45). Nerve biopsy may reveal findings consistent with axonal neuropathy (39).

Less common manifestations in CAPOS/CAOS syndrome include urinary urgency, cardiac arrhythmia, left ventricular enlargement, scoliosis, cognitive dysfunction, autistic traits (e.g., repetitive behaviors and social difficulties), bradykinesia, myoclonus, chorea, tremor, oral dyskinesias, and dystonia (39, 42, 46).

Dystonia has been reported as (1) cervical dystonia with dystonic tremor responsive to onabotulinumtoxinA (39); (2) transient upper limb dystonia with acute onset at 20 months of age, evolving years later with persistent limb dystonia (45); (3) multi-focal upper limb dystonia (42); and (4) slight focal hand dystonia-myoclonus (42).

The coexistence of CAPOS syndrome and hemiplegic migraine with *ATP1A3* p.Glu818Lys was made by Potic and colleagues (47).

Stagnaro and colleagues reported two cases, with the same *de novo* p.Arg756Cys pathogenic variant, of paroxysmal "CAPOS-like" symptoms, based on areflexia and ataxia, accompanied by dystonia and hypotonia, related to febrile episodes. However, they did not report optic atrophy or sensorio-neural hearing loss (48).

A comparison of the clinical features of the 3 classic *ATP1A3*-related syndromes is presented in **Table 1**.

Non-classical Phenotypes

Recently, an increase in the number of "non-classical" phenotypes have been reported.

Relapsing Encephalopathy With Cerebellar Ataxia

Dard and colleagues in 2015 reported a novel phenotype in a 34-year-old woman, caused by heterozygous *ATP1A3* p.Arg756Cys variant, consisting of a relapsing encephalopathy during febrile illnesses, accompanied by a prominent cerebellar syndrome, generalized dystonia, pyramidal signs, and anger outbursts. The acronym RECA (relapsing encephalopathy with cerebellar ataxia) was proposed (14).

Hully and colleagues in 2017 reported a family with two affected siblings in whom mosaic heterozygous *ATP1A3* p.Arg756Cys variant was identified.

The first infant started at the age of 9 months, after a febrile episode, with severe psychomotor regression with subsequent developmental delay. Later she developed abnormal ocular movements with severe cerebellar ataxia, choreic, and dystonic movements. Her sister, had a normal early development until the age of 22 months when she regressed during a febrile episode with acute ataxia, pyramidal signs, and hypotonia. Subsequently, she developed severe encephalopathy with cerebellar ataxia combined with nystagmus, and dystonic movements with bucco-facial involvement. She experienced two additional relapses during febrile infections at the ages of 4 and 6. Her MRI showed mild cerebellar atrophy (49).

Later in 2019 Sabouraud and colleagues described eight new RECA pediatric cases, associated with *ATP1A3* p.Arg756Cys (50).

Fever-Induced Paroxysmal Weakness and Encephalopathy

Yano and colleagues in 2017 grouped patients with *ATP1A3* p.Arg756His or *ATP1A3* p.Arg756Leu pathogenic variants, with clinical onset before the age of 3 years. Fever-Induced Paroxysmal Weakness and Encephalopathy (FIPWE) was the main phenomenon, accompanied by different combinations of oculomotor abnormalities, dysphagia, generalized hypotonia, dystonia, ataxia, or apnea (51).

Early Life Epilepsy

Paciorkowski et al. in 2015 reported two cases presenting with epilepsy. One was a child with catastrophic early life epilepsy (EE), who seized 4 h after birth. Her epilepsy continued to be intractable with recurrent episodes of status epilepticus. MRI showed progressive brain atrophy and postnatal microcephaly. She died at 16 months. The second patient had epilepsy after 6 weeks of age. His seizures were characterized by episodic, prolonged apnea, and gaze deviation. He developed postnatal microcephaly and severe developmental disability. *ATP1A3* p.Gly358Val and p.Ile363Asn were identified in these children (52).

Marzin et al. reported three children with *de novo* p.Asp742Tyr, p.Cys346Arg, and p.Asp609Tyr variants in *ATP1A3*, respectively, manifesting features close to those cases reported by Paciorkowski et al. with early-onset encephalopathy, seizures and non-epileptic attacks of movement disorders, mainly observed during infancy. No obvious plegic attacks neither mycrocephaly, were observed in these cases (53).

Holze and colleagues reported two girls presenting with unexplained severe apneic episodes around the first year of life. One patient was *ATP1A3* p.Gly89fs carrier and the other had the p.Gly706Arg variant. The authors hypothesized that the apneic episodes were symptoms of *ATP1A3*-related early onset epilepsy (54).

Hully and colleagues in 2017 reported two affected siblings in whom mosaic heterozygous *ATP1A3* p.Gly706Arg variant was identified. The siblings had neurodevelopmental delay, and seizures of varying semiology starting at 4.5–2 months of age, evolving to severe encephalopathy with autistic features, epilepsy, strabismus, nystagmus, pyramidal signs, dystonic, and ataxic gait. Brain MRI performed showed bilateral hippocampus sclerosis and cerebellar atrophy (49).

An infant carrier of trinucleotide deletion *ATP1A3* p.Asp756del manifesting at the age of 3 months with drug-resistant epileptic encephalopathy responsive to ketogenic diet, plus non-epileptic paroxysmal episodes (hypotonia, hemiplegia, apnea, monocular nystagmus) and developmental delay was reported (55).

Tran and colleagues reported a p.Val589Phe carrier whose initial presentation was an epileptic encephalopathy starting at

the age of 4 months, with subsequent AHC and then RDP symptomatology (56).

Rapid Onset Cerebellar Ataxia

Adult rapid onset cerebellar ataxia (ROA) phenotype was reported by Kathleen and colleagues in 2016. A male with normal development except for mild amblyopia, learning disability, and dyslexia, presented at age 19 with episodes of vertigo lasting for days. At age 21, he developed ataxia, progressing over 6 months requiring the use of a wheelchair. Progressive cerebellar degeneration was evident on MRI. Follow-up evaluation at age 26 revealed partial overlap with RDP syndrome. A novel *ATP1A3* p.Gly316Ser variant was identified (57).

Gusmao and colleagues reported a 28-year-old *ATP1A3* p.Gly316Ser carrier with a history of mild learning disability and migraines who developed falls at 21 years. There were no clear triggers. This progressed to prominent gait and appendicular ataxia with dysarthria, action tremor, and myoclonus. Oculomotor abnormalities included intrusions and dysmetric saccades. Within a few years, he became wheelchair-dependent. Neuroimaging demonstrated vermian cerebellar atrophy (22).

Schirinzi et al. in 2018 reported three cases of childhood ROA triggered by fever, starting before the age of 20 months. One case had dystonia, while another had self-limited episodes of hypotonus, convergent strabismus, and febrile convulsions. The authors described two different pathogenic variants in *ATP1A3* p.Arg756Cys and p.Glu818Lys, the later commonly associated to CAPOS/CAOS syndrome. These findings reinforced that ataxia may represent a peculiar, sometimes prominent or isolated, feature of *ATP1A3*-related phenotype (58).

Slowly Progressive Cerebellar Ataxia

Recently, Sasaki et al. reported two cases presenting with gradually progressive cerebellar ataxia, and mild intellectual disabilities. One of them, beginning with ataxia at the age of 1 year. At the age of 15, his neurological examination revealed intellectual disability, ataxia, and ocular motor apraxia. Brain MRI revealed cerebellar cortical atrophy mainly in the vermis. The other reported case presented early with developmental delay, manifesting later progressive gait unsteadiness and cerebellar atrophy on MRI, since the age of 7 years. None of these patients presented with paroxysmal or episodic symptoms. One patient had the *ATP1A3* p.Met154Val variant, while the other carried the p.Asp350Lys variant (59).

Childhood-Onset Schizophrenia/Autistic Spectrum Disorder

Smedemark-Margulies et al. in 2016, reported a case of Childhood-Onset Schizophrenia (COS) with a novel heterozygous pathogenic variant (p.Val129Met) in the *ATP1A3* gene (60). Subsequently in 2017 Chaumette and colleagues identified three *de novo* pathogenic variants in the *ATP1A3* gene (p. Asp801Asn; p.Glu815Lys; p. Ala813Val) in three unrelated individuals with COS, two of them also had AHC. One also had characteristics of autistic spectrum disorder (ASD) (61), which is frequently seen in COS (62).

Paroxysmal Dyskinesias

Lastly in 2019 Zúñiga-Ramírez et al. reported a pair of monozygotic twins with interictal mild generalized dystonia and paroxysmal attacks since infancy, resembling paroxysmal non-kinesigenic dyskinesias (PNKD). This attacks were triggered by weather changes, mood swings, caffeine intake, exercise, fever, and infections. Patients also presented speech arrest, and intellectual disability. After excluding known genetic causes of PNKD, whole exome sequencing showed a novel heterozygous *ATP1A3* p.Leu815Arg (63).

Interestingly Roubergue et al. in 2012 reported a family with 3 adult patients with paroxysmal exercise-induced dystonia (PED) but without plegic attacks presenting after childhood. Gene sequencing revealed the heterozygous *ATP1A3* p.Asp923Asn (64).

An *ATP1A3* p.Glu277Lys female carrier was diagnosed with mild intellectual disability, manifesting RDP symptoms when she was 9-year-old. At age of 10 she developed left lower limb paroxysmal dystonia induced by continuous exercise and mentally stressful situations. The frequency of attacks was once every 2 months, lasting 30 min (65).

Cerebral Palsy/Spastic Paraparesis

Calame et al. described four non-relatives *ATP1A3* p.Pro775Leu carriers, presenting spastic diplegia, developmental delay, epilepsy, and episodic neurological deterioration. One patient developed also static encephalopathy, microcephaly and dystonia, and one case also had sickle cell disease (66).

Dystonia, Dysmorphism, Encephalopathy, MRI Abnormalities, and no Hemiplegia (D-Demø)

Prange et al. reported a distinct phenotype in 4 carriers of *de novo ATP1A3* variants, manifesting with dystonia, dysmorphism of the face, encephalopathy with developmental delay, brain MRI abnormalities always including cerebellar hypoplasia, no hemiplegia (Ø) (D-DEMØ). In these cases, dystonia was triggered by hyperexcitation and/or physiological or psychological stressors, three of these 4 patients presented with seizures. Two presented episodes of quadriplegia, symptoms of dysautonomia, and kyphoscoliosis/scoliosis.

Dysmorphic features included a high forehead with bitemporal narrowing, broad nasal bridge and tip, narrow palpebral fissures, anteriorly facing nostrils, thickened or hypoplastic alae nasi, long philtrum, micrognathia, thin upper lip, prominent lower lip, and incompletely formed antitragus and lower part of the antihelix in the ear pinnae.

In these cases whole-exome sequencing revealed different *ATP1A3 de novo* heterozygous variants: (1) p.Thr360Arg; (2) p.Gln140His; (3) p.Gly325Asp, and 3) p.Glu324Gly (67).

Congenital Hydrocephalus

In 2019 Allocco et al. reported one patient with congenital hydrocephalus with aqueductal stenosis, craniosynostosis, open lip schizencephaly, type 1 Chiari malformation, dysgenesis of the corpus collosum, and learning disability. Routine genetic testing (FISH, microarray) was negative. Performing exome sequencing analysis, compound heterozygous *ATP1A3* variants

(p.Arg19Cys and p.Arg463Cys) were noted in the exon 2 and 11, respectively, each of which was inherited from one of the patient's unaffected parent (68). Distinctively, these variants are different from the majority of the variants identified in AHC and RDP clustered in exons 8, 14, 17, and 18 (32). Both variants were predicted to be deleterious with a disruptive effect on protein stability. Authors hypothesized that this pathogenic variants can impair CSF homeostasis and thus drive the development of hydrocephalus (68). Two observations supported this hypothesis: (1) Na+/K+-ATPase is known to regulate CSF secretion in the choroid by maintaining an osmotic gradient of Na+; (2) Immunohistochemical studies demonstrate robust ATP1A3 expression in neural stem cells, suggesting a role in regulating neural development (68). Moreover, knockdown of ATP1A3 causes ventriculomegaly in zebrafish (69).

Overlapping Phenotypes

The three neurological phenotypes RDP, AHC, and CAPOS can present with an acute onset of neurological symptoms triggered by various stimuli. However, their predominant neurological manifestations vary greatly, with early onset hemiplegic/dystonic episodes and developmental delay in AHC, ataxic encephalopathy and impairment of vision and hearing in CAPOS syndrome, and late onset of dystonia/parkinsonism in RDP (3). In addition, intermediate forms and overlapping phenotypes associated with *ATP1A3* are nowadays well-recognized. In **Figure 1**, we depicted the overlap of manifestations reported for the three classic *ATP1A3*-related syndromes.

Phenotypic overlap of AHC-CAPOS syndrome has been reported in *ATP1A3* p.Glu818Lys carriers. A previously healthy child presented at 20 months with transient afebrile episodes accompanied by abnormal ocular movement, anarthria, generalized hypotonia, paresis-dystonia predominantly of the right arm, and ataxia, with a slow improvement between episodes. At age 6 years after a fever she developed marked and persisting visual impairment. At the age of 12 year old her examination revealed marked dysarthria, bradykinesia, ataxia of gait, muscular hypotonia, mild limbs dystonia, areflexia, optic atrophy, and cochlear hearing impairment (45).

On the other hand, intermediate RDP-AHC presentations have been reported in *ATP1A3* p. p.Asp801Asn, p.Gly867Asp, p.Asp923Asn, p.Glu951Lys, p.Asp583Tyr, and p.Arg756Cys carriers (17, 70–72).

Intermediate CAPOS-RDP cases have been recently described. Chouksey and Pandey reported a case of a 12 year-old girl, who carried a heterozygous *ATP1A3* p.Glu831Lys variant. The symptoms began acutely, with transient lethargy, poor responsiveness, speech problems, and limb posturing after a febrile illness at the age of 18 months. She had a slow recovery after episodes with two relapsing separated by several years. When she was 18 year-old a CAPOS syndrome plus orolingual, cervical, and limb dystonia was established by the authors (73).

Li and colleagues reported a 31 year old man who carried a p.Pro788Leu *ATP1A3* variant. He presented with febrile convulsions yearly since he was 2 year old until the age of 5 years, concomitant with slowly progressive dystonia of the lower limbs. At the age of 26, he fulfilled the criteria for CAPOS syndrome, with atypical features such as a Babinski sign (74).

DISCUSSION

AHC, RDP, and CAPOS syndrome are considered the prototypical *ATP1A3*-related disorders. Each of these syndromes have particular diagnostic criteria and core features. The genotype-phenotype correlation is variable, with some variants presenting different phenotypes, and particular variants highly correlated with specific syndromes.

Several cases reported in the literature were characterized by overlapping phenotypes with features from the different "classical" phenotypes.

Since the discovery of RDP, AHC, CAPOS syndromes as *ATP1A3* allelic disorders, and with the availability of next-generation sequencing technologies for the genetic diagnosis, an increased number of cases with atypical features or different "non-classical" syndromes have been reported. These novel presentations still present significant clinical and genetic overlapping with the previous reported classical syndromes (for example, p.Glu818Lys in ROA and CAPOS syndrome; p.Glu815Lys in COS/ASD and AHC; or p.Asp801Asn in COS/ASD and AHC/RDP). Some cases still do not fit the reported phenotypes. On the other hand, particular phenotypes have been called with different names by different authors. In the literature RECA and FIPWE have been described as different syndromes, however since these conditions are associated to changes in the same residue (p.Arg756) with largely overlapping phenotypes and just small differences (i.e., apnea and bulbar compromise have been described more frequently in FIPWE than RECA), it appears reasonable to consider these two conditions as part of the same phenotype related to changes in p.Arg756, as other authors have suggested before (50).

It is important to emphasize that some of the novel described phenotypes are based on individual cases so far (i.e., congenital hydrocephalus related to bi-allelic variants of *ATP1A3*). For several of the reported phenotypes additional evidence is required to correlate clinical features with a specific variant. On the other hand, the reporting of atypical features (i.e., childhood onset schizophrenia) may depend on the experience of the clinician who report the case and their familiarity with those features. Moreover, as many of the phenotypes reported are based on retrospective analyses, there might be some inaccuracies regarding precise characterization. The follow-up time of cases reported is also essential, as the expression of certain clinical features is usually age-related. All these elements must be taken into consideration when discussing the genotype-phenotype correlation in *ATP1A3* variants.

Classical *ATP1A3*-related syndromes (e.g., AHC, RDP, CAPOS) manifest significant differences in their prototypical clinical pattern and type of progression.

For example, AHC typically evolve with paroxysmal attacks of weakness. On the other end, RDP evolve usually with no paroxysms and a stationary evolution. Remarkably, among the

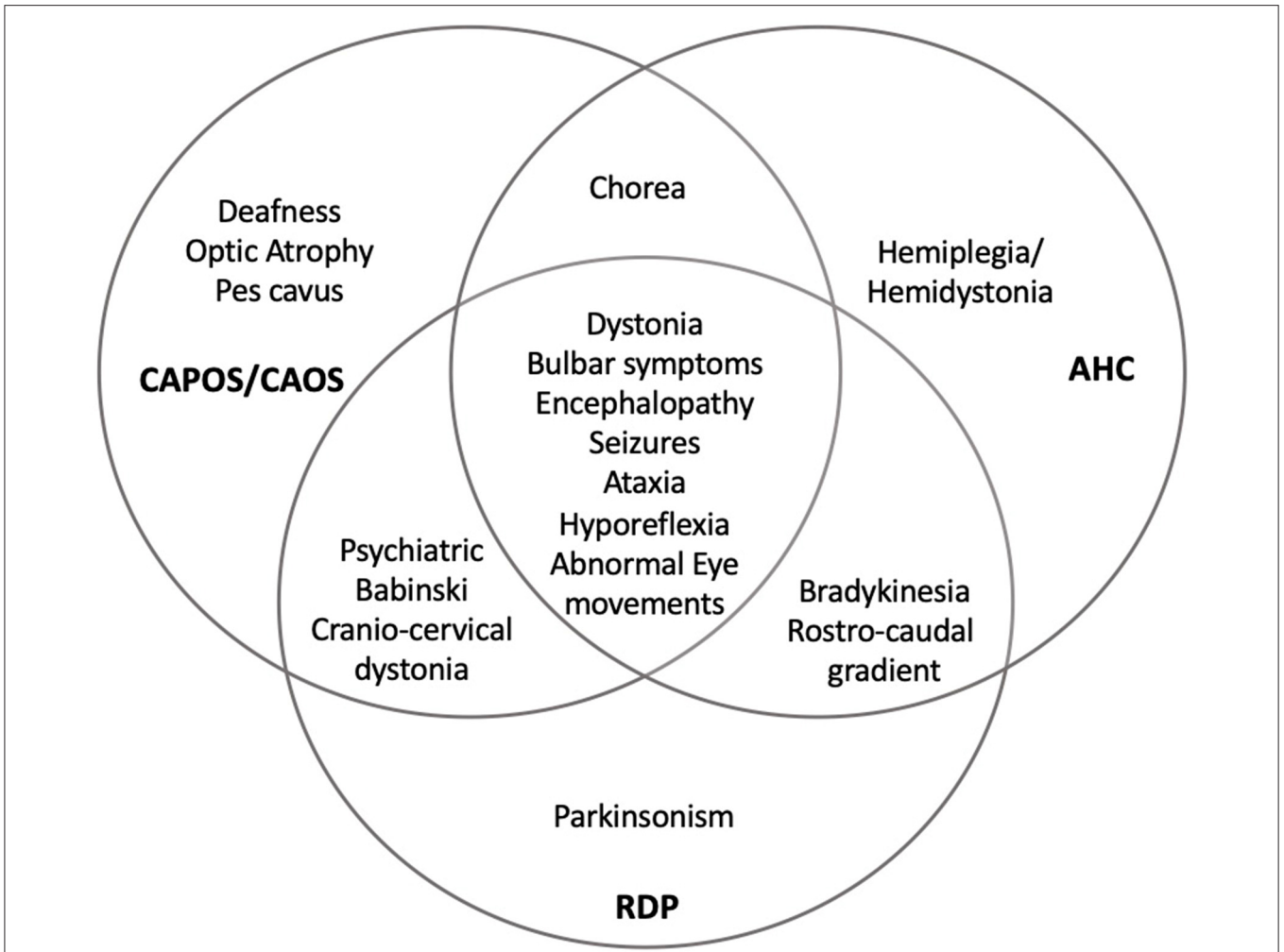

FIGURE 1 | Overlapping of ATP1A3-related disorders. RDP, rapid-onset dystonia-parkinsonism; AHC, alternating hemiplegia of childhood; CAPOS, cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss; CAOS, cerebellar ataxia, areflexia, optic atrophy, and sensorineural hearing loss. With permission from Salles and Fernandez, reference (1).

overlapping syndromes or novel phenotypes, some of them manifest no evident paroxysmal episodes, i.e., CP (p.Pro775Leu), ROA (p.Gly316Ser), COS/ASD (p.Val129Met, p.Asp801Asn, p.Glu815Lys, p.Glu815Lys), SPCA (p.Met154Val, p.Asp350Lys).

ATP1A3-related syndromes can be differentiated by severity. Those considered most severe phenotypes are defined by onset in infancy and include AHC and EE. Milder phenotypes have onset in children and adults and include CAPOS, RECA/FIPWE, ROA, and RDP (75, 76).

Genetic heterogeneity and the wide range of phenotypes and disease severity seen with *ATP1A3* variants is not yet understood. To date, almost all disease causing *ATP1A3* pathogenic variants are heterozygous, and when tested usually had loss of function or altered kinetic properties. One exception is the case included in this review, reported by Alloco et al. of a patient compound heterozygous carrying p.Arg19Cys and p.Arg463Cys. This patient presented with severe malformation of the central nervous system (68).

Clinical differences cannot been explained simply by anatomical distribution of ATP1A3, since in animal models this protein is expressed widely in neurons of the CNS and other tissues. However, neuropathologic studies suggest a possible contribution of regional neuronal degeneration and interneuron dysfunction in the mechanism of disease (52). Moreover, MRI studies in some patients with more severe phenotypes showed structural abnormalities (mainly cerebellar atrophy), which might suggest different susceptibility of certain central nervous system areas to functional or structural defects in Na^+/K^+ ATPase related with different variants.

Most common missense pathogenic variants are located mainly at highly conserved amino acid residues and seemed to interfere with ATPase activity (77). Pathogenic variants might have detrimental effect on pump activity, ion affinity, ion leakage, or biosynthesis (75). Besides, genetically defined loss-of-function pathogenic variants (frameshifts, premature stops, and deletions) are almost absent from gnomAD for *ATP1A3*

(75). On the other hand, a temperature-sensitive gain-of-function mechanism has been postulated to underlie the phenotypic consequences of disease-causing pathogenic variants in *ATP1A3* and its association with environmental triggers. However, the impact of temperature on the functional effects of AHC-and RDP-associated pathogenic variants in ATPase is unknown (8). Sweadner et al. showed that milder phenotypes in *ATP1A3* had a spread distribution, with almost no variants in the ion binding site. In contrast, the variants with severe phenotypes in *ATP1A3* were clustered around the ion binding sites. For the latter, they postulated a gain-of-function with a potential toxic effect by forming larger leaks or outward proton currents because of defective gating (75). In CAPOS the *ATP1A3* p.Glu818Lys variant affects sodium binding to, and release from, a sodium-specific cytoplasmic-facing sites of the Na^+/K^+ ATPase. This variant affect the structure of the C-terminal region. It is presumed that these changes affect propagation of membrane potential along the spiral ganglion neurons (41, 78).

Functional analysis of *ATP1A3* pathogenic variants in RDP by haplo-insufficiency determined low protein levels of the corresponding ATPase (13). On the other hand, none of the variants associated with AHC reduced protein levels, whereas both pathogenic variants of AHC and those of RDP reduced ATPase activity (31). These studies suggested that AHC related variants compromise the Na^+/K^+ ATPase function due to inhibition of ion binding.

Different phenotype severities have been reported among AHC related pathogenic variants. In general, cases with p.Asp801Asn and p.Gly947Args have a better clinical outcome than p.Glu815Lys carriers. A dominant negative mechanism has been proposed for these heterozygous variants in patients with AHC. According to Li et al. all these pathogenic variants inhibit wild type function by dominant negative interactions in a similar extent, therefore this mechanism is unlikely to explain the AHC severity spectrum (79).

Paradoxically, the severity of human symptoms have not been correlate with whether there was enough residual ATPase activity to support cell survival. Arystarkhova et al. proposed that protein misfolding and endoplasmic reticulum retention were correlated with clinical severity (76).

We carried out mapping of the variants discussed in the present review. All patient missense variants were visualized on a protein homology model (SWISS-Model repository, template: 4RET) (80) of the α3 subunit of the Na+/K+-ATPase together with control variants in the general population (**Figure 2**; see methods in **Supplementary Material** for details). Upon visual inspection, most patient variants were located more near to the center of the protein, whereas population variants tended to be localized more outside the transmembrane helices in the outer cytosolic region. Variants associated with epileptic encephalopathy tended to cluster together in the cytosolic region that is linked to the transmembrane region. These results match those of a similar analysis performed in 2019 comparing variants in three ATP1 paralogs *ATP1A1*, *ATP1A2*, and *ATP1A3* (75). Notably, all observations could not be statistically quantified likely due to the small number of patient variants and heterogeneity in the variant localization on protein structure.

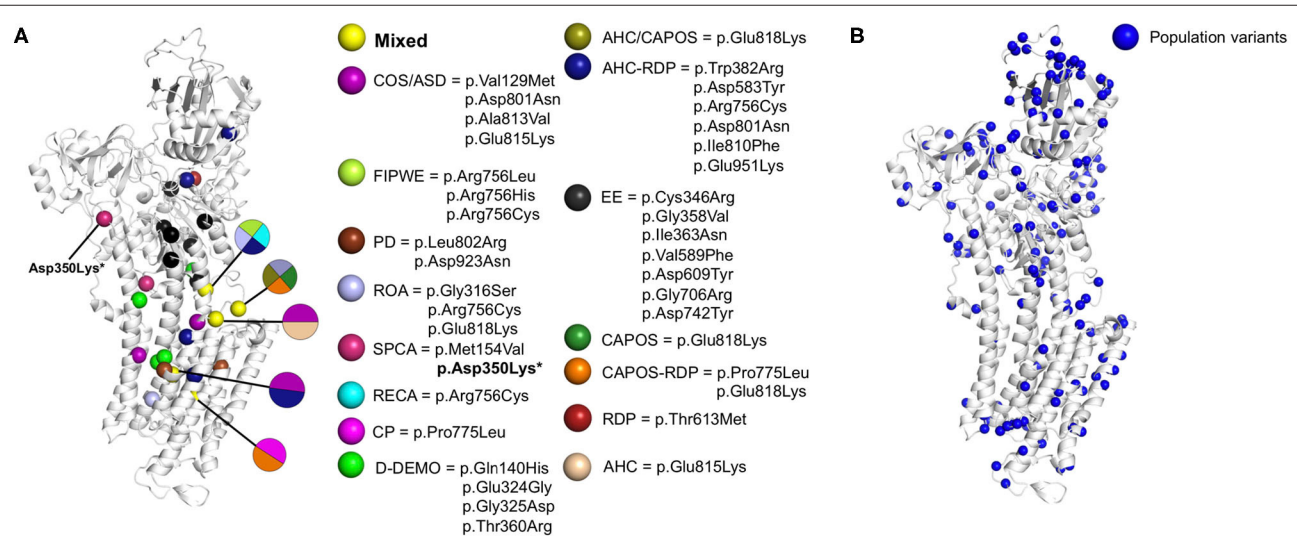

FIGURE 2 | ATP1A3 missense variants mapped on a protein structure model of the α3 subunit of the Na+/K+-ATPase. The homology model was obtained from the SWISS-Model repository (template: 4RET). **(A)** Pathogenic missense variants. Spheres are colored by disorder type. Residues associated with multiple distinct disorders were colored in yellow. **(B)** Population variants in *ATP1A3* were collected from the gnomAD database (81) and visualized as blue spheres on the homology model. *Asp350Lys did not match any protein isoform in Uniprot (82), could not be aligned to the canonical *ATP1A3* sequence and is thus not displayed in the figure. In case of AHC and RDP, we only included the pathogenic variants reported as more frequent according to the references (8, 14), respectively. AHC, alternating hemiplegia of childhood; ASD, autistic spectrum disorder; CAPOS, cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss; COS, childhood-onset schizophrenia; CP, cerebral palsy; D-DEMO dystonia, dysmorphism, encephalopathy, MRI abnormalities, and no hemiplegia; EE, early life epilepsy; FIPWE, fever-induced paroxysmal weakness and encephalopathy; PD, paroxysmal dyskinesias; RECA, relapsing encephalopathy with cerebellar ataxia; RDP, rapid-onset dystonia-parkinsonism; ROA, rapid onset cerebellar ataxia; SPCA, slowly progressive cerebellar ataxia.

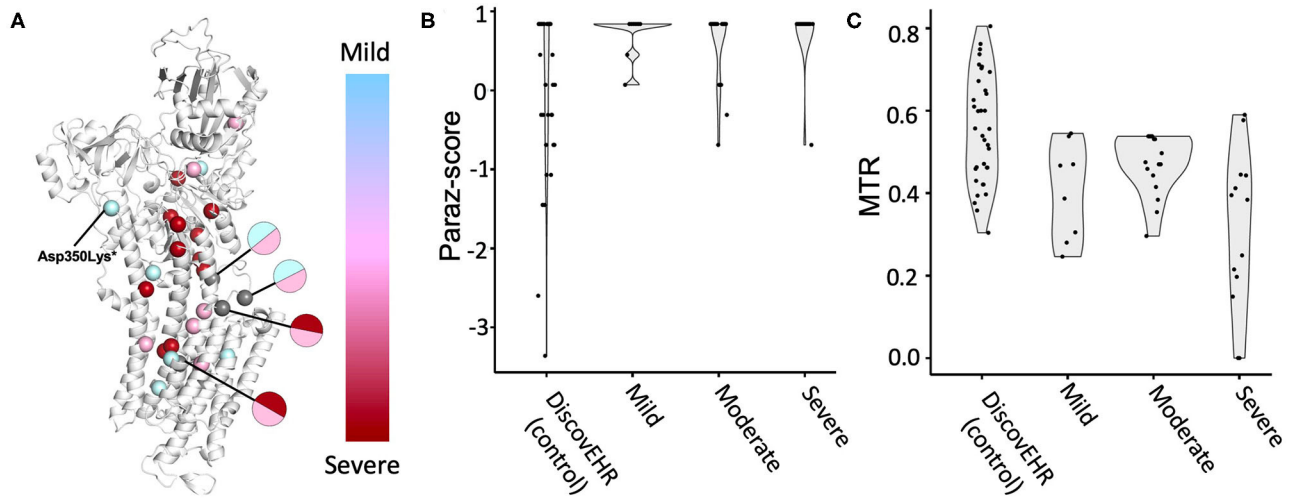

FIGURE 3 | ATP1A3 variant associated disorder severity and variant position analysis. Patients with *ATP1A3* variants were grouped by disorder severity into three groups. **(A)** The corresponding missense variants were visualized on the protein structure and colored according to grouping (mild disorder = cyan; moderate disorder = pink; severe disorder = red). Gray-coloring of spheres indicates residues where variants from multiple severity groups have been reported. Amino acid residue paralog conservation **(B)** and population constrained **(C)** were assessed for variants from each severity group together with a neutral comparison group, missense variants from the DiscovEHR database (see methods in **Supplementary Material** for details).

We also investigated whether patient variants were located at more evolutionary conserved or population constrained regions compared to a comparison group and explored the localization of the variant severity groups on protein structure spatially (**Figure 3A**; see methods in **Supplementary Material** for details). Although more variants associated with more severe disorders tend to be located near the core of the protein, no clear clusters were observed. Amino acids with patient variants were more conserved across paralogous genes compared to the comparison group ($p = 0.0075$). Similarly, patient variants are more constrained to variants from the general population ($p = 0.0081$) (**Figures 3B,C**). Next, we investigated whether variants associated with mild to severe disorders show differences in evolutionary conserved or population-constrained scores. However, no significant difference was observed as previously described (75).

The available information suggest that different molecular mechanism and complex interactions are involved in the expansive range of disease severity and clinical manifestations.

Most known patients with *ATP1A3*-related disorders fit into discrete classical syndromes with no causal variant overlapping. However, several cases show atypical features or combine features of two or more of these major phenotypes. Moreover, some pathogenic variants have been reported to manifest different phenotypes in non-related cases as well as intrafamilial. In view of the available evidence, we agree with authors that have proposed to consider *ATP1A3*-related disorders as a clinical continuum rather than distinct entities, with an age-dependent pattern of emergence and progression of different signs and symptoms (11). For example, being EE in the most severe extreme and RDP in the milder.

Despite the expanding spectrum of *ATP1A3* phenotypes some features that may guide the clinician in the diagnosis of *ATP1A3*-related disorders include an acute or rapid onset, triggered by fever or other triggers, progression with paroxysmal episodes of dystonia or attacks of weakness, developmental delay, encephalopathy, epilepsy, dysmorphism, pes cavus, hearing loss, optic atrophy, areflexia, pyramidal signs, or a wide range of movement disorders. Remarkably some of these features might differ in cases with atypical presentations. Clinical identification is important to guide molecular investigations and interpretation.

AUTHOR CONTRIBUTIONS

PS conducted the review of literature, and wrote the first manuscript. TB and DL performed all the bioinformatic analysis. IM and HF revised and edited the manuscript. All authors revised and edited the final version of the manuscript and read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.637890/full#supplementary-material

REFERENCES

1. Salles P, Fernandez HH. Untangling the complicated web of ATP1A3 mutations. *Parkinsonism Relat Disord*. (2020):1–3. doi: 10.1016/j.parkreldis.2020.09.010
2. Palmgren MG, Nissen P. P-type ATPases. *Annu Rev Biophys*. (2011) 40:243–66. doi: 10.1146/annurev.biophys.093008.131331
3. Holm TH, Lykke-Hartmann K. Insights into the pathology of the α3 Na+/K+-ATPase ion pump in neurological disorders; lessons from animal models. *Front Physiol*. (2016) 7:1–12. doi: 10.3389/fphys.2016.00209
4. Dobretsov, Maxim. Neuronal function and alpha3 isoform of the Na/K-ATPase. *Front Biosci*. (2005) 10:2373. doi: 10.2741/1704
5. Azarias G, Kruusmägi M, Connor S, Akkuratov EE, Liu X-L, Lyons D, et al. A specific and essential role for Na,K-ATPase α3 in neurons Co-expressing α1 and α3. *J Biol Chem*. (2013) 288:2734–43. doi: 10.1074/jbc.M112.425785
6. Bøttger P, Tracz Z, Heuck A, Nissen P, Romero-Ramos M, Lykke-Hartmann K. Distribution of Na/K-ATPase alpha 3 isoform, a sodium-potassium P-type pump associated with rapid-onset of dystonia parkinsonism (RDP) in the adult mouse brain. *J Comp Neurol*. (2011) 519:376–404. doi: 10.1002/cne.22524
7. McLean WJ, Smith KA, Glowatzki E, Pyott SJ. Distribution of the Na,K-ATPase α subunit in the rat spiral ganglion and organ of corti. *J Assoc Res Otolaryngol*. (2009) 10:37–49. Available from: http://link.springer.com/10.1007/s10162-008-0152-9
8. Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, et al. Distinct neurological disorders with ATP1A3 mutations. *Lancet Neurol*. (2014) 13:503–14. doi: 10.1016/S1474-4422(14)70011-0
9. Rosewich H, Sweney MT, DeBrosse S, Ess K, Ozelius L, Andermann E, et al. Research conference summary from the 2014 International task force on ATP1A3 -related disorders. *Neurol Genet*. (2017) 3:e139. doi: 10.1212/NXG.0000000000000139
10. Carecchio M, Zorzi G, Ragona F, Zibordi F, Nardocci N. ATP1A3-related disorders: an update. *Eur J Paediatr Neurol*. (2018) 22:257–63. doi: 10.1016/j.ejpn.2017.12.009
11. Capuano A, Garone G, Tiralongo G, Graziola F. Alternating hemiplegia of childhood: understanding the genotype–phenotype relationship of ATP1A3 variations. *Appl Clin Genet*. (2020) 13:71–81. doi: 10.2147/TACG.S210325
12. Dobyns WB, Ozelius LJ, Kramer PL, Brashear A, Farlow MR, Perry TR, et al. Rapid-onset dystonia-parkinsonism. *Neurology*. (1993) 43:2596–6. doi: 10.1212/WNL.43.12.2596
13. De Carvalho Aguiar P, Sweadner KJ, Penniston JT, Zaremba J, Liu L, Caton M, et al. Mutations in the Na+/K+-ATPase α3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. *Neuron*. (2004) 43:169–75. doi: 10.1016/j.neuron.2004.06.028
14. Dard R, Mignot C, Durr A, Lesca G, Sanlaville D, Roze E, et al. Relapsing encephalopathy with cerebellar ataxia related to an ATP1A3 mutation. *Dev Med Child Neurol*. (2015) 57:1183–6. doi: 10.1111/dmcn.12927
15. Brashear A, Dobyns WB, De Carvalho Aguiar P, Borg M, Frijns CJM, Gollamudi S, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the ATP1A3 gene. *Brain*. (2007) 130:828–35. doi: 10.1093/brain/awl340
16. Haq IU, Snively BM, Sweadner KJ, Suerken CK, Cook JF, Ozelius LJ, et al. Revising rapid-onset dystonia–parkinsonism: broadening indications for ATP1A3 testing. *Mov Disord*. (2019) 34:1528–36. doi: 10.1002/mds.27801
17. Termsarasab P, Yang AC, Frucht SJ. Intermediate phenotypes of ATP1A3 mutations: phenotype-genotype correlations. *Tremor Other Hyperkinet Mov*. (2015) 5:336. doi: 10.5334/tohm.255
18. Barbano RL, Hill DF, Snively BM, Light LS, Boggs N, McCall WV, et al. New triggers and non-motor findings in a family with rapid-onset dystonia-parkinsonism. *Park Relat Disord*. (2012) 18:737–41. doi: 10.1016/j.parkreldis.2012.03.020
19. Brashear A, Cook JF, Hill DF, Amponsah A, Snively BM, Light L, et al. Psychiatric disorders in rapid-Onset dystonia-Parkinsonism. *Neurology*. (2012) 79:1168–73. doi: 10.1212/WNL.0b013e3182698d6c
20. Cook JF, Hill DF, Snively BM, Boggs N, Suerken CK, Haq I, et al. Cognitive impairment in rapid-onset dystonia-parkinsonism. *Mov Disord*. (2014) 29:344–50. doi: 10.1002/mds.25790
21. Liu Y, Lu Y, Zhang X, Xie S, Wang T, Wu T, et al. A case of rapid-onset dystonia-parkinsonism accompanied by pyramidal tract impairment. *BMC Neurol*. (2016) 16:1–6. doi: 10.1186/s12883-016-0743-8
22. de Gusmao CM, Dy M, Sharma N. Beyond dystonia-parkinsonism: chorea and ataxia with ATP1A3 mutations. *Mov Disord Clin Pract*. (2016) 3:402–4. doi: 10.1002/mdc3.12317
23. Sousa AL, Alonso I, Magalhães M. A Portuguese rapid-onset dystonia-parkinsonism case with atypical features. *Neurol Sci*. (2017) 38:1713–4. doi: 10.1007/s10072-017-2996-4
24. Brashear A, Mink JW, Hill DF, Boggs N, Mccall WV, Stacy MA, et al. ATP1A3 mutations in infants: a new rapid-onset dystonia-Parkinsonism phenotype characterized by motor delay and ataxia. *Dev Med Child Neurol*. (2012) 54:1065–7. doi: 10.1111/j.1469-8749.2012.04421.x
25. Brashear A, Farlow MR, Butler IJ, Kasarskis EJ, Dobyns WB. Variable phenotype of rapid-onset dystonia-parkinsonism. *Mov Disord*. (1996) 11:151–6. doi: 10.1002/mds.870110206
26. Kamphuis DJ, Koelman H, Lees AJ, Tijsen MAJ. Sporadic rapid-onset dystonia-parkinsonism presenting as a Parkinson's disease. *Mov Disord*. (2006) 21:118–9. doi: 10.1002/mds.20695
27. Zanotti-Fregonara P, Vidailhet M, Kas A, Ozelius LJ, Clot F, Hindié E. [123I]-FP-CIT and [99mTc]-HMPAO single photon emission computed tomography in a new sporadic case of rapid-onset dystonia-parkinsonism. *J Neurol Sci*. (2008) 273:148–51. doi: 10.1016/j.jns.2008.06.033
28. Verret S, Steele JC. Alternating hemiplegia in childhood: a report of eight patients with complicated migraine beginning in infancy. *Pediatrics*. (1971) 47:675–80.
29. Panagiotakaki E, Gobbi G, Neville B, Ebinger F, Campistol J, Nevšímalová S, et al. Evidence of a non-progressive course of alternating hemiplegia of childhood: study of a large cohort of children and adults. *Brain*. (2010) 133:3598–610. doi: 10.1093/brain/awq295
30. Neville BGR, Ninan M. The treatment and management of alternating hemiplegia of childhood. *Dev Med Child Neurol*. (2007) 49:777–80. doi: 10.1111/j.1469-8749.2007.00777.x
31. Heinzen EL, Swoboda KJ, Hitomi Y, Gurrieri F, De Vries B, Tiziano FD, et al. *De novo* mutations in ATP1A3 cause alternating hemiplegia of childhood. *Nat Genet*. (2012) 44:1030–4. doi: 10.1038/ng.2358
32. Rosewich H, Thiele H, Ohlenbusch A, Maschke U, Altmüller J, Frommolt P, et al. Heterozygous de-novo mutations in ATP1A3 in patients with alternating hemiplegia of childhood: A whole-exome sequencing gene-identification study. *Lancet Neurol*. (2012) 11:764–73. doi: 10.1016/S1474-4422(12)70182-5
33. Panagiotakaki E, de Grandis E, Stagnaro M, Heinzen EL, Fons C, Sisodiya S, et al. Clinical profile of patients with ATP1A3 mutations in Alternating Hemiplegia of Childhood - A study of 155 patients. *Orphanet J Rare Dis*. (2015) 10:123. doi: 10.1186/s13023-015-0335-5
34. Giacanelli M, Petrucci A, Lispi L, Luna R, Neri G, Gurrieri F, et al. ATP1A3 mutant patient with alternating hemiplegia of childhood and brain spectroscopic abnormalities. *J Neurol Sci*. (2017) 379:36–8. doi: 10.1016/j.jns.2017.05.041
35. Kansagra S, Mikati MA, Vigevano F. Alternating hemiplegia of childhood. In: *Handbook of Clinical Neurology*, *1St ed. Vol. 112*. Elsevier (2013). p. 821–6. doi: 10.1016/B978-0-444-52910-7.00001-5
36. Novy J, McWilliams E, Sisodiya SM. Asystole in alternating hemiplegia with *de novo* ATP1A3 mutation. *Eur J Med Genet*. (2014) 57:37–9. doi: 10.1016/j.ejmg.2013.11.003
37. Gurrieri F, Tiziano FD, Zampino G, Neri G. Recognizable facial features in patients with alternating hemiplegia of childhood. *Am J Med Genet Part A*. (2016) 170:2698–705. doi: 10.1002/ajmg.a.37808
38. Nicolaides P, Appleton RE, Fryer A. Optic atrophy, and sensorineural hearing loss (CAPOS): a new syndrome. *J7 Med Genet*. (1996) 33:419–21. doi: 10.1136/jmg.33.5.419
39. Demos MK, Van Karnebeek CDM, Ross CJD, Adam S, Shen Y, Zhan SH, et al. A novel recurrent mutation in ATP1A3 causes CAPOS syndrome. *Orphanet J Rare Dis*. (2014) 9:1–9. doi: 10.1186/1750-1172-9-15
40. Heimer G, Sadaka Y, Israelian L, Feiglin A, Ruggieri A, Marshall CR, et al. CAOS-episodic cerebellar ataxia, areflexia, optic atrophy, and sensorineural hearing loss. *J Child Neurol*. (2015) 30:1749–56. doi: 10.1177/0883073815579708

41. Roenn CP, Li M, Schack VR, Forster IC, Holm R, Toustrup-Jensen MS, et al. Functional consequences of the CAPOS mutation E818K of Na,K-ATPase. *J Biol Chem*. (2019) 294:269–80. doi: 10.1074/jbc.RA118.004591
42. Maas RPPWM, Schieving JH, Schouten M, Kamsteeg EJ, Van De Warrenburg BPC. The genetic homogeneity of CAPOS syndrome: four new patients with the c.2452G>A (p.Glu818Lys) mutation in the ATP1A3 gene. *Pediatr Neurol*. (2016) 59:71–75.e1. doi: 10.1016/j.pediatrneurol.2016.02.010
43. Paquay S, Wiame E, Deggouj N, Boschi A, De Siati RD, Sznajer Y, et al. Childhood hearing loss is a key feature of CAPOS syndrome: a case report. *Int J Pediatr Otorhinolaryngol*. (2018) 104:191–4. doi: 10.1016/j.ijporl.2017.11.022
44. Han KH, Oh DY, Lee S, Lee C, Han JH, Kim MY, et al. ATP1A3 mutations can cause progressive auditory neuropathy: a new gene of auditory synaptopathy. *Sci Rep*. (2017) 7:123. doi: 10.1038/s41598-017-16676-9
45. Rosewich H, Weise D, Ohlenbusch A, Gartner J, Brockmann K. Phenotypic overlap of alternating hemiplegia of childhood and CAPOS syndrome. *Neurology*. (2014) 83:861–3. doi: 10.1212/WNL.0000000000000735
46. Hayashida T, Saito Y, Ishii A, Hirose S, Hiraiwa R, Maegaki Y, et al. Further characterization of CAPOS/CAOS syndrome with the Glu818Lys mutation in the ATP1A3 gene: a case report. *Brain Dev*. (2018) 40:576–81. doi: 10.1016/j.braindev.2018.03.004
47. Potic A, Nmezi B, Padiath QS. CAPOS syndrome and hemiplegic migraine in a novel pedigree with the specific ATP1A3 mutation. *J Neurol Sci*. (2015) 358:453–6. doi: 10.1016/j.jns.2015.10.002
48. Stagnaro M, Pisciotta L, Gherzi M, Di Rocco M, Gurrieri F, Parrini E, et al. ATP1A3 spectrum disorders: a video-documented history of 7 genetically confirmed early onset cases. *Eur J Paediatr Neurol*. (2018) 22:264–71. doi: 10.1016/j.ejpn.2018.01.010
49. Hully M, Ropars J, Hubert L, Boddaert N, Rio M, Bernardelli M, et al. Mosaicism in ATP1A3-related disorders: not just a theoretical risk. *Neurogenetics*. (2017) 18:23–8. doi: 10.1007/s10048-016-0498-9
50. Sabouraud P, Riquet A, Spitz MA, Deiva K, Nevsimalova S, Mignot C, et al. Relapsing encephalopathy with cerebellar ataxia are caused by variants involving p.Arg756 in ATP1A3. *Eur J Paediatr Neurol*. (2019) 23:448–55. doi: 10.1016/j.ejpn.2019.02.004
51. Yano ST, Silver K, Young R, DeBrosse SD, Ebel RS, Swoboda KJ, et al. Fever-induced paroxysmal weakness and encephalopathy, a new phenotype of ATP1A3 mutation. *Pediatr Neurol*. (2017) 73:101–5. doi: 10.1016/j.pediatrneurol.2017.04.022
52. Paciorkowski AR, McDaniel SS, Jansen LA, Tully H, Tuttle E, Ghoneim DH, et al. Novel mutations in ATP1A3 associated with catastrophic early life epilepsy, episodic prolonged apnea, and postnatal microcephaly. *Epilepsia*. (2015) 56:422–30. doi: 10.1111/epi.12914
53. Marzin P, Mignot C, Dorison N, Dufour L, Ville D, Kaminska A, et al. Early-onset encephalopathy with paroxysmal movement disorders and epileptic seizures without hemiplegic attacks: about three children with novel ATP1A3 mutations. *Brain Dev*. (2018) 40:768–74. doi: 10.1016/j.braindev.2018.05.008
54. Holze N, Baalen A Van, Stephani U, Helbig I, Muhle H. Variants in the ATP1A3 gene mutations within severe apnea starting in early infancy: an observational study of two cases with a possible relation to epileptic activity. *Neuropediatrics*. (2018) 49:342–6. doi: 10.1055/s-0038-1653978
55. Schirinzi T, Graziola F, Cusmai R, Fusco L, Nicita F, Elia M, et al. ATP1A3-related epileptic encephalopathy responding to ketogenic diet. *Brain Dev*. (2018) 40:433–8. doi: 10.1016/j.braindev.2018.01.002
56. Tran L, Richards J, McDonald M, McConkie-Rosell A, Stong N, Jasien J, et al. Epileptic encephalopathy with features of rapid-onset dystonia Parkinsonism and alternating hemiplegia of childhood: a novel combination phenotype associated with ATP1A3 mutation. *Epileptic Disord*. (2020) 22:103–9. doi: 10.1684/epd.2020.1127
57. Sweadner KJ, Toro C, Whitlow CT, Snively BM, Cook JF, Ozelius LJ, et al. ATP1A3 mutation in adult rapid-onset ataxia. *PLoS ONE*. (2016) 11:51429. doi: 10.1371/journal.pone.0151429
58. Schirinzi T, Graziola F, Nicita F, Travaglini L, Stregapede F, Valeriani M, et al. Childhood rapid-onset ataxia: expanding the phenotypic spectrum of ATP1A3 mutations. *Cerebellum*. (2018) 17:489–93. doi: 10.1007/s12311-018-0920-y
59. Sasaki M, Sumitomo N, Shimizu-Motohashi Y, Takeshita E, Kurosawa K, Kosaki K, et al. ATP1A3 variants and slowly progressive cerebellar ataxia without paroxysmal or episodic symptoms in children. *Dev Med Child Neurol*. (2021) 63:111–5. doi: 10.1111/dmcn.14666
60. Smedemark-Margulies N, Brownstein CA, Vargas S, Tembulkar SK, Towne MC, Shi J, et al. A novel *de novo* mutation in ATP1A3 and childhood-onset schizophrenia. *Mol Case Stud*. (2016) 2:a001008. doi: 10.1101/mcs.a001008
61. Chaumette B, Ferrafiat V, Ambalavanan A, Goldenberg A, Dionne-Laporte A, Spiegelman D, et al. Missense variants in ATP1A3 and FXYD gene family are associated with childhood-onset schizophrenia. *Mol Psychiatr*. (2020) 25:821–30. doi: 10.1038/s41380-018-0103-8
62. Forsyth JK, Asarnow RF. Genetics of childhood-onset schizophrenia 2019 update. *Child Adolesc Psychiatr Clin N Am*. (2020) 29:157–70. doi: 10.1016/j.chc.2019.08.007
63. Zúñiga-Ramírez C, Kramis-Hollands M, Mercado-Pimentel R, González-Usigli HA, Sáenz-Farret M, Soto-Escageda A, et al. Generalized dystonia and paroxysmal dystonic attacks due to a novel ATP1A3 variant. *Tremor Other Hyperkinet Mov*. (2019) 9:1–5. doi: 10.5334/tohm.490
64. Roubergue A, Roze E, Vuillaumier-Barrot S, Fontenille MJ, Méneret A, Vidailhet M, et al. The multiple faces of the ATP1A3-related dystonic movement disorder. *Mov Disord*. (2013) 28:1457–9. doi: 10.1002/mds.25396
65. Nomura S, Kashiwagi M, Tanabe T, Oba C, Yanagi K, Kaname T, et al. Rapid-onset dystonia-parkinsonism with ATP1A3 mutation and left lower limb paroxysmal dystonia. *Brain Dev*. (2021). Available online at: https://linkinghub.elsevier.com/retrieve/pii/S0387760420303338
66. Calame D, Shinawi M, Cohen J, Person R, Telegrafi A, Lotze T, et al. Atypical phenotypes caused by the ATP1A3 variant p.P775L (1946). *Neurology*. (2020) 94(15 Suppl):1946.
67. Prange L, Pratt M, Herman K, Schiffmann R, Mueller DM, McLean M, et al. D-DEMØ, a distinct phenotype caused by ATP1A3 mutations. *Neurol Genet*. (2020) 6:e466. doi: 10.1212/NXG.0000000000000466
68. Allocco AA, Jin SC, Duy PQ, Furey CG, Zeng X, Dong W, et al. Recessive inheritance of congenital hydrocephalus with other structural brain abnormalities caused by compound heterozygous mutations in ATP1A3. *Front Cell Neurosci*. (2019) 13:1–8. doi: 10.3389/fncel.2019.00425
69. Doganli C, Beck HC, Ribera AB, Oxvig C, Lykke-Hartmann K. α3Na+/K+-ATPase deficiency causes brain ventricle dilation and abrupt embryonic motility in zebrafish*. *J Biol Chem*. (2013) 288:8862–74. doi: 10.1074/jbc.M112.421529
70. Rosewich H, Ohlenbusch A, Huppke P, Schlotawa L, Baethmann M, Carrilho I, et al. The expanding clinical and genetic spectrum of ATP1A3-related disorders. *Neurology*. (2014) 82:945–55. doi: 10.1212/WNL.0000000000000212
71. Sasaki M, Ishii A, Saito Y, Hirose S. Intermediate form between alternating hemiplegia of childhood and rapid-onset dystonia-parkinsonism. *Mov Disord*. (2014) 29:153–4. doi: 10.1002/mds.25659
72. Nicita F, Travaglini L, Sabatini S, Garavaglia B, Panteghini C, Valeriani M, et al. Childhood-onset ATP1A3-related conditions: report of two new cases of phenotypic spectrum. *Park Relat Disord*. (2016) 30:81–2. doi: 10.1016/j.parkreldis.2016.05.029
73. Chouksey A, Pandey S. ATP1A3 mutation presenting as CAPOS syndrome + dystonia phenotype. *Park Relat Disord*. (2020) 78:192–4. doi: 10.1016/j.parkreldis.2020.05.029
74. Li X, Hong Y, Li S, Wang L, Wan X. Movement disorders rounds: atypical cases in two Chinese families with novel variants in ATP1A3. *Park Relat Disord*. (2020) 76:46–8 doi: 10.1016/j.parkreldis.2020.05.030
75. Sweadner KJ, Arystarkhova E, Penniston JT, Swoboda KJ, Brashear A, Ozelius LJ. Genotype-structure-phenotype relationships diverge in paralogs ATP1A1, ATP1A2, and ATP1A3. *Neurol Genet*. (2019) 5:e303. doi: 10.1212/NXG.0000000000000303
76. Arystarkhova E, Haq IU, Luebbert T, Mochel F, Saunders-Pullman R, Bressman SB, et al. Factors in the disease severity of ATP1A3 mutations: Impairment, misfolding, and allele competition. *Neurobiol Dis*. (2019) 132:104577. doi: 10.1016/j.nbd.2019.104577
77. Ishii A, Saito Y, Mitsui J, Ishiura H, Yoshimura J, Arai H, et al. Identification of ATP1A3 mutations by exome sequencing as the cause of alternating hemiplegia of childhood in japanese patients. Speletas M, editor. *PLoS ONE*. (2013) 8:e56120. doi: 10.1371/journal.pone.0056120

78. Tranebjærg L, Strenzke N, Lindholm S, Rendtorff ND, Poulsen H, Khandelia H, et al. Correction to: the CAPOS mutation in ATP1A3 alters Na/K-ATPase function and results in auditory neuropathy which has implications for management. *Hum Genet*. (2018) 137:279–80. doi: 10.1007/s00439-018-1870-7
79. Li M, Jazayeri D, Corry B, McSweeney KM, Heinzen EL, Goldstein DB, et al. A functional correlate of severity in alternating hemiplegia of childhood. *Neurobiol Dis*. (2015) 77:88–93. doi: 10.1016/j.nbd.2015.02.002
80. Bienert S, Waterhouse A, de Beer TAP, Tauriello G, Studer G, Bordoli L, et al. The SWISS-MODEL repository—new features and functionality. *Nucleic Acids Res*. (2017) 45:D313–9. doi: 10.1093/nar/gkw1132
81. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. (2020) 581:434–43. doi: 10.1530/ey.17.14.3
82. The UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res*. (2019) 47:D506–15. doi: 10.1093/nar/gky1049

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Variable Effects of PD-Risk Associated SNPs and Variants in Parkinsonism-Associated Genes on Disease Phenotype in a Community-Based Cohort

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Genetic risk factors for Parkinson's disease (PD) risk and progression have been identified from genome-wide association studies (GWAS), as well as studies of familial forms of PD, implicating common variants at more than 90 loci and pathogenic or likely pathogenic variants at 16 loci. With the goal of understanding whether genetic variants at these PD-risk loci/genes differentially contribute to individual clinical phenotypic characteristics of PD, we used structured clinical documentation tools within the electronic medical record in an effort to provide a standardized and detailed clinical phenotypic characterization at the point of care in a cohort of 856 PD patients. We analyzed common SNPs identified in previous GWAS studies, as well as low-frequency and rare variants at parkinsonism-associated genes in the MDSgene database for their association with individual clinical characteristics and test scores at baseline assessment in our community-based PD patient cohort: age at onset, disease duration, Unified Parkinson's Disease Rating Scale I-VI, cognitive status, initial and baseline motor and non-motor symptoms, complications of levodopa therapy, comorbidities and family history of neurological disease with one or more than one affected family members. We find that in most cases an individual common PD-risk SNP identified in GWAS is associated with only a single clinical feature or test score, while gene-level tests assessing low-frequency and rare variants reveal genes associated in either a unique or partially overlapping manner with the different clinical features and test scores. Protein-protein interaction network analysis of the identified genes reveals that while some of these genes are members of already identified protein networks others are not. These findings indicate that genetic risk factors for PD differentially affect the phenotypic presentation and that genes associated with PD risk are also differentially associated with individual disease phenotypic characteristics at baseline. These findings raise the intriguing possibility that different SNPs/gene effects impact discrete phenotypic characteristics. Furthermore,

they support the hypothesis that different gene and protein-protein interaction networks that underlie PD risk, the PD phenotype, and the neurodegenerative process leading to the disease phenotype, and point to the significance of the genetic background on disease phenotype.

Keywords: phenotype, genetic association, protein interaction network, gene level tests, community cohort, Parkinson's disease

INTRODUCTION

Parkinson's disease (PD), the second most common neurodegenerative disease, has an insidious onset and a long pre-symptomatic and symptomatic course. Four cardinal features that include resting tremor, bradykinesia, rigidity, and postural instability define the motor aspects of the disease. The constellation of clinical symptoms however is variable both in terms of symptom combination and temporal profile. This variability has led to phenotypic classification according to different disease characteristics. A commonly accepted classification is based on motor symptoms: disease subtypes include a tremor-predominant, akinetic/rigid, and mixed subtype (1). More recently, additional classifications have emerged based on different clinical features such as non-motor features, disease progression, a combination of motor and non-motor features, combination of clinical features and comorbidities, multimodal imaging and genetic burden. More specifically, Sauerbier et al. (2) in their review proposed the existence of a distinct non-motor subtype (NMS) of NMS-dominant PD based on the burden of non-motor symptoms in early PD including cognitive dysfunction, anosmia, anxiety, depression, sleep disorders, and autonomic dysfunction observed either alone or in varying combinations. Simuni et al. (3) reported that, for the Primary Progression Markers Initiative (PPMI) PD cohort, higher baseline non-motor scores were associated with female sex and a more severe motor phenotype. Longitudinal increase in non-motor score severity was associated with older age and lower CSF aβ1–42 at baseline. Lawton et al. (4) identified four phenotypic clusters in their cohort: (1) fast motor progression, (2) mild motor and non-motor disease, (3) severe motor disease, poor psychological well-being and poor sleep with intermediate motor progression, and (4) slow motor progression with tremor-dominant unilateral disease. Mollenhauer et al. (5) in their analysis of the *De Novo* Parkinson (DeNOPA) cohort, reported that baseline predictors of worse progression of motor symptoms included male sex, orthostatic blood pressure drop, diagnosis of coronary artery disease, arterial hypertension, elevated serum uric acid, and CSF neurofilament light chain.

A variable temporal profile of motor symptom appearance and progression has been reported in different cohorts that have been followed longitudinally for different lengths of time and identified predictors of disease progression and phenotypic clusters. In the DeNOPA cohort, predictors of cognitive decline in PD included previous heavy alcohol abuse, current diagnoses of diabetes mellitus, arterial hypertension, elevated periodic limb movement index during sleep, decreased hippocampal volume by MRI, and higher baseline levels of uric acid, C-reactive protein, high density lipoprotein (HDL) cholesterol, and glucose. In their cohort, risk markers for faster disease progression included cardiovascular risk factors, deregulated blood glucose, uric acid metabolism and inflammation. In the PPMI cohort, Aleksovski et al. (6) reported that the postural instability gait disorder (PIGD) subtype, compared to the tremor-predominant subtype, was characterized by more severe disease manifestations at diagnosis, greater cognitive progression, and more frequent psychosis (5). In the PPMI cohort, Latourelle et al. (7) found that higher baseline MDS-UPDRS motor score, male sex, and increased age, as well as a novel Parkinson's disease-specific epistatic interaction, were indicative of faster motor progression. In their retrospective review of a cohort of 100 autopsy confirmed PD cases, Pablo-Fernandez at al. (8) reported that the presence of autonomic dysfunction defined as autonomic failure on autonomic testing or the presence of at least two symptoms such as urinary symptoms, constipation, orthostatic hypotension, or sweating abnormalities was associated with a more rapid progression and shorter survival.

Other classifications of disease subtypes have been proposed in addition to motor, non-motor symptom and disease course-based classifications. Inguanzo et al. (9) employed a radiomics and hybrid machine learning approach to identify mild, intermediate and severe disease subtypes based on a combination of dopaminergic deficit by imaging and escalating motor and non-motor manifestations.

In the last two decades, genome-wide association studies (GWAS) of common genetic variants and dissection of the low frequency and rare variants contributing to familial forms of PD has implicated an increasing number of genetic loci in disease risk and severity. This has cemented the view that PD is a complex and heterogeneous genetic disorder, with variants at many genes impacting disease phenotype and course. We are just beginning to understand whether PD-risk variants are differentially associated with baseline features or disease subtype. Tan et al. (10) performed a GWAS of motor and cognitive progression in PD and reported that *ATPBB2*, a phospholipid transporter related to vesicle formation, is associated with motor progression, and that variants at *APOE* drive cognitive progression, whereas there was no overlap of variants associated with PD risk and PD age-at-onset with disease progression. Iwaki et al. (11) demonstrated sex-specific SNP associations with features of the PD phenotype: female patients had a higher risk of developing dyskinesias and a lower risk of developing cognitive impairment. Periñán et al. (12) reported an association of the TT genotype at the *PICALM* SNP rs3851179 with a decreased risk of cognitive impairment in PD. *GBA* variants have been associated with PD and generally are associated with faster progression

and more severe phenotypes (13, 14). Blauwendraat et al. (15) reported that in a large PD patient cohort, *GBA* risk variants decrease age at onset in PD.

Genetic factors that increase the risk of PD and genetic factors that affect disease severity and progression are not necessarily identical. Furthermore, individual genetic factors that influence disease severity and progression may not have an immediately identifiable impact in the clinical practice setting. It is therefore important to consider the predictive ability and significance of the impact of genetic variation on individual phenotypic characteristics and parameters that are clinically relevant and may have treatment implications (16–18). If one or a set of genetic variants contribute differentially to a particular phenotypic characteristic, it will be challenging to discover them using GWAS or gene-level association tests in a genome-wide screen since phenotypically well-characterized cohorts are typically modest in size, making it unlikely to discover genome-wide significant associations. We have therefore taken a focused approach, choosing to evaluate possible associations with SNPs that have been previously demonstrated to show significant associations with PD using large GWAS and low frequency and rare variants at parkinsonism-associated genes identified in the MDSgene database (19), hypothesizing that these genetic variants may differentially contribute to baseline clinical parameters/symptoms. Under this hypothesis, evaluating their association in a smaller cohort of subjects where individual clinical symptoms and objective test scores are obtained at baseline using structured clinical documentation support (SCDS) tools embedded in the electronic medical record (EMR) in a routine clinical practice setting (20) could allow for the discovery of significant associations. This would not be possible in the context of a case-control GWAS.

Indeed, we find that common SNPs from PD-risk genes identified in GWAS are individually associated with a range of clinical features: family history of dementia, the presence of hallucinations, bradykinesia, depression, orthostatism, disease subtype, and complications of levodopa therapy. When low-frequency and rare variants at PD-risk genes and parkinsonism-associated genes are analyzed in gene-level tests, associations with clinical characteristics such as presence of bradykinesia, depression, autonomic symptoms (orthostatism, constipation) UPDRS motor scores, mentation, complications of therapy scores, H&Y stage, and a family history of dementia are identified. All of the associations we report survive Bonferroni correction and some approach or reach genome-wide significance. It is interesting to note that the gene associations identified from the analysis of individual common SNPs do not always overlap with those identified in gene-level tests using low-frequency and rare variants suggesting an important role of the genetic background on the phenotypic manifestations.

METHODS

Subjects and Clinical Information

Eight hundred and fifty-six subjects with clinically definite or clinically probably Parkinson's disease (Bower criteria) (21) enrolled in two previously described patient cohorts [Molecular Epidemiology of Parkinson's Disease, MEPD (22), $N = 201$; DodoNA (23), $N = 655$] were included in this study. All patients in these cohorts had a diagnosis of PD at study entry and were residents of Cook and Lake Counties in Illinois, USA. Though both cohorts include individuals with diverse ancestries, the filtering described in the following section restricted the analysis to 786 individuals of European ancestry: 504 males, 282 females. Blood samples were collected in the majority of cases at an initial baseline visit or within a 3-month window following the initial visit. Data on clinical parameters were obtained from SCDS developed to standardize clinical assessment and retained within the EMR as described (20, 23). Given the community-based practice setting, our cohort included both *de novo* and previously diagnosed PD patients.

The following phenotypic characteristics were analyzed in our cohort: initial motor and non-motor symptoms as reported by the patient, as well as motor and non-motor symptoms identified by the clinician at their baseline encounter. Objective clinical assessment at the baseline encounter included scores on the Mini-mental Status Evaluation (MMSE) / Montreal Cognitive Assessment (MoCA) or Short Test of Mental Status (STMS) (24–26). Due to copyright limitations, cognitive status was assessed initially using the MMSE, at a later time-point the MoCA, and finally the STMS. The individual test scores on the MoCA and STMS were converted to MMSE scores using established normograms prior to analysis (26, 27). Objective clinical assessments at the baseline encounter also included scores on the Unified Parkinson's Disease Rating Scale (UPDRS) (28) [I – Mentation, Behavior and Mood; II – Activities of Daily Living; III – Motor Examination; IV – Complications of Therapy; V – Hoehn &Yahr stage; VI – Schwab & England Activities of Daily Living Scale], Epworth sleepiness scale (ESS) (29) and Geriatric Depression scale (GDS) (30), information on family history of PD, dementia, stroke, epilepsy, multiple sclerosis, and neuropathy, as well as information on comorbidities including diabetes, cardiovascular disease, migraine, schizophrenia, anxiety, depression, peripheral neuropathy and sleep apnea. **Supplementary Table 1** presents the list of clinical parameters and descriptive statistics for these parameters. Treatment details including medical and surgical therapy were collected but not included in the analysis presented here.

Genotyping and Quality Control Measures

Blood samples were stored at −80°C until DNA was extracted. Genotypes were obtained by interrogating an Affymetrix Axiom™ genome-wide human array containing 531,674 variants that included custom content, specifically variants at genes associated with PD and other neurological disorders. Prior to imputation using IMPUTE2 (31) against the 1,000 Genomes Phase 3 CEU genome, subjects were filtered in PLINK 1.07 (32) or 1.9 (33) for low overall genotyping rates (<95%) and sex-discordance, and variants with >5% missing calls were removed from the analysis. Imputed SNPs were retained only if $R^2 \geq 0.90$. Only subjects with European ancestry were retained by using principal components one and two (PC1 and PC2) from a principal components analysis (PCA) with 103 ancestry

informative markers (AIMs). For association tests with single variants, variants were also filtered by Hardy-Weinberg test statistic (1×10^{-4}) and to have a minor allele frequency (MAF) $> 1\%$.

Association Tests

Genes and variants initially identified for testing association with clinical parameters were selected based on a prior demonstrated association with PD/parkinsonism or disease progression [MDSgene.org; (10–12, 34–38)], or because the gene harbors pathogenic variants that cause PD/parkinsonism [for review, see (39, 40)]. Of 168 variants with a previously reported association and a MAF > 1%, 138 variants (**Supplementary Table 2**) were present in our data after filtering as described above. These were tested using PLINK for association applying logistic regression for binomial variables if at least 3% of subjects ($N = 24$) displayed the clinical parameter, or linear regression with standardized (mean 0, standard deviation 1) scaled variables and reverse scoring the MMSE so that worse scores indicate poorer performance. Associations were evaluated for both sexes jointly and for each sex separately. Sex, age-at-encounter and, since our community-based cohort includes both *de novo* and previously diagnosed patients, years-from-diagnosis were included as covariates for associations evaluated in both sexes, age-at-encounter, and years-from-diagnosis as covariates for associations evaluated in just one sex, and years-of-education added as an additional covariate for tests of association with cognitive measures (MMSE).

After using PLINK 1.9 to convert binary files to a VCF format, CHECKVCF (https://github.com/zhanxw/checkVCF) was used to verify the quality of the VCF file and TABANNO (https://github.com/zhanxw/anno) was used to annotate genes relative to NCBI build 37 (hg19). Gene-level association testing performed was using the sequence kernel association test (SKAT) (41) as implemented in RVTESTS (42), with and without the covariates: sex, age-at-encounter, years-from-diagnosis, and for MMSE, years of education (https://github.com/zhanxw/rvtests), using default parameters [significance evaluated using 10,000 permutations at alpha = 0.05, weight = Beta (beta1 = 1.00, beta2 = 25.00), missing genotypes imputed to mean], variant filtering to include non-synonymous, start-gain, stop-gain, stop-loss, start-loss, frameshift, codon-gain, codon-loss, codon-region, insertion, deletion, essential-splice-site, normal-splice-site, and structural-variation variants, filtering to include both rare (<1% MAF) and low-frequency (1–5% MAF) variants or only rare variants, and including only genes with at least two variants ($N = 117$ for MAF $\leq 5\%$, $N = 106$ for MAF $\leq 1\%$, **Supplementary Table 3**). An association was considered significant if the Bonferroni-corrected p-value was < 0.05.

Protein-Protein Interaction Network Evaluation

To evaluate whether the genes whose variants exhibited significant associations with clinical parameters identify protein products that are members of a functional protein-protein interaction network, those genes were entered into the Search Tool for the Retrieval of Interacting Genes, STRING, v.11 (43).

RESULTS

We hypothesized that SNPs which have been previously demonstrated to show significant associations with PD-risk using large GWAS and low frequency and rare variants at parkinsonism-associated genes identified in the MDSgene database (19) differentially contribute to discrete baseline clinical parameters/symptoms. To test this hypothesis, we evaluated their association in two well-characterized patient cohorts [MEPD (20) and DodoNA (23)] where individual clinical symptoms and objective test scores were obtained at baseline using SCDS tools embedded in the EMR. The findings are presented in the following two sections.

Single SNP Association Analyses

We initially evaluated whether common SNPs that have been previously associated with PD-risk in large GWAS are also associated with distinct binomial clinical phenotypic features of PD at their baseline presentation. We find significant associations that are at times sex-specific, and that the significant SNPs are typically located in non-overlapping genes/regions (**Table 1**).

Using an additive model, female PD patients carrying the minor allele (T) at SNP rs429358 at *APOE*, or having an *APOE* ε4 allele have an ∼8-fold increased risk of having a positive family history of more than one family member with dementia. Individuals with the minor allele (T) at SNP rs3431186 at *TMEM175*, which encodes a potassium channel that regulates lysosomal membrane potential and pH stability in neurons (44), are about twice as likely to have reduced arm swing, a manifestation of bradykinesia. Male PD patients with the minor allele (T) at SNP rs5396167 in *KPNA1*, which encodes importin α5 and is involved in lysosomal biogenesis and autophagy (45, 46), have a 2.8-fold increased risk to have hallucinations at baseline. Males with the minor (T) allele at SNP rs12528068 108.6 kb from the *RIMS1* gene, which encodes one of four isoforms of presynaptic scaffolding proteins involved in synaptic transmission (47), have a 2.1-fold increased risk of a history of essential tremor. Individuals carrying the minor allele (G) at SNP rs186798 in *ELOVL7* have a 3.8-fold increased risk to also have a prior diagnosis of peripheral neuropathy. The *ELOVL7* gene is a PD risk factor that also confers regional vulnerability, i.e., it is a Braak stage-related gene with an altered expression pattern in the brains of PD cases, with down regulated expression in endothelial cells and oligodendrocytes (48) (**Table 1**).

Additional associations are identified using the GENO-2DF model, which considers both additive and dominance effects. SNP rs2280194 in *BIN3* and rs10253857 in an intergenic region near *SNX13* are associated with a family history of dementia. SNP rs2074404 in *WNT3* is associated with a family history of stroke. SNP rs2694528 in *NDUFAF2*, which is near *ELOVL7*, is associated with the presence of neuropathy. SNPs rs8192591 in *NOTCH4* and rs1293298 in *CTSB* are associated with bradykinesia as an initial motor symptom. SNPs rs117615688

TABLE 1 | Associations of binomial traits with individual common PD-risk SNPs*.

Clinical feature	SNP	Nearest gene	Distance (kb)	MAF this study	Sex	Model	unadjusted *p*-value	Bonferroni corrected *p*-value	OR	95% CI
Family history of dementia	rs2280104	*BIN3*	0	33.0	Males	GENO-2DF	1.64×10^{-4}	0.0186	NA	
$n = 1$	rs10253857	*SNX13*	116.9	23.6	Both	GENO-2DF	3.55×10^{-4}	0.0415	NA	
Family history of dementia	rs429358	*APOE*	0	12.4	Females	Additive	7.36×10^{-5}	0.00375	8.92	3.02–26.3
$n \geq 2$					Females	GENO-2DF	3.86×10^{-4}	0.0197	NA	
	APOE ε4 dose			11.1	Females	Additive	7.26×10^{-5}	0.00370	7.42	2.72–20.3
					Females	GENO-2DF	4.75×10^{-4}	0.0242	NA	
Family history of dementia	rs2280104	*BIN3*	0	33.0	Males	Additive	2.91×10^{-4}	0.0340	1.76	1.30–2.39
$n \geq 1$					Males	GENO-2DF	8.95×10^{-5}	0.0105	NA	
Family history of stroke $n = 1$	rs2074404	*WNT3*	0	4.07	Males	GENO-2DF	1.28×10^{-6}	1.42×10^{-4}	NA	
Presence of neuropathy	rs1867598	*ELOVL7*	0	13.5	Both	Additive	3.02×10^{-5}	0.00296	3.8	2.04–7.18
					Both	GENO-2DF	1.33×10^{-4}	0.0130	NA	
					Females	Additive	3.00×10^{-4}	0.0242	5.92	2.26–15.5
	rs2694528	*NDUFAF2*	0	13.4	Both	Additive	2.97×10^{-5}	0.00291	3.8	2.04–7.19
					Both	GENO-2DF	1.30×10^{-4}	0.0130	NA	
History of essential tremor	rs12528068	*RIMS1*	108.6	13.5	Males	Additive	4.51×10^{-4}	0.0491	2.06	1.38–3.09
Initial motor symptoms										
Bradykinesia – any	rs8192591	*NOTCH4*	0	3.18	Both	GENO-2DF	1.71×10^{-5}	0.00206	NA	
Bradykinesia – reduced dexterity	rs1293298	*CTSB*	0	26.8	Both	GENO-2DF	5.20×10^{-4}	0.0499	NA	
Bradykinesia – reduced arm swing	rs34311866	*TMEM175*	0.5	25.3	Both	Additive	1.82×10^{-4}	0.0160	2.31	1.50–3.57
Initial non-motor symptoms										
Depression	rs61169879	*BRIP1*	0	14.9	Males	Additive	2.84×10^{-4}	0.0304	2.60	1.55–4.37
Non-motor symptoms at baseline										
Hallucinations	rs55961674	*KPNA1*	0	19.9	Males	Additive	1.72×10^{-4}	0.0161	2.84	1.65–4.90
Insomnia	rs117615688	*CRHR1*	0	5.92	Females	GENO-2DF	1.87×10^{-4}	0.0179	NA	
Restless leg syndrome	rs382940	*SLC44A1*	0	6.79	Both	GENO-2DF	5.14×10^{-4}	0.0483	NA	
Orthostatism	rs34025766	*LCORL*	0	17.0	Males	Additive	2.14×10^{-4}	0.0218	2.91	1.65–5.12
Disease subtype										
Tremor-predominant subtype	rs9468199**	*LOC100507172*	3.2	17.4	Both	Additive	5.55×10^{-4}	0.0765	2.10	1.37–3.20

Covariates: sex, age-at-encounter, years-since-diagnosis, and for MMSE, years of education.

***Fails to sustain significance if years-from-diagnosis is included as a covariate.*

in *CRHR1* and rs382940 in *SLC44A1* are associated the non-motor symptoms of insomnia and restless leg syndrome (RLS), respectively (**Table 1**).

We also identified significant associations between common SNPs conferring risk of PD in GWAS and test scores that reflect an objective assessment of the PD patient (**Table 2**). The minor allele (T) at SNP rs12528068 in an intergenic region 108.6 kb from *RIMS1* that is associated with a history of essential tremor in males is also associated with increased dyskinesia scores in females. SNPs rs113343 and rs6497339 at *SYT17*, which encodes synaptotagmin-17, are associated with higher GDS scores. SNP rs12283611 at *DLG2*, which functions in the clustering of receptors, ion channels and associated signaling proteins, is associated with lower UPDRS-VI scores.

We included years-from-diagnosis as a covariate in the above analyses since our community-based cohort includes previously diagnosed patients. It is interesting that some results that trended toward significance survive Bonferonni correction if this measure of disease duration is not included as a covariate (**Supplementary Tables 4**, **5**). Individuals with the minor (A) allele at the SNP rs9468199 in an intergenic region 3.2 kilobases (kb) from *LOC1005071*, an uncharacterized non-coding RNA, are twice more likely to present with the tremor-predominant PD subtype and not the akinetic/rigid or mixed disease subtype. The minor (C) allele at SNP rs12813102 in *GPR19*, which encodes a proton-sensing G-protein coupled receptor abundant in skin and brain (49), has a relatively strong effect on higher H&Y stage (β ∼1.7 on standardized H&Y scores) in both sexes or just males.

In contrast, other SNPs have less strong effect sizes (β range 0.24–0.47 on standardized scores). The presence of the minor allele (C) at SNP rs823118 in *NUCKS1*, which is involved in homologous recombination DNA repair (50), is associated with higher MMSE baseline scores only in males. Its small effect is not unexpected given that early in the disease process, cognitive impairment is not prominent in typical PD. Finally, the minor allele (T) at SNP rs224750 located 167.5 kb from *PARD3* is associated with higher UPDRS-IVc scores only in females. *PARD3* is a gene involved in the regulation of cellular junction formation in ependymal cells, cilia, tumor suppression (49). It will be useful to evaluate these variants in longitudinal follow-up studies.

In summary, these results collectively demonstrate that some of the PD-risk SNPs identified in case-control GWAS are also associated with the differential presentation of PD and discrete phenotypic characteristics at baseline.

Gene-Level Association Analysis

We employed gene-level association tests (sequence kernel association tests) to evaluate whether the set of rare (MAF < 1%) or both rare and less common (MAF < 5%) variants present in the PD-associated genes of our cohorts also exert differential effects on baseline clinical features. Significant findings from these gene-level association tests in our cohorts are presented in **Table 3**. The following findings are notable: *LRRK2* is associated with a prior diagnosis of essential tremor (ET). *NUCKS1*, a gene that shows allele-specific gene expression in the human brain (51), is significantly associated with UPDRS-III motor scores and

TABLE 2 | Significant associations of test scores with common PD-risk SNPs*.

Clinical feature	SNP	Nearest gene	Distance to nearest gene (kb)	MAF this study	Sex	Model	Unadjusted *p*-value	Bonferroni corrected *p*-value	*β*	95% CI
GDS score	rs11343	*SYT17*	0	43.1	Males	GENO-2DF	1.35×10^{-4}	0.0166	NA	
					Both	GENO-2DF	7.17×10^{-5}	0.00910	NA	
	rs6497339	*SYT17*	0	43.8	Both	GENO-2DF	2.22×10^{-4}	0.0281	NA	
UPDRS IV- dyskinesia subscore	rs12528068	*RIMS1*	108.6	28.6	Females	Additive	5.25×10^{-5}	0.00635	0.254	0.132–0.375
						GENO-2DF	1.33×10^{-4}	0.0161	NA	
UPDRS VI-Schwab and England	rs12283611	*DLG2*	0	44.0	Males	GENO-2DF	1.98×10^{-4}	0.0243	NA	

*Covariates: sex, age-at-encounter, years-since-diagnosis, and for MMSE, years of education.

with UPDRS-V (H&Y stage). Of note, the PD-risk SNP rs823118 in the same gene was associated with higher MMSE scores in males when disease duration was not included as a covariate (**Supplementary Table 5**).

TOX3, a transcriptional co-activator (52) previously associated with periodic leg movements during sleep (53), and *SULT1C2*, a cytosolic sulfotransferase (54), are associated with the UPDRS-IV total score. *TRIM40*, a gene whose protein product may function as a E3 ubiquitin-protein ligase (55) and inhibit NF-kB activity, is associated with UPDRS-VI (Schwab & England score). *SNCA* and *FAM184A* are associated with dyskinesias at baseline encounter, and *SNCA* is also associated with cognitive impairment. *CHD9*, which encodes a transcriptional activator (56) and *GPNMB*, which encodes a transmembrane glycoprotein (57), are associated with the initial motor symptoms of micrographia (bradykinesia manifestation) and rigidity, respectively. *GPNMB,* demonstrating genome-wide significance, is also associated with bradykinesia at baseline, as are *THSD4*, which attenuates TGFβ signaling, and *MCCC1,* which is used in NFκB signaling (58). *STK39*, which encodes a protein kinase that may mediate stress-activated signals (51), is associated with RLS.

Certain comorbid conditions often seen in PD patients are associated with the different genes. *BIN3,* which encodes a protein involved in cytokinesis (59) is associated with anxiety disorder. *VAMP4* (60) is associated with sleep apnea. *PET117*, which encodes a mitochondrial protein homolog (61), is associated with traumatic brain injury.

Similar results are obtained when sequence kernel association tests are performed without including sex, age at encounter, disease duration and, for MMSE, years of education, as covariates (**Supplementary Table 6**). In these analyses, different measures of complications of levodopa therapy are associated with some of the genes described above: *TOX3* and *SULT1C2* are associated with the UPDRS-IVa-Dyskinesia subscore; *SULT1C2, MCCC1, TOX3,* and *BAG3* are associated with the UPDRS-IVb-Fluctuations subscore; and *STBD1* is associated with the UPDRS-IVc-Other subscore.

In summary, these results demonstrate that variants in PD-associated genes are differentially associated with the following phenotypic features: history of essential tremor, initial motor and non-motor symptoms, test scores, motor and non-motor symptoms at baseline study entry, family history of essential tremor and of dementia, and comorbidities including anxiety, sleep apnea, and traumatic brain injury (TBI). These associations raise the possibility of underlying links between PD, essential tremor, mood disorders, and TBI.

Protein-Protein Interaction Network Analysis

The protein products of the genes included in this analysis s are involved in many different cellular processes implicated in neurodegeneration. To assess whether the significant associations between SNPs/genes with baseline clinical parameters identified here reflect functional interactions between the genes, we entered all of the genes identified as having significant associations with a phenotypic feature (i.e., all genes listed in **Tables 1–3**, **Supplementary Tables 4–6**) into the Search Tool for the Retrieval of Interacting Genes (STRING) and evaluated their participation in protein-protein interaction (PPI) networks. The network shown in **Figure 1** was obtained using 0.4 as the minimum required interaction score (medium confidence) and allowed up to 20 second-shell interactions to reveal indirect interactions among these proteins. The network contains 69 nodes and 113 edges (*cf.* 46 expected) with an average node degree of 3.28 and an enrichment p-value of 1.10×10^{-16}. Sixteen nodes are unconnected to the protein-interaction network.

The top 30 Gene Ontology (GO) processes in which these genes and their interactors are implicated are shown in **Table 4**, with the genes having significant SNP or gene-level associations highlighted in bold. It is interesting to note that this analysis reveals three interaction patterns: one in which proteins encoded by genes such as *APOE, KPNA1, LRRK2, TMEM175, MCCC1, FAM49B,* and *SNCA* are members of closely interacting networks, a second one in which genes such as *PARD3* or *NUCKS1* are members of more remotely interacting networks, and a third one in which genes such as *ELOVL7, GPR19, LCORL, FAM184A,* and *BIN3* are not nodes in these protein-interaction networks.

Several genes occupy central nodes in the protein network: *APOE* occupies a central node in the protein network and in our analysis is associated with the family history of dementia. *APOE* is a well-established AD risk factor (62) with an important role in normal brain function (63) and the *APOE* e4 allele has been associated with cognitive decline in PD (10, 64, 65). *LRRK2* is also occupying a central node in the protein network: *LRRK2* has a dual role as a PD risk factor and a gene involved in PD pathogenesis (66, 67) and encodes a protein kinase involved in autophagy. *SNCA* also occupies a central node in the PPI and is a key player in PD pathogenesis (68).

Taken together with the results of the association analyses, these results are consistent with the hypothesis that genetic variation that affects the functioning of protein-protein interaction networks can contribute to the differential presentation of PD symptoms. In addition, it is important to note that a number of these genes are members of known networks and hubs, whereas others are not.

DISCUSSION

Here we present the results of association analyses of baseline clinical features in PD with genetic variants that have been shown to be significant in prior case-control GWAS to confer PD risk or have been identified as PD-associated genes in the MDSgene database. We analyzed discrete clinical phenotypic features and test scores in a two-pronged approach: in the first, we evaluated their association with individual common SNPs that have been demonstrated in case-control GWAS to confer PD-risk; in the second we used gene-level tests to evaluate the association of these phenotypic features with low frequency (1–5% MAF) and rare (<1% MAF) variants in both pathogenic PD genes and the genes conferring PD-risk identified by case-control GWAS. The rationale of this approach is based on the

TABLE 3 | Significant associations in gene-based sequence kernel association tests*.

Clinical feature	Analysis *N*	*N* with condition in this study (%)	Variant MAF criteria	Gene	Number of variants	Unadjusted *p* (permutation *p*)	Bonferroni corrected *p*
Clinical history							
Essential tremor	779	144 (18.3)	≤5%	*LRRK2*	37	3.93×10^{-5} (0)	0.00460
Test scores							
MMSE	783	-	≤1%	*FAM171A1*	15	2.99×10^{-4} (0.0102)	0.0317
UPDRS III	743	-	≤1%	*NUCKS1*	8	3.87×10^{-4} (0.0012)	0.0410
UPDRS -I	768	-	≤1%	*TMEM163*	19	3.33×10^{-4} (0.0012)	0.0353
UPDRS IV-total score	786	-	≤1%	*TOX3*	16	3.55×10^{-5} (0.0014)	0.00376
			≤1%	*SULT1C2*	6	3.59×10^{-4} (0.0036)	0.0381
UPDRS-V-H&Y stage	773	-	≤1%	*NUCKS1*	8	1.34×10^{-4} (0.0003)	0.0223
			≤5%	*NUCKS1*	11	1.90×10^{-4} (0.0002)	0.0142
UPDRS-VI-Schwab & England	773	-	≤1%	*TRIM40*	2	1.22×10^{-6} (0.0004)	0.000130
Dyskinesia at baseline: chorea	675	61 (7.7)	≤5%	*FAM184A*	60	3.55×10^{-4} (0.0005)	0.0416
Dyskinesia severity at baseline: severe	784	61 (7.7)	≤5%	*FAM184A*	60	1.71×10^{-4} (0.0012)	0.00201
Dyskinesia distribution at baseline: generalized	786	30 (3.8)	≤1%	*SNCA*	7	2.17×10^{-4} (0.0351)	0.0230
			≤5%	*FAM184A*	60	1.29×10^{-5} (0.0003)	0.00151
Initial motor symptoms							
Bradykinesia (reduced dexterity)	786	40 (5.1))	≤1%	*HLA-DQB1*	1	4.40×10^{-4} (0.0108)	0.0466
Bradykinesia (generalized)	786	65 (8.2)	≤1%	*SCAF11*	15	4.43×10^{-4} (0.0149)	0.0469
Bradykinesia (micrographia)	786	30 (3.8)	≤1%	*CHD9*	30	2.40×10^{-5} (0.0037)	0.00254
Postural tremor	786	140 (17.8)	≤1%	*FAM49B*	15	3.62×10^{-5} (0.0005)	0.00389
Rigidity	786	681 (86.4)	≤5%	*GPNMB*	20	2.26×10^{-4} (0.0082)	0.0265
Motor symptoms at baseline							
Bradykinesia	786	779 (98.9)	≤1%	*GPNMB*	17	1.18×10^{-8} (0.0072)	1.25×10^{-6}
			≤5%	*GPNMB*	20	5.48×10^{-5} (0.0135)	0.00641
			≤1%	*MCCC1*	14	6.61×10^{-5} (0.0180)	0.00700
			≤1%	*THSD4*	133	3.08×10^{-4} (0.0066)	0.0361
Initial non-motor symptom							
Depression	786	125 (15.9)	≤1%	*ITGA8*	26	2.47×10^{-4} (0.0017)	0.0262
Non-motor symptoms at baseline							
Cognitive impairment	786	106 (13.5)	≤5%	*SNCA*	19	2.90×10^{-5} (0.0001)	0.00391
Constipation	786	157 (19.9)	≤1%	*ITGA8*	26	1.5×10^{-5} (0.0001)	0.00444
Orthostatism	786	56 (7.1)	≤5%	*PET117*	4	1.74×10^{-4} (0.0217))	0.0238
UPDRS IV-Orthostasis	786	62 (7.9)	≤5%	*STBD1*	2	7.40×10^{-5} (0.0014)	0.0419
			≤1%	*STBD1*	2	7.40×10^{-5} (0.002)	0.00784
Hallucinations	786	49 (6.2)	≤5%	*LRRK2*	36	1.68×10^{-5} (0.0001)	0.0419
Dysphagia	786	47 (6.0)	≤1%	*FAM49B*	15	1.81×10^{-4} (0.0052)	0.0192
Anxiety	786	50 (6.3)	≤1%	*CATSPER3*	7	1.58×10^{-7} (0.0008)	1.68×10^{-5}
Unexplained weight loss	786	26 (3.3)	≤1%	*SQRDL*	6	1.48×10^{-4} (0.0091)	0.01566
				RIMS1	72	4.68×10^{-5} (0.0027)	0.0496
Restless leg syndrome	786	31 (3.9)	≤1%	*STK39*	20	2.24×10^{-5} (0.0021)	0.00238
Excess daytime sleepiness	786	60 (7.6)	≤1%	*ITGA8*	26	1.83×10^{-4} (0.0028)	0.0194
Family history							
Dementia (1 family member)	786	160 (20.3)	≤1%	*SLC44A1*	16	1.48×10^{-4} (0.0009)	0.0156
Dementia (≥ 2 family members)	786	24 (3.0)	≤5%	*ASXL3*	27	1.50×10^{-5} (0.0044)	0.00176
				LAMB2	5	4.19×10^{-4} (0.0105)	0.0490
Dementia (≥ 1 family member)	786	184 (23.4)	≤1%	*ASXL3*	27	5.00×10^{-5} (0.0003)	0.00530
				SLC44A1	16	4.47×10^{-4} (0.0006)	0.0474
Tremor (≥ 2 family members)	786	31 (3.9)	≤1%	*GAK*	20	8.70×10^{-5} (0.0067)	0.00922
Tremor (≥ 1 family member)	786	121 (15.4)	≤1%	*BIN3*	10	1.35×10^{-4} (0.0003)	0.014
Comorbidities							
Anxiety disorder	786	33 (4.2)	≤1%	*BIN3*	10	3.12×10^{-4} (0.0057)	0.033
Sleep apnea	786	68 (8.6)	≤1%	*VAMP4*	4	3.01×10^{-4} (0.0041)	0.0319
Traumatic brain injury	786	41 (5.2)	≤5%	PET117	4	6.87×10^{-7} (0.0109)	8.03×10^{-5}

**Covariates: sex, age-at-encounter, years-since-diagnosis, and for MMSE, years of education.*

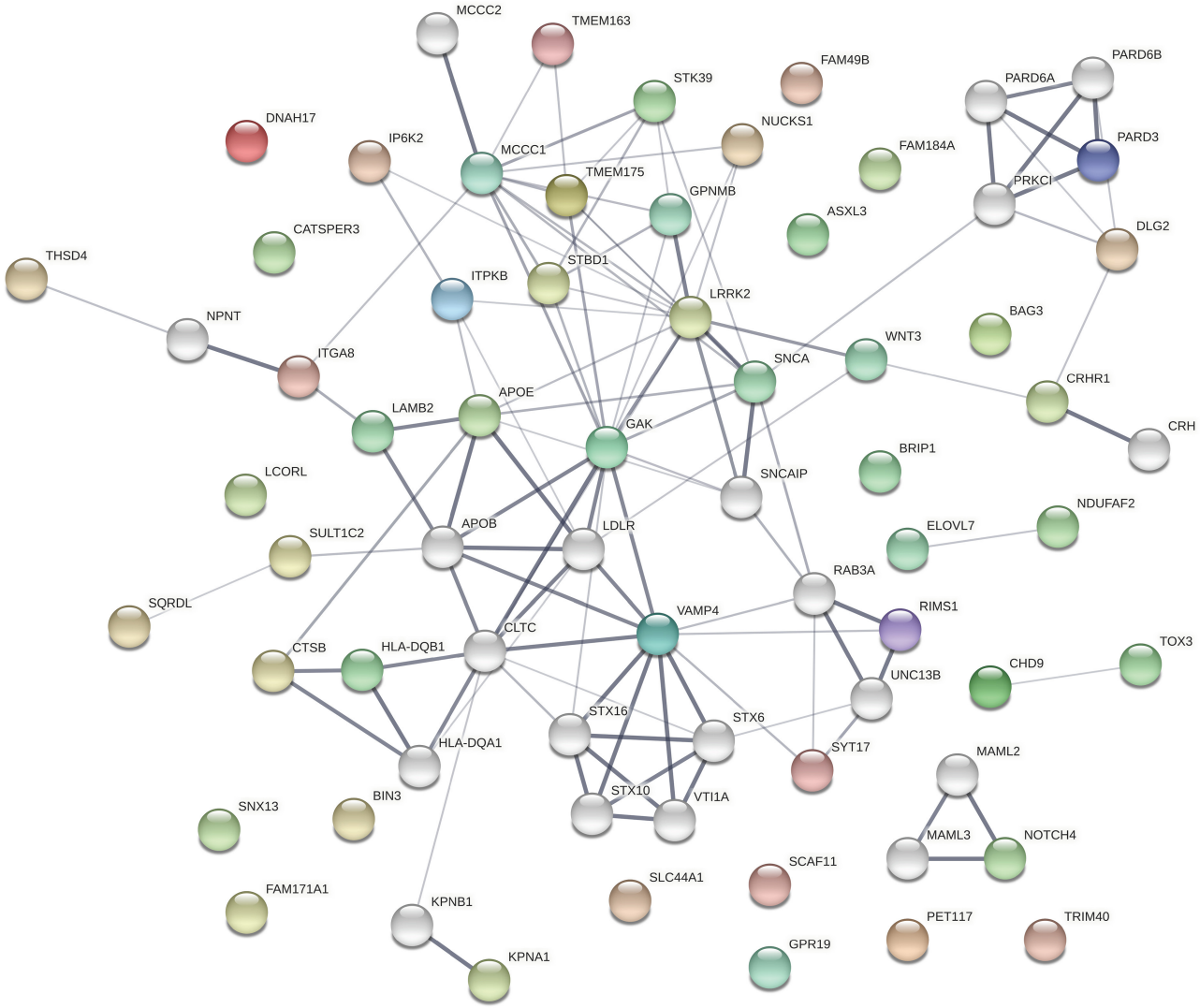

FIGURE 1 | The network shown was obtained using 0.4 as the minimum required interaction score (medium confidence). In the network, the thickness of the lines corresponds to the confidence of the interactions, the colored spheres represent the 49 proteins of the genes identified in the association analyses, and uncolored spheres represent up to 20 second-shell interactions that reveal indirect interactions among the proteins. The network contains 69 nodes and 113 edges (46 expected) with an average node degree of 3.28 and an enrichment *p*-value of 1.11×10^{-16}. Sixteen nodes are unconnected to the protein-interaction network.

hypothesis that individual discrete phenotypic characteristics may be differentially affected by the action of individual SNPs that tag a particular PD-risk haplotype, and/or multiple variants at a particular gene. Furthermore, the observed associations may reflect the effects of variants with different MAF. The alternative to this hypothesis is that the single SNPs and variants within a gene that confer PD-risk affect groups of clinical features or test scores more uniformly.

Our results support this hypothesis: individual common SNPs conferring PD risk are associated with phenotypic traits mostly in a non-overlapping manner, and gene-level tests reveal associations with individual clinical features and test scores that are often differentially affected, though at times have overlapping effects. This raises the intriguing possibility that individual phenotypic characteristics of a neurodegenerative disease such as PD that are associated with a specific gene may be related with the same phenotypic characteristic in a different neurodegenerative disease/syndrome. This may allow for the development of a "polyphenic" risk score to complement polygenic composite risk scores that already have been developed for Alzheimer's disease and other diseases (69).

It is interesting to point out certain associations that may hint to pathogenetic links between PD and other disorders. The relationship between PD and ET has long been a matter of debate (70). In our cohort, gene-based tests reveal an association between LRRK2 and history of essential tremor. This finding suggests that genetic variation at *LRRK2* may provide a link between long-standing ET and the development of PD at least

TABLE 4 | Top gene ontology enrichment processes in the PPI.

Gene ontology term description	Observed gene count	Background gene count	Strength	False discovery rate	Matching proteins in network
Vesicle fusion	7	95	1.32	8.84×10^{-5}	**VAMP4**, STX6, **TMEM175**, **SYT17**, STX16, VTI1A, STX10
Cellular component organization	38	5163	0.32	8.84×10^{-5}	PARD6A, RAB3A, **WNT3**, APOB, **VAMP4**, **APOE**, STX6, **BRIP1**, **TMEM175**, **MCCC1**, **BIN3**, KPNB1, PRKCI, **NDUFAF2**, **LRRK2**, **GAK**, **SNCA**, MCCC2, **KPNA1**, **THSD4**, **SYT17**, **NUCKS1**, **SCAF11**, STX16, PARD6B, **PARD3**, **NOTCH4**, **ITGA8**, UNC13B, VTI1A, **LAMB2**, NPNT, **PET117**, **RIMS1**, LDLR, **CHD9**, STX10, CLTC
Vesicle-mediated transport	22	1699	0.56	8.84×10^{-5}	RAB3A, APOB, **VAMP4**, **STBD1**, **APOE**, STX6, **TMEM175**, KPNB1, PRKCI, **LRRK2**, **GAK**, **SNCA**, **CTSB**, **SYT17**, STX16, UNC13B, VTI1A, **RIMS1**, **FAM49B**, LDLR, STX10, CLTC
Regulation of localization	26	2524	0.47	8.84×10^{-5}	PARD6A, RAB3A, **WNT3**, APOB, **APOE**, STX6, SNCAIP, CRH, **CATSPER3**, PRKCI, **NDUFAF2**, **LRRK2**, **SNCA**, **SYT17**, **STK39**, **BAG3**, PARD6B, **PARD3**, UNC13B, **GPNMB**, **TRIM40**, **CRHR1**, **RIMS1**, LDLR, STX10, CLTC
Intracellular transport	20	1390	0.61	8.84×10^{-5}	RAB3A, **STBD1**, **APOE**, STX6, KPNB1, PRKCI, **GAK**, **SNCA**, **KPNA1**, **SYT17**, STX16, **PARD3**, **DLG2**, UNC13B, VTI1A, **SNX13**, **RIMS1**, LDLR, STX10, CLTC
Regulation of neurotransmitter secretion	6	55	1.49	8.84×10^{-5}	RAB3A, SNCAIP, **LRRK2**, **SNCA**, UNC13B, **RIMS1**
Regulation of neurotransmitter transport	7	92	1.33	8.84×10^{-5}	RAB3A, SNCAIP, CRH, **LRRK2**, **SNCA**, UNC13B, **RIMS1**
Regulation of cellular localization	15	766	0.74	8.84×10^{-5}	PARD6A, RAB3A, STX6, SNCAIP, CRH, PRKCI, **NDUFAF2**, **LRRK2**, **SNCA**, **BAG3**, **PARD3**, UNC13B, **TRIM40**, **RIMS1**, CLTC
Membrane organization	15	729	0.77	8.84×10^{-5}	APOB, **VAMP4**, **APOE**, STX6, **TMEM175**, PRKCI, **GAK**, **SNCA**, **SYT17**, STX16, VTI1A, **RIMS1**, LDLR, STX10, CLTC
Membrane fusion	8	170	1.13	8.84×10^{-5}	**VAMP4**, STX6, **TMEM175**, **SYT17**, STX16, VTI1A, **RIMS1**, STX10
Golgi ribbon formation	4	11	2.01	8.84×10^{-5}	**VAMP4**, STX6, STX16, VTI1A
Regulation of synaptic vesicle cycle	5	46	1.49	0.00016	RAB3A, **LRRK2**, **SNCA**, UNC13B, **RIMS1**
Golgi organization	6	95	1.25	0.00022	**VAMP4**, STX6, **LRRK2**, **GAK**, STX16, VTI1A
Chemical synaptic transmission	10	402	0.85	0.00024	RAB3A, **APOE**, CRH, **LRRK2**, **SNCA**, **SYT17**, **DLG2**, UNC13B, **RIMS1**, **GPR19**
Vesicle organization	9	318	0.9	0.00024	RAB3A, **VAMP4**, STX6, **TMEM175**, PRKCI, **SYT17**, STX16, VTI1A, STX10
Synaptic vesicle cycle	6	100	1.23	0.00024	RAB3A, **GAK**, **SNCA**, **SYT17**, UNC13B, **RIMS1**
Regulation of synaptic vesicle exocytosis	4	24	1.67	0.00028	RAB3A, **LRRK2**, UNC13B, **RIMS1**
Cell-cell signaling	15	1073	0.6	0.00042	PARD6A, RAB3A, **WNT3**, **APOE**, CRH, **LRRK2**, **SNCA**, **SYT17**, **DLG2**, UNC13B, **GPNMB**, **CRHR1**, **RIMS1**, **GPR19**, CLTC
Regulation of synaptic vesicle transport	4	28	1.61	0.00044	RAB3A, **LRRK2**, UNC13B, **RIMS1**
Regulation of calcium ion-dependent exocytosis	5	66	1.33	0.00045	RAB3A, **LRRK2**, **SYT17**, UNC13B, **RIMS1**
Establishment of localization	32	4248	0.33	0.00045	RAB3A, APOB, **VAMP4**, **STBD1**, **APOE**, STX6, **TMEM175**, **CATSPER3**, KPNB1, PRKCI, **LRRK2**, **GAK**, **IP6K2**, **SNCA**, **KPNA1**, **CTSB**, **SYT17**, STX16, **SLC44A1**, **PARD3**, **DLG2**, **ITGA8**, UNC13B, VTI1A, **CRHR1**, NPNT, **SNX13**, **RIMS1**, **FAM49B**, LDLR, STX10, CLTC
Chylomicron remnant clearance	3	8	2.03	0.00057	APOB, **APOE**, LDLR
Bicellular tight junction assembly	4	32	1.55	0.0006	PARD6A, PRKCI, PARD6B, **PARD3**

(Continued)

TABLE 4 | Continued

Gene ontology term description	Observed gene count	Background gene count	Strength	False discovery rate	Matching proteins in network
Cytosolic transport	6	132	1.11	0.00065	STX6, **GAK**, STX16, VTI1A, STX10, CLTC
Protein localization	20	1966	0.46	0.00071	RAB3A, APOB, **APOE**, STX6, **BIN3**, KPNB1, PRKCI, **LRRK2**, **GAK**, **KPNA1**, STX16, **PARD3**, **DLG2**, **ITGA8**, VTI1A, NPNT, **SNX13**, **RIMS1**, STX10, CLTC
Regulation of neurotransmitter levels	8	295	0.89	0.00073	RAB3A, SNCAIP, **LRRK2**, **SNCA**, **SYT17**, **SLC44A1**, UNC13B, **RIMS1**
Cellular component assembly	22	2343	0.43	0.00073	PARD6A, APOB, **VAMP4**, **APOE**, **BRIP1**, **TMEM175**, **MCCC1**, **BIN3**, KPNB1, PRKCI, **NDUFAF2**, **LRRK2**, **GAK**, MCCC2, **THSD4**, **SCAF11**, PARD6B, **PARD3**, UNC13B, **PET117**, **RIMS1**, CLTC
Retrograde transport, endosome to Golgi	5	79	1.25	0.00073	STX6, STX16, VTI1A, STX10, CLTC
Establishment of protein localization	17	1467	0.52	0.00073	RAB3A, APOB, **APOE**, STX6, KPNB1, PRKCI, **KPNA1**, STX16, **PARD3**, **DLG2**, **ITGA8**, VTI1A, NPNT, **SNX13**, **RIMS1**, STX10, CLTC
Cellular localization	21	2180	0.44	0.0008	RAB3A, **STBD1**, **APOE**, STX6, KPNB1, PRKCI, **LRRK2**, **GAK**, **SNCA**, **KPNA1**, **SYT17**, STX16, **PARD3**, **DLG2**, UNC13B, VTI1A, **SNX13**, **RIMS1**, LDLR, STX10, CLTC

The protein products of genes with significant associations are highlighted in bold.

in some cohorts. The presence of neuropathy in our cohorts is associated with variants in the *ELOVL7* and *NDUFAF2* genes that are located in the same region on chromosome 5. Clinically, peripheral neuropathy has been reported in PD, however, its cause remains unclear, potentially reflecting medication adverse effects (71).

Another striking association in our cohort is that of *SNCA* with cognitive impairment. The role of common variants at *SNCA* as PD risk factors, as well as rare gene variants as pathogenic mutations has been clearly demonstrated over the last two decades. Our findings suggest that multiple, less common variants at SNCA, not necessarily pathogenic variants, may affect cognition in PD patients.

The reported prevalence and incidence estimates in PD show a 1.5:1 male to female ratio (72). Here we find that sex often differentially affects an association with a particular phenotypic trait, either in the form of a symptom or a test score: some of the associations are significant for males or females, whereas others in both sexes. This suggests that sex may have a differential effect on the phenotypic manifestation of genetic PD risk.

As would be expected from our current understanding of the genetic mechanisms underlying PD, protein-protein interaction network analysis demonstrates that about two-thirds of the genes with significant associations are members of previously identified networks. However, about a third of the genes appear unconnected to these networks. This raises the interesting possibility that as yet unidentified gene networks and connections may be implicated in phenotypic manifestations, in either a deleterious or protective role.

It is important to stress that the analyses presented here are based on patient-reported initial symptoms and symptoms at baseline encounter, as well as objective test scores determined at the baseline encounter. Longitudinal evaluation of this and other cohorts through a standardized assessment at annual intervals will enable the extension of this analysis to determine whether the impact of the genotypes on the clinical phenotype and test scores is among other factors dependent on disease subtype, severity and duration. It also will be informative to undertake additional analyses that cluster individual symptoms and analyze their associations with genetic risk factors.

One limitation to our study is the inclusion of both *de novo* and previously diagnosed patients. Therefore, our cohort is likely more heterogeneous than an exclusively *de novo* cohort such as the PPMI cohort. However, given that the study participation originates in a community-based cohort, it is likely more representative of the phenotypic spectrum that is typically observed in clinician practices. Furthermore, the PD diagnosis in our cohort according to published diagnostic criteria (21) is ascertained at the baseline visit and can also be reliably ascertained at annual intervals using the EMR-based SCDS, thus providing high clinical diagnostic accuracy. In addition, the use of SCDS allows for detailed and accurate clinical data collection in a routine clinical practice, thus more accurately reflecting the clinical course.

A second limitation of this study is that the sample size of our cohort limits its power to detect associations. While none of the associations with common PD-risk SNPs reach genome-wide significance ($\sim 5 \times 10^{-8}$) (**Tables 1**, **2**, **Supplementary Tables 4**, **5**), gene-level tests using rare variants identify four associations with baseline clinical features that approach or reach significance for the number of mapped genes (2.81×10^{-6}): *TOX3* and *SULT1C2* with UPDRS IV-total score, *GPNMB* with bradykinesia, *CATSPER3* (73) with anxiety (**Table 3**, **Supplementary Table 6**). It is important to point out in this context that the genes included in this analysis have

been previously clearly associated with PD-risk in case-control GWAS. Nevertheless, given the size of our cohort, it will be informative to evaluate the reproducibility of our findings in other cohorts.

In summary, our analysis shows that common SNPs conferring PD-risk, as well as low-frequency and rare variants in genes implicated in PD/parkinsonism are associated with distinct phenotypic characteristics at baseline presentation in our PD cohorts, supporting the hypothesis that the genetic background significantly affects disease presentation and raising the possibility that it also affects disease course and severity. The associations observed are often, but not always, dependent on sex. It is conceivable that this is related to the observed PD prevalence and incidence estimates that point to PD-risk differences based on sex. Finally, this analysis identifies different patterns in protein interaction networks that may underlie disease phenotype and pathogenesis. Longitudinal studies of this and other PD cohorts using this approach can provide insights on the impact of genetic risk factors on disease severity and progression, and enhance our understanding of the underlying pathogenetic mechanisms contributing to PD.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because this would jeopardize patient confidentiality. Additional information can be made available to qualified researchers after completing a material transfer agreement that maintains patient confidentiality with NorthShore University HealthSystem. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NorthsShore University HealthSystem Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KM designed the study and wrote the manuscript. BC performed data analysis and contributed to the writing. KM, DM, and RF designed clinical instruments used in the study. KM, DM, APP, BS, and NK provided the clinical assessment. AP, LG, and RV provided research assistance. JW, AE, and HY processed genomic and clinical data. KM, BC, RF, and DM edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.662278/full#supplementary-material

REFERENCES

1. Thenganatt MA, Jankovic J. Parkinson disease subtypes. *JAMA Neurol*. (2014) 71:499–504. doi: 10.1001/jamaneurol.2013.6233
2. Sauerbier A, Jenner P, Todorova A, Chaudhuri KR. Non motor subtypes and Parkinson's disease. *Parkinsonism Relat Disord*. (2016) 22(Suppl.1):S41–6. doi: 10.1016/j.parkreldis.2015.09.027
3. Simuni T, Caspell-Garcia C, Coffey CS, Weintraub D, Mollenhauer B, Lasch S, et al., Baseline prevalence and longitudinal evolution of non-motor symptoms in early Parkinson's disease: the PPMI cohort. Version 2. *J Neurol Neurosurg Psychiatry*. (2018) 89:78–88. doi: 10.1136/jnnp-2017-316213
4. Lawton M, Ben-Shlomo Y, May MT, Baig F, Barber TR, Klein JC, et al., Developing and validating Parkinson's disease subtypes and their motor and cognitive progression. *J Neurol Neurosurg Psychiatry*. (2018) 89:1279–87. doi: 10.1136/jnnp-2018-318337
5. Mollenhauer B, Zimmermann J, Sixel-Döring F, Focke NK, Wicke T, Ebentheuer J, et al., Baseline predictors for progression 4 years after Parkinson's disease diagnosis in the *de novo* Parkinson Cohort (DeNoPa). *Mov Disord*. (2019) 34:67–77. doi: 10.1002/mds.27492
6. Aleksovski D, Miljkovic D, Bravi D, Antonini A. Disease progression in Parkinson subtypes: the PPMI dataset. *Neurol Sci*. (2018) 39:1971–6. doi: 10.1007/s10072-018-3522-z
7. Latourelle JC, Beste MT, Hadzi TC, Miller RE, Oppenheim JN, Valko MP, et al. Large-scale identification of clinical and genetic predictors of motor progression in patients with newly diagnosed Parkinson's disease: a longitudinal cohort study and validation. *Lancet Neurol*. (2017) 16:908–16. doi: 10.1016/S1474-4422(17)30328-9
8. De Pablo-Fernandez E, Tur C, Revesz T, Lees AJ, Holton JL, Warner TT. Association of autonomic dysfunction with disease progression and survival in Parkinson disease. *JAMA Neurol*. (2017) 74:970–6. doi: 10.1001/jamaneurol.2017.1125
9. Inguanzo A, Sala-Llonch R, Segura B, Erostarbe H, Abos A, Campabadal A, et al. Hierarchical cluster analysis of multimodal imaging data identifies brain atrophy and cognitive patterns in Parkinson's disease. *Parkinsonism Relat Disord*. (2020) 82:16–23. doi: 10.1016/j.parkreldis.2020.11.010
10. Tan MMX, Lawton MA, Jabbari E, Reynolds RH, Iwaki H, Blauwendraat C, et al. Genome-wide association studies of cognitive and motor progression in Parkinson's disease. *Mov Disord*. (2020). 36:424–33. doi: 10.1002/mds.28342
11. Iwaki H, Blauwendraat C, Leonard HL, Kim JJ, Liu G, Maple-Grødem J, et al. Genome-wide association study of Parkinson's disease clinical biomarkers in 12 longitudinal patients' cohorts. *Mov Disord*. (2019) 34:1839–50. doi: 10.1002/mds.27845
12. Periñán MT, Macías-García D, Labrador-Espinosa MÁ, Jesús S, Buiza-Rueda D, Adarmes-Gómez AD, et al. Association of PICALM with cognitive impairment in Parkinson's disease. *Mov Disord*. (2020) 36:118–23. doi: 10.1002/mds.28283
13. Sidransky E, Lopez G. The link between the GBA gene and Parkinsonism. *Lancet Neurol*. (2012) 11:986–98. doi: 10.1016/S1474-4422(12)70190-4
14. Migdalska-Richards A, Schapira AH. The relationship between glucocerebrosidase mutations and Parkinson disease. *J Neurochem*. (2016) 139(Suppl.1):77–90. doi: 10.1111/jnc.13385
15. Blauwendraat C, Reed X, Krohn L, Heilbron K, Bandres-Ciga S, Tan M, et al. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. *Brain*. (2020) 143:234–48. doi: 10.1093/brain/awz350

16. Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. *Lancet Neurol*. (2017) 16:66–75. doi: 10.1016/S1474-4422(16)30328-3
17. Puschmann A, Brighina L, Markopoulou K, Aasly J, Chung SJ, Frigerio R, et al., Clinically meaningful parameters of progression and long-term outcome of Parkinson disease: an international consensus statement. *Parkinsonism Relat Disord*. (2015) 21:675–82. doi: 10.1016/j.parkreldis.2015.04.029
18. Andrews SJ, Fulton-Howard B, Goate A. Interpretation of risk loci from genome-wide association studies of Alzheimer's disease. *Lancet Neurol*. (2020) 19:326–35. doi: 10.1016/S1474-4422(19)30435-1
19. Lill CM, Mashychev A, Hartmann C, Lohmann K, Marras C, Lang AE, et al. Launching the movement disorders society genetic mutation database (MDSGene). *Mov Disord*. (2016) 31:607–9. doi: 10.1002/mds.26651
20. Maraganore DM, Frigerio R, Kazmi N, Meyers SL, Sefa M, Walters SA, et al. Quality improvement and practice-based research in neurology using the electronic medical record. *Neurol Clin Pract*. (2015) 5:419–29. doi: 10.1212/CPJ.0000000000000176
21. Bower JH, Maraganore DM, McDonnell SK, Rocca WA. Influence of strict, intermediate, and broad diagnostic criteria on the age- and sex-specific incidence of Parkinson's disease. *Mov Disord*. (2000) 15:819–25. doi: 10.1002/1531-8257(200009)15:5<819::AID-MDS1009>3.0.CO;2-P
22. Markopoulou K, Biernacka JM, Armasu SM, Anderson KJ, Ahlskog JE, Chase BA, et al. Does α-synuclein have a dual and opposing effect in preclinical vs. clinical Parkinson's disease? *Parkinsonism Relat Disord*. (2014) 20:584–9. doi: 10.1016/j.parkreldis.2014.02.021
23. Markopoulou K, Aasly J, Chung SJ, Dardiotis E, Wirdefeldt K, Premkumar AP, et al. Longitudinal monitoring of Parkinson's disease in different ethnic cohorts: the DodoNA and LONG-PD study. *Front Neurol*. (2020) 11:548. doi: 10.3389/fneur.2020.00548
24. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. (2005) 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x
25. Kokmen E, Naessens JM, Offord KP, A short test of mental status: description and preliminary results. *Mayo Clin Proc*. (1987) 62:281–8. doi: 10.1016/S0025-6196(12)61905-3
26. Roalf DR, Moberg PJ, Xie SX, Wolk DA, Moelter ST, Arnold SE. Comparative accuracies of two common screening instruments for classification of Alzheimer's disease, mild cognitive impairment, and healthy aging. *Alzheimer's Dement*. (2013) 9:529–37. doi: 10.1016/j.jalz.2012.10.001
27. Townley RA, Syrjanen JA, Botha H, Kremers WK, Aakre JA, Fields JA, et al. Comparison of the short test of mental status and the montreal cognitive assessment across the cognitive spectrum. *Mayo Clin Proc*. (2019) 94:1516–23. doi: 10.1016/j.mayocp.2019.01.043
28. Fahn S, Elton R, Members of the UPDRS Development Committee. The unified parkinson's disease rating scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, editors, *Recent Developments in Parkinson's Disease, Vol 2*. Florham Park, NJ: Macmillan Health Care Information (1987). p. 153–163, 293–304.
29. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*. (1991) 14:540–5. doi: 10.1093/sleep/14.6.540
30. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res*. (1983) 17:37–49. doi: 10.1016/0022-3956(82)90033-4
31. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. (2009) 5:e1000529. doi: 10.1371/journal.pgen.1000529
32. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a toolset for whole-genome association and population-based linkage analysis. *Am J Hum Genet*. (2007) 81:519795. doi: 10.1086/519795
33. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. (2015) 4:7. doi: 10.1186/s13742-015-0047-8
34. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol*. (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
35. Daida K, Nishioka K, Li Y, Yoshino H, Shimada T, Dougu N, et al. PLA2G6 variants associated with the number of affected alleles in Parkinson's disease in Japan. *Neurobiol Aging*. (2021) 97:147.e1–9. doi: 10.1016/j.neurobiolaging.2020.07.004
36. Oji Y, Hatano T, Ueno SI, Funayama M, Ishikawa KI, Okuzumi A, et al. Variants in saposin D domain of prosaposin gene linked to Parkinson's disease. *Brain*. (2020) 143:1190–205. doi: 10.1093/brain/awaa064
37. Foo JN, Tan LC, Irwan ID, Au WL, Low HQ, Prakash KM, et al. Genome-wide association study of Parkinson's disease in East Asians. *Hum Mol Genet*. (2017) 26:226–32. doi: 10.1093/hmg/ddw379
38. Iwaki H, Blauwendraat C, Leonard HL, Liu G, Maple-Grødem J, Corvol JC, et al. Genetic risk of Parkinson disease and progression: an analysis of 13 longitudinal cohorts. *Neurol Genet*. (2019) 5:e348. doi: 10.1212/NXG.0000000000000354
39. Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. *Lancet Neurol*. (2020) 19:170–8. doi: 10.1016/S1474-4422(19)30287-X
40. Cherian A, Divya KP. Genetics of Parkinson's disease. *Acta Neurol Belg*. (2020) 120:1297–305. doi: 10.1007/s13760-020-01473-5
41. Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet*. (2011) 89:82–93. doi: 10.1016/j.ajhg.2011.05.029
42. Zhan X, Hu Y, Li B, Abecasis GR, Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. *Bioinformatics*. (2016) 32:1423–6. doi: 10.1093/bioinformatics/btw079
43. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucl Acids Res*. (2019) 47:D607–13. doi: 10.1093/nar/gky1131
44. Zhang M, Lu H, Xie X, Shen H, Li X, Zhang Y, et al. TMEM175 mediates Lysosomal function and participates in neuronal injury induced by cerebral ischemia-reperfusion. *Mol Brain*. (2020) 13:113. doi: 10.1186/s13041-020-00651-z
45. Fielhaber JA, Tan J, Joung KB, Attias O, Huegel S, Bader M, et al. Regulation of karyopherin α1 and nuclear import by mammalian target of rapamycin. *J Biol Chem*. (2012) 287:14325–35. doi: 10.1074/jbc.M111.246785
46. Li X, Yu W, Qian X, Xia Y, Zheng Y, Lee JH, et al. Nucleus-translocated ACSS2 promotes gene transcription for lysosomal biogenesis and autophagy. *Mol Cell*. (2017) 66:684–97.e9. doi: 10.1016/j.molcel.2017.04.026
47. Kupferschmidt DA, Augustin SM, Johnson KA, Lovinger DM. Active zone proteins RIM1αβ are required for normal corticostriatal transmission and action control. *J Neurosci*. (2019) 39:1457–70. doi: 10.1523/JNEUROSCI.1940-18.2018
48. Keo A, Mahfouz A, Ingrassia AMT, Meneboo JP, Villenet C, Mutez E, et al. Transcriptomic signatures of brain regional vulnerability to Parkinson's disease. *Commun Biol*. (2020) 3:101. doi: 10.1038/s42003-020-0804-9
49. Ear J, Saklecha A, Rajapakse N, Choi J, Ghassemian M, Kufareva I, et al. Tyrosine-based signals regulate the assembly of daple·PARD3 complex at cell-cell junctions. *iScience*. (2020) 23:100859. doi: 10.1016/j.isci.2020.100859
50. Maranon DG, Sharma N, Huang Y, Selemenakis P, Wang M, Altina N, et al. NUCKS1 promotes RAD54 activity in homologous recombination DNA repair. *J Cell Biol*. (2020) 219:e201911049. doi: 10.1083/jcb.201911049
51. Langmyhr M, Henriksen SP, Cappelletti C, van de Berg WDJ, Pihlstrøm L, Toft M. Allele-specific expression of Parkinson's disease susceptibility genes in human brain. *Sci Rep*. (2021) 11:504. doi: 10.1038/s41598-020-79990-9
52. O'Flaherty E, Kaye J. TOX defines a conserved subfamily of HMG-box proteins. *BMC Genom*. (2003) 4:13. doi: 10.1186/1471-2164-4-13
53. Moore H, Winkelmann J, Lin L, Finn L, Peppard P, Mignot E. Periodic leg movements during sleep are associated with polymorphisms in BTBD9, TOX3/BC034767, MEIS1, MAP2K5/SKOR1, and PTPRD. *Sleep*. (2014) 37:1535–42. doi: 10.5665/sleep.4006
54. Freimuth RR, Raftogianis RB, Wood TC, Moon E, Kim UJ, Xu J, et al. Human sulfotransferases SULT1C1 and SULT1C2: cDNA characterization, gene cloning, and chromosomal localization. *Genomics*. (2000) 65:157–65. doi: 10.1006/geno.2000.6150
55. Zhao C, Jia M, Song H, Yu Z, Wang W, Li Q, et al. The E3 ubiquitin ligase TRIM40 attenuates antiviral immune responses by targeting MDA5 and RIG-I. *Cell Rep*. (2017) 21:1613–23. doi: 10.1016/j.celrep.2017.10.020

56. Schlachetzki JCM, Prots I, Tao J, Chun HB, Saijo K, Gosselin D, et al. A monocyte gene expression signature in the early clinical course of Parkinson's disease. *Sci Rep*. (2018) 8:10757. doi: 10.1038/s41598-018-28986-7
57. de Dieuleveult M, Yen K, Hmitou I, Depaux A, Boussouar F, Bou Dargham D, et al. Genome-wide nucleosome specificity and function of chromatin remodellers in ES cells. *Nature*. (2016) 530:113–6. doi: 10.1038/nature16505
58. Cao Z, Xia Z, Zhou Y, Yang X, Hao H, Peng N, et al. Methylcrotonoyl-CoA carboxylase 1 potentiates RLR-induced NF-κB signaling by targeting MAVS complex. *Sci Rep*. (2016) 6:33557. doi: 10.1038/srep33557
59. Cosgrove MS, Ding Y, Rennie WA, Lane MJ, Hanes SD. The Bin3 RNA methyltransferase targets 7SK RNA to control transcription and translation. *Wiley Interdiscip Rev RNA*. (2012) 3:633–47. doi: 10.1002/wrna.1123
60. Brown EE, Blauwendraat C, Trinh J, Rizig M, Nalls MA, Leveille E, et al. Analysis of DNM3 and VAMP4 as genetic modifiers of LRRK2 Parkinson's disease. *Neurobiol Aging*. (2021) 97:148.e17–24. doi: 10.1016/j.neurobiolaging.2020.07.002
61. Taylor NG, Swenson S, Harris NJ, Germany EM, Fox JL, Khalimonchuk O. The assembly factor Pet117 couples heme a synthase activity to cytochrome oxidase assembly. *J Biol Chem*. (2017) 292:1815–25. doi: 10.1074/jbc.M116.766980
62. Rodriguez-Vieitez E, Nielsen HM. Associations between APOE variants, tau and α-synuclein. *Adv Exp Med Biol*. (2019) 1184:177–86. doi: 10.1007/978-981-32-9358-8_15
63. Flowers SA, Rebeck GW. APOE in the normal brain. *Neurobiol Dis*. (2020) 136:104724. doi: 10.1016/j.nbd.2019.104724
64. Shahid M, Kim J, Leaver K, Hendershott T, Zhu D, Cholerton B, et al. An increased rate of longitudinal cognitive decline is observed in Parkinson's disease patients with low CSF Aß42 and an APOE ε4 allele. *Neurobiol Dis*. (2019) 127:278–86. doi: 10.1016/j.nbd.2019.02.023
65. Paslawski W, Zareba-Paslawska J, Zhang X, Hölzl K, Wadensten H, Shariatgorji M, et al. α-synuclein-lipoprotein interactions and elevated ApoE level in cerebrospinal fluid from Parkinson's disease patients. *Proc Natl Acad Sci USA*. (2019) 116:15226–35. doi: 10.1073/pnas.1821409116
66. Senkevich K, Gan-Or Z. Autophagy lysosomal pathway dysfunction in Parkinson's dise evidence from human genetics. *Parkinsonism Relat Disord*. (2020) 73:60–71. doi: 10.1016/j.parkreldis.2019.11.015
67. Jeong GR, Lee BD. Pathological functions of LRRK2 in Parkinson's disease. *Cells*. (2020) 9:2565. doi: 10.3390/cells9122565
68. Erskine D, Koss D, Korolchuk VI, Outeiro TF, Attems J, McKeith I. Lipids, lysosomes and mitochondria: insights into Lewy body formation from rare monogenic disorders. *Acta Neuropathol*. (2021) 141:511–526. doi: 10.1007/s00401-021-02266-7
69. Chasioti D, Yan J, Nho K, Saykin AJ. Progress in polygenic composite scores in Alzheimer's and other complex diseases. *Trends Genet*. (2019) 35:371–82. doi: 10.1016/j.tig.2019.02.005
70. Wang XX, Feng Y, Li X, Zhu XY, Truong D, Ondo WG, et al. Prodromal markers of Parkinson's disease in patients with essential tremor. *Front Neurol*. (2020) 11:874. doi: 10.3389/fneur.2020.00874
71. Zis P, Grünewald RA, Chaudhuri RK, Hadjivassiliou M. Peripheral neuropathy in idiopathic Parkinson's disease: a systematic review. *J Neurol Sci*. (2017) 378:204–9. doi: 10.1016/j.jns.2017.05.023
72. Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol*. (2011) 26(Suppl.1):S1–58. doi: 10.1007/s10654-011-9581-6
73. Qi H, Moran MM, Navarro B, Chong JA, Krapivinsky G, Krapivinsky L, et al. All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. *Proc Natl Acad Sci USA*. (2007) 104:1219–23. doi: 10.1073/pnas.0610286104

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REVIEW
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Targeting of Lysosomal Pathway Genes for Parkinson's Disease Modification: Insights From Cellular and Animal Models

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Previous genetic studies on hereditary Parkinson's disease (PD) have identified a set of pathogenic gene mutations that have strong impacts on the pathogenicity of PD. In addition, genome-wide association studies (GWAS) targeted to sporadic PD have nominated an increasing number of genetic variants that influence PD susceptibility. Although the clinical and pathological characteristics in hereditary PD are not identical to those in sporadic PD, α-synuclein, and LRRK2 are definitely associated with both types of PD, with *LRRK2* mutations being the most frequent cause of autosomal-dominant PD. On the other hand, a significant portion of risk genes identified from GWAS have been associated with lysosomal functions, pointing to a critical role of lysosomes in PD pathogenesis. Experimental studies have suggested that the maintenance or upregulation of lysosomal activity may protect against neuronal dysfunction or degeneration. Here we focus on the roles of representative PD gene products that are implicated in lysosomal pathway, namely LRRK2, VPS35, ATP13A2, and glucocerebrosidase, and provide an overview of their disease-associated functions as well as their cooperative actions in the pathogenesis of PD, based on the evidence from cellular and animal models. We also discuss future perspectives of targeting lysosomal activation as a possible strategy to treat neurodegeneration.

Keywords: lysosome, α-synuclein, LRRK2, VPS35, ATP13A2, GBA

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, affecting about 10 million people worldwide. PD is clinically characterized by bradykinesia, tremor, rigidity, and postural instability as well as olfactory abnormalities and sleep disturbances. The motor symptoms of PD are mainly attributable to the selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), causing dopamine deficiency (1). An important pathological hallmark in PD lesions is the intraneuronal inclusions called Lewy bodies that consist of aggregated α-synuclein phosphorylated at Ser129 residue (2–4). It is widely accepted that α-synuclein aggregates or oligomeric species spread to interconnected brain regions in a prion-like manner, although the processes are not fully understood (5).

Although the majority of PD cases (∼90%) are sporadic, some forms of PD are hereditary and the responsible genes have been identified. *SNCA* encoding α-synuclein was the first gene identified, and the mutations in other genes such as leucine-rich repeat kinase 2 (*LRRK2*) and vacuolar protein

sorting-associated protein 35 (*VPS35*) are also established as the cause for autosomal-dominant PD. On the other hand, genes associated with autosomal-recessive PD include *PRKN*, *PINK1,* and *ATP13A2* (6). Importantly, accumulating evidence has pointed to a greater contribution of genetic determinants in sporadic PD (7, 8). Especially, past meta-analyses of genome-wide association studies (GWAS) targeting sporadic PD have repeatedly identified two of the above familial PD genes—*LRRK2* and *SNCA*—as major risk factors, indicating that the impact of these two genes is more common in the general population (9–11). These GWAS for sporadic PD have succeeded in nominating a number of additional genes that were not identified from linkage analyses of familial PD cases, and *GBA1* in particular is the most representative of such genes.

Importantly, a significant proportion of PD-associated genes (*e.g., LRRK2*, *GBA1*, *ATP13A2*, *VPS35,* and *TMEM175*) have been functionally implicated in the endolysosomal system in cells (12–16). Especially, *GBA1* is well-known as a responsible gene for Gaucher disease, the most common lysosomal storage disorder. Moreover, the recent expansion of genetic, transcriptomic, and epigenetic studies in sporadic PD has nominated an increasing number of lysosomal pathway genes as a risk factor for PD (17–19). Endolysosomal dysfunctions are also frequently described in other neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), all of which accompany neuronal accumulation of misfolded proteins (20, 21).

In addition to the evidence from genetics, the involvement of lysosomal dysfunction in PD has been implicated from pathological and biochemical studies using postmortem disease samples. The reduction in the immunoreactivity of lysosomal markers, such as LAMP1 and cathepsin D, was detected in PD and Lewy body disease (22, 23), and lysosomal breakdown, autophagosomal accumulation and the colocalization of autophagosomal markers with Lewy bodies were also detected in PD brains (24). Cathepsin D immunoreactivity has been shown to colocalize with α-synuclein pre-aggregates in nigral neurons in PD (25). The levels of lysosomal enzymes have been reported to be altered in cerebrospinal fluid and blood samples from PD patients (26–28). Thus, the role of lysosomes in PD pathogenesis is receiving increasing attention.

However, the detailed mechanisms on how lysosomal dysfunction leads to the neurodegeneration in PD remain largely elusive. There is a wide range of functions of PD-causative genes that are related to lysosomes, and much research has been focused on the elucidation of disease-related functions as well as the relationship among these genes. A common mechanism assumed by many researchers is that lysosomal dysfunction ultimately leads to α-synuclein accumulation and propagation in neurons. In fact, the role of lysosomes in α-synuclein degradation has long been attracted attention, and many studies on PD genes have also examined their effects on α-synuclein intracellular dynamics (i.e., metabolism, aggregation, secretion, and internalization).

In this article, we first summarize the current knowledge about the mechanisms of α-synuclein degradation in lysosomes, and then focus on the roles of other well-analyzed PD gene products, namely LRRK2, VPS35, ATP13A2 and GBA, in terms of their individual and co-operative regulations of endolysosomes and α-synuclein dynamics. Finally, we will discuss the potential of targeting endolysosomal system, especially the strategies to enhance lysosomal activity, in the future treatment of PD.

α-SYNUCLEIN: THE CENTRAL EFFECTOR DEGRADED IN LYSOSOMES

Missense mutation in *SNCA* gene encoding α-synuclein was first identified in 1997 as a cause of autosomal-dominant PD (29). Later on, more mutations in *SNCA* gene have been identified to date, including A53T, A30P, E46K, H50Q, G51D, and A53E (29–34). Furthermore, gene triplication and duplication of *SNCA* locus without missense mutations have also been reported as a cause of familial PD (35–37). This means that the increase of α-synuclein level by itself is sufficient to develop PD, and therefore proper clearance of α-synuclein is required for the prevention of disease onset. Multiple lines of evidence have suggested that α-synuclein is degraded in two major proteolytic pathways: the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) (38, 39). The metabolism in ALP has been the focus of much attention, especially in relation to the clearance of aggregated α-synuclein species.

Previous studies have shown that both extracellular and intracellular α-synuclein species are transported into lysosomes via the endosomal system or autophagy (40). It has been reported that α-synuclein is mainly degraded by cathepsins, especially cathepsin D, in lysosomes (41, 42). Cathepsin D level is shown to influence α-synuclein aggregation and toxicity *in vivo* (43). Treatment of cells with a lysosomal inhibitor bafilomycin A_1 has been reported to not only affect α-synuclein metabolism but also to promote its propagation (44, 45).

Conversely, it has also been shown that the aggregated α-synuclein itself inhibits the function of lysosomes as well as other organelles. For example, α-synuclein pre-formed fibrils (PFFs) act on lysosomal membranes and cause its rupture (46–48). Another study has reported that α-synuclein impedes the lysosomal stress response mediated by the SNARE protein ykt6 (49). ykt6 is known as a regulator of ER-Golgi trafficking that is also reported to be disrupted by accumulated α-synuclein (50, 51), suggesting the possibility that the effect of α-synuclein on lysosomes is not necessarily direct. Collectively, it is assumed that lysosome inhibition exacerbates α-synuclein toxicity and α-synuclein accumulation in turn inhibits lysosomes, forming a vicious cycle that leads to the development of the disease.

Autophagy has also been established as a key mechanism regulating α-synuclein metabolism and toxicity. Macroautophagy is a major autophagy machinery that processes the degradation of a large portion of the cytoplasmic components through the formation of double-membrane structures called autophagosomes. The autophagosomes fuse with primary lysosomes to form autolysosomes where their contents are degraded and then either disposed or recycled back to the cell (52, 53). Inhibition of autophagosome-lysosome fusion by treatment with bafilomycin A_1 or chloroquine enhanced

α-synuclein release and transfer in human neuroglioma cells and rat primary cortical neurons (54, 55). In a mouse model of PD expressing human α-synuclein, impairment of macroautophagy under DA neuron-specifc knockout of *Atg7* gene caused the aggravation of neuropathology, although the behavior of mice was paradoxically improved (56). In humans, it has been reported that the majority of Lewy bodies (∼80%) composed of α-syuclein in the SNpc of PD patients were strongly immonoreactive for LC3 (24), and similar observation for LC3 immunoreactivity was observed in Lewy bodies of dementia with Lewy bodies (DLB) patients (57). These reports collectively implicate the impaired macroautophagy in the pathogenetic processes involving α-synuclein, although we should note that there is little direct evidence of α-synuclein degradation by macroautophagy.

On the other hand, another type of autophagy called chaperone-mediated autophagy (CMA) has been considered as a possible mechanism of PD (58). CMA mediates the lysosomal degradation of a specific subset of soluble cytosolic proteins containing a KFERQ-like motif, which can be recognized by the cytosolic chaperone heat shock cognate protein 70 (Hsc70). Proteins targeted by Hsc70 are directly transported into the lysosomes for degradation through association with lysosome-associated membrane protein 2A (LAMP2A). It has been shown that wild-type α-synuclein can be degraded in CMA whereas mutant α-synuclein interferes with the lysosomal transport process in CMA, suggesting a possible link between defective CMA activity and PD (59).

Accumulating evidence has suggested that these ALP machineries may be modified by several PD-associated gene products, including LRRK2, VPS35, ATP13A2, and GBA. In the following sections, we will discuss the possible roles of these proteins in ALP and α-synuclein metabolism, focusing on the pathological relevance in PD (**Figure 1**).

LRRK2: A MULTIFACETED KINASE IN THE ENDOLYSOSOMAL SYSTEM

Mutations in *LRRK2* gene have been identified as the most common cause of autosomal-dominant PD (60, 61). LRRK2 is a large ∼280 kDa protein and is widely expressed in human tissues including brains, although the expression is higher in the kidney, lung and immune cells (61–64). LRRK2 protein consists of multiple enzymatic and protein interaction domains including armadillo repeats (ARM), ankyrin repeats (ANK), leucine-rich repeats (LRR), Ras of complex (Roc), C-terminal of Roc (COR), kinase, and WD40 domains (61, 65, 66), suggesting that LRRK2 has diverse binding partners in distinct cellular pathways. LRRK2 has an ability to bind GTP through its ROC domain, and PD-associated mutations in LRRK2 have been shown to cause alterations in GTP binding and/or GTPase activity (64, 67, 68). A number of mutations in *LRRK2* gene have been reported so far (69), and the following mutations are well-validated: N1437H, R1441C/G/H, Y1699C, G2019S, and I2020T. These mutations are located either in the ROC domain (N1437H, R1441C/G/H), COR domain (Y1699C) or kinase domain (G2019S, I2020T). Among these, G2019S is the most prevalent, followed by R1441C/G/H (60, 61, 69–72). It has been shown that G2019S mutation in LRRK2 increases its intrinsic kinase activity (73), whereas ROC/COR domain mutants affect GTPase activity or GTP binding (64, 68). These findings implicate the important roles of both GTPase/GTP binding and kinase activities of LRRK2 in PD pathomechanisms. Recent structural analyses of LRRK2 on microtubules using cryo-electron tomography/microscopy have shown that the kinase and GTPase domains are in close proximity (74, 75), suggesting that the activities of both domains are not independent but influence each other.

Recent studies have accumulated evidence that LRRK2 phosphorylates a subset of Rab family GTPases, including Rab3, Rab8, Rab10, Rab29, and Rab35, in their switch-II regions (76–80). Rab GTPases are the major regulators of intracellular membrane trafficking (81). It has been shown that LRRK2 and its substrate Rab GTPases, especially Rab8 and Rab10, are readily recruited onto lysosomes that are stressed by lysosomal overload (82, 83) or by membrane damage (84, 85). Under lysosomal overload stress, LRRK2 and Rab8 act against lysosomal hypertrophy, whereas LRRK2 and Rab10 facilitate the release of lysosomal contents. Under lysosomal membrane-damaging stress, LRRK2 recruits the ESCRT-III component CHMP4B via Rab8a (85) or the motor adaptor protein JIP4 via Rab10/Rab35 (84) to damaged lysosomes for membrane repair. The lysosomal recruitment of LRRK2 is further regulated by Rab29 (also known as Rab7L1), another interactor and substrate of LRRK2 (82, 83). Studies in *C. elegans* neurons have suggested that the orthologs of LRRK2 and Rab29 both regulate axon termination, and double mutant analysis has revealed their functions in a same genetic pathway that involves the clathrin adaptor protein complex 3 (AP-3), an important regulator of Golgi-lysosome transport of lysosomal membrane proteins (86).

A variety of studies have also reported the relationship between LRRK2 and autophagy. Studies of *Lrrk2* KO mice have demonstrated the altered autophagic markers such as the autophagosome marker LC3-II and the autophagy substrate p62 (87, 88). The levels of these autophagic markers were changed in age-dependent and bi-phasic manners; LC3-II level was increased at 7 months of age but decreased at 20 months in *Lrrk2* KO mice, whereas p62 was decreased at 7 months and increased at 20 months (87). *In vitro* studies have shown that the knockdown of LRRK2 in neuroblastoma SH-SY5Y cells caused a marked increase in LC3-II and p62 levels (89). In contrast, another study has shown that the knockdown of endogenous LRRK2 in macrophage or microglial cells decreased LC3-II levels and autophagy flux (90). Thus, although these changes in the levels of autophagic markers indicate the important role of LRRK2 in the proper regulation of autophagic flux, the effects of LRRK2 on autophagy depend on the conditions such as cell type and experimental methodology, and the mechanism of how LRRK2 regulates autophagy still remains unclear.

As for the relationship between LRRK2 and CMA, it has been reported that LRRK2-G2019S inhibits CMA by affecting LAMP2A-mediated internalization of the substrate proteins like α-synuclein into lysosomes, which results in α-synuclein accumulation in neurons (91). Consistently, a significant reduction in CMA or lysosomal markers such as LAMP1,

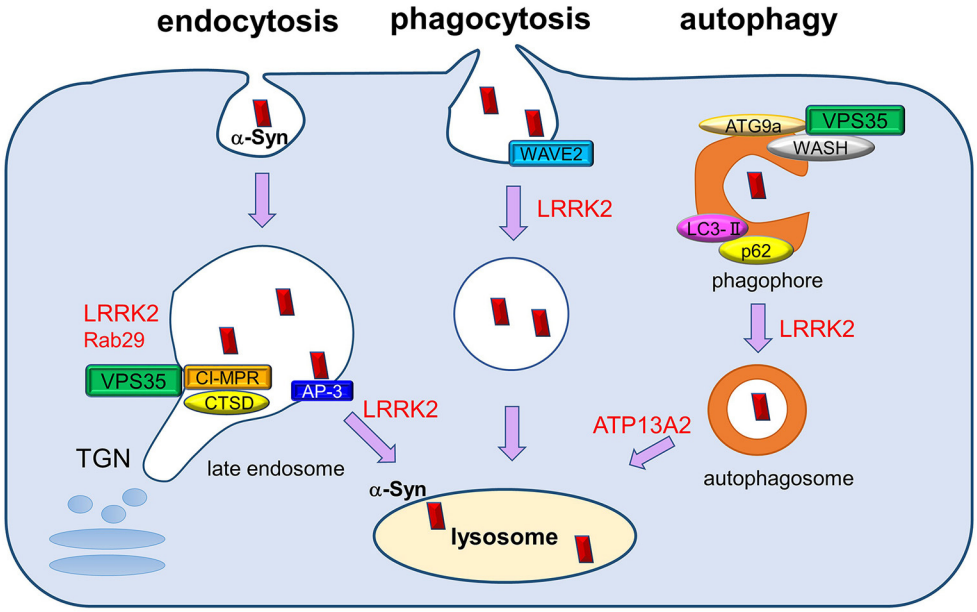

FIGURE 1 | The roles of PD-associated proteins in endolysosomal pathways responsible for α-synuclein degradation. Extracellular and intracellular α-synuclein species (both soluble and aggregated) are transported into lysosomes for degradation through several pathways, including endocytosis, phagocytosis, and autophagy. PD-associated proteins VPS35, LRRK2, and Rab29 influence multiple steps of these degradation pathways, both individually and cooperatively. The retromer complex component VPS35 regulates the recycling of cathepsin D (CATD), the main lysosomal hydrolase responsible for α-synuclein degradation, by retrieving the lysosomal hydrolase receptor CI-MPR from endosome to the TGN. VPS35 pathogenic mutation may affect the recycling of CATD and thus impair α-synuclein degradation. LRRK2 and Rab29 further interact with VPS35 and regulate its function cooperatively. In endocytosis pathway, LRRK2 regulates AP-3-mediated intracellular recycling of lysosomal membrane proteins, whereas LRRK2 modulates the phagocytic activity by interacting with actin-cytoskeletal regulator WAVE2. VPS35 has been shown to function in macroautophagy pathway together with its interactors WASH complex and ATG9a, thereby regulating the transport of LC3-positive compartments. LRRK2 also regulates the autophagic flux, and ATP13A2 influences the clearance of autophagosomes. The perturbation of macroautophagy pathway is thought to contribute to the impaired degradation of α-synuclein, especially those of aggregated species.

LAMP2A, Hsc70, and cathepsin D has been described in whole brains or SNpc of PD patients (22, 24, 92, 93). LRRK2 may additionally regulate the phagocytic activity in myeloid cells, where LRRK2 binds and phosphorylates the actin remodeling protein Wiskott-Aldrich syndrome protein family verprolin-homologous protein 2 (WAVE2), which is important for the efficient promotion of phagocytosis (94).

In neurons, LRRK2 physically and functionally interacts with the retromer complex component VPS35, which is also known as a causative gene product for hereditary PD. Retromer complex functions on endosomes to selectively transport cargo proteins to the *trans*-Golgi network (TGN) or plasma membranes (95), and intirectly regulates lysosomal functions, as described later. The LRRK2-VPS35 functional interaction in various experimental context was further modulated by a LRRK2-binding protein Rab29 (96). Another report has demonstrated that a pathogenic VPS35 mutation (D620N) influences LRRK2 kinase activity with unknown mechanism; that is, LRRK2 activity to phosphorylate its substrate Rab GTPases was significantly enhanced in VPS35[D620N] knock-in cells compared to those without VPS35 mutation (97). Collectively, there is considerable evidence that LRRK2 acts on endolysosomal system, although further analysis is needed to determine which of these functions is particularly important in PD pathogenesis.

VPS35: AN INDIRECT REGULATOR OF LYSOSOMES

Mutations in vacuolar protein sorting-associated protein 35 (*VPS35*) gene are the genetic cause in *PARK17*, a locus for autosomal-dominant familial PD. Two independent groups have investigated Austrian and Swiss kindreds that develop PD and identified D620N mutation in VPS35 as the cause of the disease (98, 99). Patients with VPS35 D620N mutation have a mean age of onset in the 50s, and their clinical manifestations are similar to those of sporadic PD, such as resting tremor, bradycardia and L-DOPA reactivity (100, 101). Thus, although the presence of Lewy bodies in patient brains has not been confirmed, PD with *VPS35* mutation and sporadic PD are thought to share some common pathogenetic mechanisms.

The *VPS35* gene encodes a 796 amino acid protein that acts as a crucial component of the retromer complex, a mediator of the retrograde transport of endosomal proteins to TGN or plasma membranes (102–104). Retromer contains two subprotein complexes: a cargo recognition complex composed of VPS26–VPS29–VPS35 heterotrimer and a membrane-targeting dimer of sorting nexins (SNX1, SNX2, SNX5, SNX6, and SNX32) (105–108). VPS35 is located at the center of the complex and is important for the recognition and binding of the cytoplasmic

domain of cargoes for retrograde transport (109). Particularly, retromer is responsible for the retrograde transport of cation-independent mannose 6-phosphate receptor (CI-MPR), a sorting receptor of lysosomal hydrolases including cathepsin D (110). Therefore, the dysfunction of VPS35 or retromer is thought to affect lysosomal activity through impaired delivery of lysosomal hydrolases, and this may also affect α-synuclein clearance, as cathepsin D is one of major enzymes responsible for the degaradation of α-synuclein (41, 43). In relation to PD, it has been reported that PD-associated D620N mutation in VPS35 causes defects in sorting of CI-MPR (111). Also, D620N mutation in VPS35 has been shown to affect retromer binding to the actin-nucleating Wiskott-Aldrich syndrome and SCAR homolog (WASH) complex, an important functional partner of retromer (16, 112).

VPS35 has also been associated with other cellular processes such as autophagy (102). It has been shown that VPS35 regulates macroautophagy by controlling the endosomal localization of WASH complex as well as ATG9a, a multipass transmembrane protein that is considered to regulate the early steps of autophagosome formation (16). Specifically, the transport of ATG9a is affected by D620N mutant VPS35, which then causes the impairment of autophagosome formation. Another study has suggested the role of VPS35 in CMA, where VPS35 mediates endosome-to-Golgi retrieval of LAMP2A receptor (113). Mice with reduced Vps35 level or D620N mutation showed alterations in lysosomal morphology with a decrease in the level of LAMP2A. This may be due to impaired recovery of LAMP2A from the endosome to the Golgi, which then leads to the enhanced degradation at the lysosomes. This reduction in LAMP2A level is expected to cause a decrease in CMA-mediated α-synuclein degradation. Actually, *Vps35*-deficient mice showed multiple PD-like phenotypes such as the accumulation of α-synuclein in DA neurons, reduced level of the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH) and DA transmitters, dystrophic TH-positive neurites/axons, and impaired motor behaviors (113). Another group has reported that lentivirus-mediated overexpression of human wild-type VPS35, but not PD-linked P316S mutant, rescues α-synuclein accumulation as well as α-synuclein-mediated neuronal loss and astrogliosis in α-synuclein transgenic mice (114). In humans, the alterations in the protein levels of CMA markers (LAMP2A and Hsc70) are documented in SNpc and amygdala of PD patients (115). These findings collectively suggest the role of VPS35 as an indirect controller of lysosomes through the regulation of intracellular trafficking of lysosomal enzyme adaptors or multiple autophagic regulators.

ATP13A2: A UNIQUE CATION TRANSPORTER ON LYSOSOMES

Recessive mutations in *ATP13A2* (polyamine-transporting ATPase 13A2), a gene located in a PD-associated locus *PARK9*, have been identified as the genetic cause for Kufor-rakeb syndrome (KRS), which is a type of Parkinsonian syndromes. KRS is clinically characterized by L-DOPA-responsive juvenile parkinsonism as well as cognitive impairment and myoclonus (116), and pathologically characterized by diffuse cerebral and cerebellar atrophy (117). Loss-of-function mutations in *ATP13A2* have additionally been reported to cause neuronal ceroid lipofuscinosis (118, 119). ATP13A2 is a lysosomal P5-type transport ATPase that is involved in the transport of divalent metal cations as well as polyamines on lysosomal membranes (120). Loss of ATP13A2 causes lysosomal accumulation of polyamines (e.g., spermine) and lysosomal rupture, leading to cell toxicity (121). ATP13A2 has also been suggested to regulate multiple cellular functions related to lysosomes, including heavy metal homeostasis and mitochondrial homeostasis (15, 122). For example, a recent study using SH-SY5Y cells, patient-derived fibroblasts and the nematode *C. elegans* has identified a conserved cell protective pathway that counters mitochondrial oxidative stress via ATP13A2-mediated lysosomal spermine export (123).

A number of previous studies have pointed to the essential role of ATP13A2 in the homeostasis of lysosomal function (124). Studies with PD patient-derived mutant ATP13A2 fibroblasts and ATP13A2-knockdown DA neurons have shown that PD-linked mutations in ATP13A2 lead to several lysosomal alterations, including impaired lysosomal acidification, decreased activity of lysosomal enzymes, reduced degradation of lysosomal substrates and defective clearance of autophagosomes (125). Conversely, overexpression of wild-type ATP13A2 in *ATP13A2*-deficient cells restores lysosomal function and prevents cell death (125). Other studies have demonstrated that ATP13A2 regulates endolysosomal cargo sorting through its cytosolic N terminal domain, independent of its catalytic activity (126), and ATP13A2 regulates mTORC1-TFEB pathway together with another PD-associated gene product synaptotagmin 11 (SYT11) to induce autophagy as well as α-synuclein clearance (127). ATP13A2 deficiency and mutation have also been shown to cause the reduction in the level of cathepsin D, a main α-synuclein-degrading enzyme in lysosomes, in human neuroblastoma SH-SY5Y cells and in medaka fish (128).

The relevance of ATP13A2 defects to α-synuclein accumulation has been more directly demonstrated from other studies. Depletion of ATP13A2 in primary cortical neurons using a short hairpin RNA promoted the aggregation of α-synuclein by reducing lysosomal activity, which ultimately led to cell death (15, 129). On the other hand, overexpression of ATP13A2 in α-synuclein-stable SH-SY5Y cells lowered intracellular α-synuclein levels and instead promoted extracellular secretion of α-synuclein (130). Another study has reported that overexpression of ATP13A2 rescued DA neuron degeneration caused by overexpressed α-synuclein in rat primary midbrain cultures and in *C. elegans* (131).

In vivo, *Atp13a2* knockout mice exhibit a neuronal ceroid lipofuscinosis-like phenotype, accumulation of mitochondrial ATP synthase subunit C (132), α-synuclein accumulation, dopaminergic pathology and late-onset sensorimotor deficits (133, 134). More specifically, ATP13A2 deficiency causes dysfunctions in the fusion of autophagic vacuoles with lysosomes as well as the impairment of lysosome-mediated degradation of proteins including α-synuclein (135). Analyses of postmortem

PD patient brains have shown the presence of ATP13A2 in the Lewy bodies and a decrease in the levels of lysosomal components including ATP13A2 in DA neurons (125, 136). Although the mutations in *ATP13A2* are rare in humans, these studies have collectively pointed to the important roles of ATP13A2 in ALP that may be involved in the neurodegenerative processes.

GLUCOCEREBROSIDASE: THE LYSOSOMAL ENZYME LINKED TO SPORADIC PD

Homozygous or compound heterozygous mutations in *GBA1* gene are well-known to cause Gaucher disease (GD), a lysosomal storage disorder, whereas heterozygous mutations that in the homozygous state lead to GD have been reported to increase the risk for developing PD (137–139). Also, a higher incidence of Parkinsonism in patients with GD harboring *GBA1* homozygous mutations has been reported (140, 141). Moreover, a number of genome-wide association studies (GWAS) have identified *GBA1* as a most common genetic risk factor for idiopathic PD (9, 11, 142). Compared to non-*GBA1*-associated PD, *GBA1*-associated PD shows an earlier onset of the disease and a higher prevalence of non-motor symptoms, such as cognitive decline. They also tend to have a family history of dementia, and non-motor symptoms often manifest before the onset of motor symptoms (143, 144). *GBA1* mutations are also a risk factor for dementia with Lewy bodies (DLB) (145, 146), and PD patients with *GBA1* mutation have about a 3-fold higher risk of progressing to dementia than those without mutation (147). They also exhibit a faster progression of visual hallucinations, dysautonomia and motor symptoms, with a resultant decrease in survival rate (143, 145, 148).

GBA1 gene encodes the lysosomal enzyme glucocerebrosidase (GCase) that hydrolyzes glucosylceramide (GlcCer) to ceramide and glucose. *GBA1* mutations have been shown to cause the reduction in the enzymatic activity of GCase (149, 150) and prevent GCase from reaching the lysosome, causing the accumulation of GlcCer in neurons (151–153). The significant correlation between the severity of the specific *GBA1* mutation and that of clinical phenotypes (e.g., odds ratios for PD, age at onset, risk for dementia) has been reported (145, 147, 154), suggesting major impact of GCase activity in the pathogenetic processes. Importantly, idiopathic PD patients without *GBA1* mutations also showed lower enzymatic activity and levels of GCase in brain tissue samples (155–157) and in dried blood spots (149). The reduction in GCase activity was further demonstrated in PD patient-derived DA neurons without *GBA1* mutations (158, 159). These observations suggest that GCase dysfunction is a common pathogenic mechanism in idiopathic PD.

The reduced function of GCase are expected to contribute to the accumulation of α-synuclein in PD lesions (160). Indeed, treatment with a GCase inhibitor Conduritol B epoxide (CBE) caused a large increase in the levels of α-synuclein, without increasing α-synuclein mRNA, in human neuroblastoma SH-SY5Y cells and in mice (161). The association between reduced GCase and increased α-synuclein is further implicated in human PD postmortem brains (157). The accumulation of GlcCer in neurons as a result of GCase deficiency is thought to promote the formation of toxic α-synuclein aggregates (162), as lipids like GlcCer may strongly interact with α-synuclein and accelerate its fibril formation (163, 164). Another study has suggested a model where α-synuclein deposition and reduced GCase activity may influence each other and form a positive feedback loop that leads to a vicious cycle of disease progression (156).

On the other hand, the activity and function of GCase in microglia or related phagocytic cells have also been focused, as GCase is highly expressed in monocyte lineage cells. In mice, genetic depletion or pharmacological inhibition of GCase caused microglial activation (165, 166). Lower GCase activity was detected in monocytes, but not lymphocytes, from PD patients, when compared with those from healthy subjects (167). Importantly, such reduction in GCase activity was detected in those from patients without *GBA1* mutations. As monocyte lineage cells contain a large number of well-developed lysosomes, it is possible to assume that the dysfunction of lysosomal GCase in these cells greatly influences α-synuclein metabolism.

Recently, much attention has been paid to the relationship between *GBA1* and *LRRK2*. An increasing number of patients harboring both *GBA1* and *LRRK2* mutations have been reported, and these patients tend to develop PD at an earlier age than carriers of *LRRK2* or *GBA1* mutation alone (168–170). These reports suggest the cooperative effect of *GBA1* and *LRRK2* mutations for the development of PD. In experiments using DA neurons derived from PD patients, reduced GCase activity was observed in cells with LRRK2 mutations, and the inhibition of LRRK2 kinase activity restored GCase activity (171). Furthermore, treatment of *GBA1* mutant knock-in astrocytes with LRRK2 kinase inhibitor rescued the lysosomal abnormalities such as pH increase and the reduction in cathepsin B activity (172). These observations collectively suggest that the functions of LRRK2 and GCase in terms of lysosomal regulation are closely interrelated.

PERSPECTIVES ON THE THERAPEUTIC STRATEGIES TARGETING LYSOSOMES

As described above, ALP can be regulated by PD-associated genes *LRRK2*, *VPS35*, *ATP13A2,* and *GBA1* not only individually but also cooperatively. Especially, cooperative maintenance of lysosomes by these genes is considered as one of key mechanisms related to PD (**Figure 2**). For example, lysosomal morphology under lysosomal overload stress is maintained by LRRK2 kinase activity (82) that is enhanced in cells harboring *VPS35* pathogenic mutation, although the mechanism of enhancement is unclear (97). As lysosomes apparently play important roles in the accumulation and toxicity of α-synuclein, a number of studies have focused on enhancing ALP as a possible therapeutic strategy for α-synucleinopathies (173).

Enhancement of lysosomal activity is one of plausible approaches to facilitate α-synuclein degradation. Among the

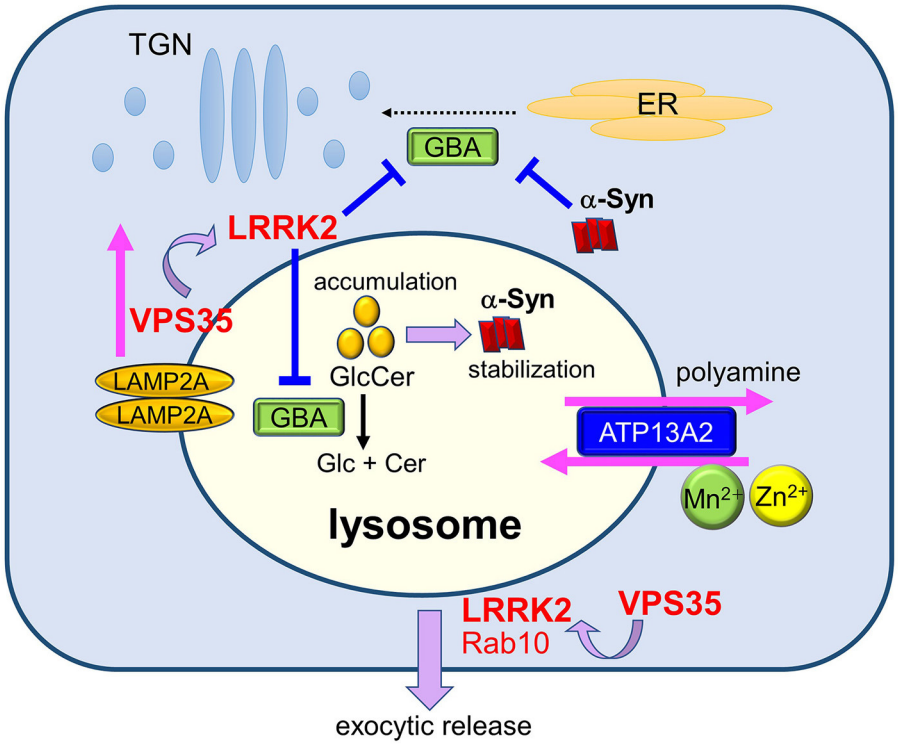

FIGURE 2 | Maintenance of lysosomes by PD gene products and its relevance to PD. Lysosomal homeostasis is regulated by several PD genes such as ATP13A2, LRRK2, GBA, and VPS35. Deficiency in ATP13A2, a P5-type ATPase localized to the lysosomal membranes, is expected to affect lysosomal functions via dysregulated transport of several divalent metal cations and polyamines. LRRK2 functions in the maintenance of stressed lysosomes by facilitating the exocytic release of lysosomal contents together with its substrate Rab10 under lysosomal overload stress. LRRK2 may also negatively regulate the activity of lysosomal hydrolase glucocerebrosidase (GCase). Decreased activity of GCase causes the accumulation of its substrate glucosylceramide (GlcCer), which stabilizes toxic α-synuclein species. Accumulation of α-synuclein has been shown to block ER-to-Golgi trafficking of GCase, causing a further decrease in lysosomal GCase. The retromer component VPS35 mediates the retrieval of a CMA receptor LAMP2A on endolysosomal membranes, and mutation in VPS35 leads to the enhanced degradation of LAMP2A at the lysosomes, causing CMA defects and α-synuclein accumulation. VPS35 mutation also causes the enhancement of LRRK2 kinase activity, which may then affect the lysosomal maintenance and GCase activity.

PD-associated gene products introduced above, GCase has been the most well-studied as a target that contributes to lysosomal activation and α-synuclein metabolism. It has been shown that lysosomal GCase activity can be enhanced by treatment with ambroxol hydrochloride, a clinically used expectorant drug and an effective pharmacological chaperone for GCase (174–176). Oral administration of ambroxol to wild-type and α-synuclein transgenic mice caused the increase in brain GCase activity as well as the reduction in the levels of total and phosphorylated α-synuclein (177). Amboxol administration in rats also resulted in the restoration of decreased GCase activity and the decrease of α-synuclein pathology that were induced by 6-hydroxydopamine (6-OHDA) treatment (178). Additionally, oral administration of another molecular chaperone for GCase, AT2101 (afegostat-tartrate, isofagomine), to α-synuclein transgenic mice improved motor and non-motor function, abolished microglial response in the substantia nigra, and reduced the number of small α-synuclein aggregates (179). Adeno-associated virus (AAV)-mediated overexpression of GCase in hippocampus ameliorated α-synuclein accumulation as well as cognitive impairment in transgenic mice expressing mutant GCase (D409V/D409V) or A53T α-synucein (180, 181). Using the same mice models, the researchers have also shown that the administration of a brain-penetrant inhibitor of GlcCer synthase (GCS), GZ667161, ameliorated α-synuclein accumulation and cognitive deficits (182). These reports indicated that proper GlcCer metabolism is important to control α-synuclein accumulation.

Farnesyltransferease inhibitors (FTIs) are recently attracting significant attention as a promising lysosomal activator. It has been reported that FTI treatment in α-synuclein transgenic mice enhanced GCase activity and rescued pathological α-synuclein aggregation (49). FTI treatment has also been reported to reduce tau pathology in tauopathy model mice by activating lysosomes (183). Importantly, one of FTIs, lonafarnib, has been approved by FDA very recently for the treatment of Hutchinson-Gilford progeria syndrome, a rare and fatal premature aging disease (184). Thus, it will be of particular interest to see if such

TABLE 1 | Strategies to enhance lysosomal activity for the modulation of PD-related phenotypes *in vivo*.

Target	Strategy	Effects	Reference
GCase	Oral administration of GCase chaperones (ambroxol, AT2101)	Reduction of total- and phospho-α-synuclein Decrease of 6-OHDA-induced α-syn pathology Reduction of small α-syn aggregates (AT2101)	(177) (178) (179)
GCase	Overexpression of GCase	Amelioration of α-syn accumulation and cognitive impairment in Gba1-D409V or α-syn-A53T Tg mice	(180) (181)
GlcCer synthase	GlcCer synthase inhibitor (GZ667161) administration	Amelioration of α-syn accumulation and cognitive impairment in Gba1-D409V or α-syn-A53T Tg mice	(182)
Farnesyltransferase	Farnesyltransferase inhibitor (FTI) treatment	sReduction of pathological α-synuclein in Tg mice Increase of GCase activity Reduction of tau pathology	(49) (183)
TFEB	Overexpression of TFEB	Protection of DA neurons from α-syn toxicity in Tg rats	(185)
mTOR	Rapamycin treatment	Reduction of α-synuclein accumulation (2 weeks) Improvement of motor function (24 weeks)	(189) (190)
Autophagy-AMPK	Trehalose treatment	Reduction of insoluble α-synuclein (1 week) Attenuation of motor deficits, degeneration and α-syn deposition (6-weeks)	(197) (198)
Autophagy-AMPK	Nilotinib treatment	Reduction of α-syn levels, suppression of DA neuron loss and motor deficits in α-syn-A53T Tg mice	(200)
CMA	Overexpression of LAMP2A	Complete restoration of α-syn-mediated nigrostriatal degeneration in AAV-α-syn rats	(201)
CMA	Geniposide treatment	Decrease of α-syn levels and increase of LAMP2A in MPTP-treated mice	(204)

therapeutic strategies are actually effective in the treatment of PD or related neurodegenerative disorders.

Another plausible approach to activate lysosomes is the expression of transcription factor EB (TFEB), a master transcriptional regulator of ALP. Overexpression of TFEB has been shown to rescue midbrain DA neurons from α-synuclein-induced toxicity in transgenic rat models (185). In addition to α-synuclein, overexpression of constitutively active TFEB has been shown to reduce protein aggregates in old quiescent neural stem cells (qNSCs) (186) and in p53-induced senescent fibroblast cells (187). Nuclear translocation of TFEB is induced by inhibition of mammalian target of rapamycin (mTOR) (188), a well-known negative regulator of macroautophagy and ALP, and therefore mTOR inhibition has also been focused as a promising strategy. Intra-cerebral infusion of an mTOR inhibitor rapamycin for 2 weeks in α-synuclein transgenic mice resulted in reduced accumulation of α-synuclein (189), and long-term feeding a rapamycin diet (∼24 weeks) improved motor performance in A53T α-synuclein transgenic mice (190). However, due to the side effects of rapamycin that have been noted to be problematic with long-term use (191), the use of rapamycin in the treatment of PD is expected to be challenging.

On the other hand, an mTOR-independent activator of autophagy, trehalose, has been shown to activate macroautophagy and enhance the clearance of wild-type or mutant forms of α-synuclein (192–195). Mechanistically, trehalose has been shown to activate macroautophagy by inhibiting the glucose transporter SLC2A, which ultimately leads to the activation of an energy-sensing kinase AMPK that stimulates autophagy (196). Oral administration of trehalose to A53T α-synuclein transgenic mice for 1 week induced macroautophagy and reduced the level of insoluble α-synuclein in the brain (197). Similarly, oral administration of trahalose to AAV-based rat model expressing A53T α-synuclein for 6 weeks caused a significant attenuation in α-synuclein-mediated motor deficits and DA neurodegeneration as well as α-synuclein accumulation (198). In addition to trehalose, a tyrosine kinase inhibitor nilotinib is another drug that stimulates autophagy by activating AMPK (199); chronic administration of nilotinib for 3–6 weeks in human A53T α-synuclein transgenic mice resulted in the decrease of α-synuclein levels, suppression of DA neuronal loss and improvement of motor behavior (200).

Activation of the CMA pathway is considered as an alternative strategy to increase the clearance of α-synuclein. Overexpression of LAMP2A has been shown to upregulate CMA, decrease α-synuclein accumulation and protect against α-synuclein toxicity in human neuroblastoma SH-SY5Y cells, rat primary cortical neurons, and nigral dopaminergic neurons *in vivo* (201). Inhibition of signaling through retinoic acid receptor α (RARα), a negative regulator of CMA, has also been focused; treatment with the RARα inhibitor all-*trans*-retinoic acid and its synthetic derivatives has been shown to activate CMA and protect against oxidative stress and proteotoxicity in cells (202). A specific subset of miRNAs that downregulate CMA has also been identified (203), and treatment with Geniposide, a bioactive iridoid glycoside that acts as a down-regulator of miRNAs especially for miR-21, increased LAMP2A expression and reduced α-synuclein levels in SH-SY5Y cells and MPTP-treated PD model mice (204).

In conclusion, a variety of strategies that aim to activate ALP have been developed and shown to modulate α-synuclein accumulation as well as PD-related phenotypes. The strategies that were tested for *in vivo* phenotypic modulation are summarized in **Table 1**. Several of the compounds used in these strategies are now being examined in clinical trials for PD and

related disorders [e.g., ambroxol (205) and nilotinib (206, 207), *see ClinicalTrials.gov*]. These compounds or related products with similar mechanisms is expected to be available in the future as disease-modifying therapies. Moreover, as overviewed above, ALP is regulated in various ways by PD gene products—including LRRK2, VPS35, ATP13A2, GCase, and other risk factors not mentioned in this review—and among these, not only GCase (activator, ambroxol) but also LRRK2 (inhibitor) are being targeted in clinical trials (208). Further clarification of the functional relationships among PD-causing genes and their regulation to ALP may lead to the proposal of new therapeutic targets. It is hoped that further basic analysis of cellular and animal models, such as those described in this review, will accelerate the development of fundamental therapeutic agents.

AUTHOR CONTRIBUTIONS

TA and TK conceived and wrote the article. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Braak H, Braak E. Pathoanatomy of Parkinson's disease. *J Neurol.* (2000) 247(Suppl 2):II3–10. doi: 10.1007/PL00007758
2. Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, et al. Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am J Pathol.* (1998) 152:879–84.
3. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, et al. α-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol.* (2002) 4:160–4. doi: 10.1038/ncb748
4. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. α-synuclein in Lewy bodies. *Nature.* (1997) 388:839–40. doi: 10.1038/42166
5. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
6. Klein C, Westenberger A. Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med.* (2012) 2:a008888. doi: 10.1101/cshperspect.a008888
7. Lubbe S, Morris HR. Recent advances in Parkinson's disease genetics. *J Neurol.* (2014) 261:259–66. doi: 10.1007/s00415-013-7003-2
8. Zhang PL, Chen Y, Zhang CH, Wang YX, Fernandez-Funez P. Genetics of Parkinson's disease and related disorders. *J Med Genet.* (2018) 55:73–80. doi: 10.1136/jmedgenet-2017-105047
9. Chang D, Nalls MA, Hallgrimsdottir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet.* (2017) 49:1511–6. doi: 10.1038/ng.3955
10. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
11. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* (2014) 46:989–93. doi: 10.1038/ng.3043
12. Jinn S, Drolet RE, Cramer PE, Wong AH, Toolan DM, Gretzula CA, et al. TMEM175 deficiency impairs lysosomal and mitochondrial function and increases α -synuclein aggregation. *Proc Natl Acad Sci USA.* (2017) 114:2389–94. doi: 10.1073/pnas.1616332114
13. Schapansky J, Khasnavis S, DeAndrade MP, Nardozzi JD, Falkson SR, Boyd JD, et al. Familial knockin mutation of LRRK2 causes lysosomal dysfunction and accumulation of endogenous insoluble α-synuclein in neurons. *Neurobiol Dis.* (2018) 111:26–35. doi: 10.1016/j.nbd.2017.12.005
14. Taguchi YV, Liu J, Ruan J, Pacheco J, Zhang X, Abbasi J, et al. Glucosylsphingosine promotes α-Synuclein pathology in mutant GBA-associated Parkinson's Disease. *J Neurosci.* (2017) 37:9617–31. doi: 10.1523/JNEUROSCI.1525-17.2017
15. Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D. Deficiency of ATP13A2 leads to lysosomal dysfunction, α-synuclein accumulation, and neurotoxicity. *J Neurosci.* (2012) 32:4240–6. doi: 10.1523/JNEUROSCI.5575-11.2012
16. Zavodszky E, Seaman MN, Moreau K, Jimenez-Sanchez M, Breusegem SY, Harbour ME, et al. Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. *Nat Commun.* (2014) 5:3828. doi: 10.1038/ncomms4828
17. Bandres-Ciga S, Saez-Atienzar S, Bonet-Ponce L, Billingsley K, Vitale D, Blauwendraat C, et al. The endocytic membrane trafficking pathway plays a major role in the risk of Parkinson's disease. *Mov Disord.* (2019) 34:460–8. doi: 10.1002/mds.27614
18. Hopfner F, Mueller SH, Szymczak S, Junge O, Tittmann L, May S, et al. Rare variants in specific lysosomal genes are associated with Parkinson's disease. *Mov Disord.* (2020) 35:1245–8. doi: 10.1002/mds.28037
19. Kia DA, Zhang D, Guelfi S, Manzoni C, Hubbard L, Reynolds RH, et al. Identification of candidate Parkinson disease genes by integrating genome-wide association study, expression, and epigenetic data sets. *JAMA Neurol.* (2021) 78:464–72. doi: 10.1001/jamaneurol.2020.5257
20. Bourdenx M, Koulakiotis NS, Sanoudou D, Bezard E, Dehay B, Tsarbopoulos A. Protein aggregation and neurodegeneration in prototypical neurodegenerative diseases: examples of amyloidopathies, tauopathies and synucleinopathies. *Prog Neurobiol.* (2017) 155:171–93. doi: 10.1016/j.pneurobio.2015.07.003
21. Soria FN, Pampliega O, Bourdenx M, Meissner WG, Bezard E, Dehay B. Exosomes, an unmasked culprit in neurodegenerative diseases. *Front Neurosci.* (2017) 11:26. doi: 10.3389/fnins.2017.00026
22. Chu Y, Dodiya H, Aebischer P, Olanow CW, Kordower JH. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to α-synuclein inclusions. *Neurobiol Dis.* (2009) 35:385–98. doi: 10.1016/j.nbd.2009.05.023
23. Gurney R, Davidson YS, Robinson AC, Richardson A, Jones M, Snowden JS, et al. Lysosomes, autophagosomes and Alzheimer pathology in dementia with Lewy body disease. *Neuropathology.* (2018) 38:347–60. doi: 10.1111/neup.12472
24. Dehay B, Bove J, Rodriguez-Muela N, Perier C, Recasens A, Boya P, et al. Pathogenic lysosomal depletion in Parkinson's disease. *J Neurosci.* (2010) 30:12535–44. doi: 10.1523/JNEUROSCI.1920-10.2010
25. Puska G, Lutz MI, Molnar K, Regelsberger G, Ricken G, Pirker W, et al. Lysosomal response in relation to α-synuclein pathology differs between Parkinson's disease and multiple system atrophy. *Neurobiol Dis.* (2018) 114:140–52. doi: 10.1016/j.nbd.2018.02.019
26. Niimi Y, Ito S, Mizutani Y, Murate K, Shima S, Ueda A, et al. Altered regulation of serum lysosomal acid hydrolase activities in Parkinson's disease: a potential peripheral biomarker? *Parkinsonism Relat Disord.* (2019) 61:132–7. doi: 10.1016/j.parkreldis.2018.10.032
27. Parnetti L, Chiasserini D, Persichetti E, Eusebi P, Varghese S, Qureshi MM, et al. Cerebrospinal fluid lysosomal enzymes and α-synuclein in Parkinson's disease. *Mov Disord.* (2014) 29:1019–27. doi: 10.1002/mds.25772

28. van Dijk KD, Persichetti E, Chiasserini D, Eusebi P, Beccari T, Calabresi P, et al. Changes in endolysosomal enzyme activities in cerebrospinal fluid of patients with Parkinson's disease. *Mov Disord.* (2013) 28:747–54. doi: 10.1002/mds.25495
29. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science.* (1997) 276:2045–7. doi: 10.1126/science.276.5321.2045
30. Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, et al. α-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Mov Disord.* (2013) 28:811–3. doi: 10.1002/mds.25421
31. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nat Genet.* (1998) 18:106–8. doi: 10.1038/ng0298-106
32. Lesage S, Anheim M, Letournel F, Bousset L, Honore A, Rozas N, et al. G51D α-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. *Ann Neurol.* (2013) 73:459–71. doi: 10.1002/ana.23894
33. Pasanen P, Myllykangas L, Siitonen M, Raunio A, Kaakkola S, Lyytinen J, et al. Novel α-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. *Neurobiol Aging.* (2014) 35:2180.e2181–5. doi: 10.1016/j.neurobiolaging.2014.03.024
34. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of α-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol.* (2004) 55:164–73. doi: 10.1002/ana.10795
35. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, et al. α-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet.* (2004) 364:1167–9. doi: 10.1016/S0140-6736(04)17103-1
36. Ibanez P, Bonnet AM, Debarges B, Lohmann E, Tison F, Pollak P, et al. Causal relation between α-synuclein gene duplication and familial Parkinson's disease. *Lancet.* (2004) 364:1169–71. doi: 10.1016/S0140-6736(04)17104-3
37. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. α-Synuclein locus triplication causes Parkinson's disease. *Science.* (2003) 302:841. doi: 10.1126/science.1090278
38. Stefanis L, Emmanouilidou E, Pantazopoulou M, Kirik D, Vekrellis K, Tofaris GK. How is α-synuclein cleared from the cell? *J Neurochem.* (2019) 150:577–90. doi: 10.1111/jnc.14704
39. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. α-Synuclein is degraded by both autophagy and the proteasome. *J Biol Chem.* (2003) 278:25009–13. doi: 10.1074/jbc.M300227200
40. Xilouri M, Brekk OR, Stefanis L. α-Synuclein and protein degradation systems: a reciprocal relationship. *Mol Neurobiol.* (2013) 47:537–51. doi: 10.1007/s12035-012-8341-2
41. McGlinchey RP, Lee JC. Cysteine cathepsins are essential in lysosomal degradation of α-synuclein. *Proc Natl Acad Sci USA.* (2015) 112:9322–7. doi: 10.1073/pnas.1500937112
42. Sevlever D, Jiang P, Yen SH. Cathepsin D is the main lysosomal enzyme involved in the degradation of α-synuclein and generation of its carboxy-terminally truncated species. *Biochemistry.* (2008) 47:9678–87. doi: 10.1021/bi800699v
43. Cullen V, Lindfors M, Ng J, Paetau A, Swinton E, Kolodziej P, et al. Cathepsin D expression level affects α-synuclein processing, aggregation, and toxicity *in vivo*. *Mol Brain.* (2009) 2:5. doi: 10.1186/1756-6606-2-5
44. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. *Proc Natl Acad Sci USA.* (2009) 106:13010–5. doi: 10.1073/pnas.0903691106
45. Jang A, Lee HJ, Suk JE, Jung JW, Kim KP, Lee SJ. Non-classical exocytosis of α-synuclein is sensitive to folding states and promoted under stress conditions. *J Neurochem.* (2010) 113:1263–74. doi: 10.1111/j.1471-4159.2010.06695.x
46. Flavin WP, Bousset L, Green ZC, Chu Y, Skarpathiotis S, Chaney MJ, et al. Endocytic vesicle rupture is a conserved mechanism of cellular invasion by amyloid proteins. *Acta Neuropathol.* (2017) 134:629–53. doi: 10.1007/s00401-017-1722-x
47. Freeman D, Cedillos R, Choyke S, Lukic Z, McGuire K, Marvin S, et al. α-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. *PLoS ONE.* (2013) 8:e62143. doi: 10.1371/journal.pone.0062143
48. Samuel F, Flavin WP, Iqbal S, Pacelli C, Sri Renganathan SD, Trudeau LE, et al. Effects of Serine 129 Phosphorylation on α-synuclein aggregation, membrane association, and internalization. *J Biol Chem.* (2016) 291:4374–85. doi: 10.1074/jbc.M115.705095
49. Cuddy LK, Wani WY, Morella ML, Pitcairn C, Tsutsumi K, Fredriksen K, et al. Stress-induced cellular clearance is mediated by the SNARE protein ykt6 and disrupted by α-Synuclein. *Neuron.* (2019) 104:869–84.e811. doi: 10.1016/j.neuron.2019.09.001
50. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, et al. α-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science.* (2006) 313:324–8. doi: 10.1126/science.1129462
51. Thayanidhi N, Helm JR, Nycz DC, Bentley M, Liang Y, Hay JC. α-synuclein delays endoplasmic reticulum (ER)-to-Golgi transport in mammalian cells by antagonizing ER/Golgi SNAREs. *Mol Biol Cell.* (2010) 21:1850–63. doi: 10.1091/mbc.e09-09-0801
52. Klionsky DJ, Ohsumi Y. Vacuolar import of proteins and organelles from the cytoplasm. *Annu Rev Cell Dev Biol.* (1999) 15:1–32. doi: 10.1146/annurev.cellbio.15.1.1
53. Xilouri M, Brekk OR, Stefanis L. Autophagy and α-synuclein: relevance to Parkinson's disease and related synucleopathies. *Mov Disord.* (2016) 31:178–92. doi: 10.1002/mds.26477
54. Minakaki G, Menges S, Kittel A, Emmanouilidou E, Schaeffner I, Barkovits K, et al. Autophagy inhibition promotes SNCA/ α-synuclein release and transfer via extracellular vesicles with a hybrid autophagosome-exosome-like phenotype. *Autophagy.* (2018) 14:98–119. doi: 10.1080/15548627.2017.1395992
55. Poehler AM, Xiang W, Spitzer P, May VE, Meixner H, Rockenstein E, et al. Autophagy modulates SNCA/ α-synuclein release, thereby generating a hostile microenvironment. *Autophagy.* (2014) 10:2171–92. doi: 10.4161/auto.36436
56. Hunn BHM, Vingill S, Threlfell S, Alegre-Abarrategui J, Magdelyns M, Deltheil T, et al. Impairment of macroautophagy in dopamine neurons has opposing effects on Parkinsonian pathology and behavior. *Cell Rep.* (2019) 29:920–31.e927. doi: 10.1016/j.celrep.2019.09.029
57. Tanji K, Mori F, Kakita A, Takahashi H, Wakabayashi K. Alteration of autophagosomal proteins (LC3, GABARAP and GATE-16) in Lewy body disease. *Neurobiol Dis.* (2011) 43:690–7. doi: 10.1016/j.nbd.2011.05.022
58. Cuervo AM, Wong E. Chaperone-mediated autophagy: roles in disease and aging. *Cell Res.* (2014) 24:92–104. doi: 10.1038/cr.2013.153
59. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. *Science.* (2004) 305:1292–5. doi: 10.1126/science.1101738
60. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* (2004) 44:595–600. doi: 10.1016/j.neuron.2004.10.023
61. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron.* (2004) 44:601–7. doi: 10.1016/j.neuron.2004.11.005
62. Biskup S, Moore DJ, Rea A, Lorenz-Deperieux B, Coombes CE, Dawson VL, et al. Dynamic and redundant regulation of LRRK2 and LRRK1 expression. *BMC Neurosci.* (2007) 8:102. doi: 10.1186/1471-2202-8-102
63. Hakimi M, Selvanantham T, Swinton E, Padmore RF, Tong Y, Kabbach G, et al. Parkinson's disease-linked LRRK2 is expressed in circulating and tissue immune cells and upregulated following recognition of microbial structures. *J Neural Transm.* (2011) 118:795–808. doi: 10.1007/s00702-011-0653-2
64. Li X, Tan YC, Poulose S, Olanow CW, Huang XY, Yue Z. Leucine-rich repeat kinase 2 (LRRK2)/PARK8 possesses GTPase activity that is altered in familial Parkinson's disease R1441C/G mutants. *J Neurochem.* (2007) 103:238–247. doi: 10.1111/j.1471-4159.2007.04743.x
65. Marin I. The Parkinson disease gene LRRK2: evolutionary and structural insights. *Mol Biol Evol.* (2006) 23:2423–33. doi: 10.1093/molbev/msl114
66. Rideout HJ, Stefanis L. The neurobiology of LRRK2 and its role in the pathogenesis of Parkinson's disease. *Neurochem Res.* (2014) 39:576–92. doi: 10.1007/s11064-013-1073-5
67. Ito G, Okai T, Fujino G, Takeda K, Ichijo H, Katada T, et al. GTP binding is essential to the protein kinase activity of LRRK2, a causative gene

product for familial Parkinson's disease. *Biochemistry*. (2007) 46:1380–8. doi: 10.1021/bi061960m
68. Lewis PA, Greggio E, Beilina A, Jain S, Baker A, Cookson MR. The R1441C mutation of LRRK2 disrupts GTP hydrolysis. *Biochem Biophys Res Commun.* (2007) 357:668–71. doi: 10.1016/j.bbrc.2007.04.006
69. Di Fonzo A, Rohe CF, Ferreira J, Chien HF, Vacca L, Stocchi F, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet.* (2005) 365:412–5. doi: 10.1016/S0140-6736(05)17829-5
70. Aasly JO, Vilarino-Guell C, Dachsel JC, Webber PJ, West AB, Haugarvoll K, et al. Novel pathogenic LRRK2 p.Asn1437His substitution in familial Parkinson's disease. *Mov Disord.* (2010) 25:2156–63. doi: 10.1002/mds.23265
71. Funayama M, Hasegawa K, Ohta E, Kawashima N, Komiyama M, Kowa H, et al. An LRRK2 mutation as a cause for the parkinsonism in the original PARK8 family. *Ann Neurol.* (2005) 57:918–21. doi: 10.1002/ana.20484
72. Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, et al. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. *Neurology*. (2005) 65:741–4. doi: 10.1212/01.WNL.0000172630.22804.73
73. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci USA*. (2005) 102:16842–7. doi: 10.1073/pnas.0507360102
74. Deniston CK, Salogiannis J, Mathea S, Snead DM, Lahiri I, Matyszewski M, et al. Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. *Nature.* (2020) 588:344–9. doi: 10.1038/s41586-020-2673-2
75. Watanabe R, Buschauer R, Bohning J, Audagnotto M, Lasker K, Lu TW, et al. The *in situ* structure of Parkinson's disease-linked LRRK2. *Cell.* (2020) 182:1508–18.e1516. doi: 10.1016/j.bpj.2019.11.2690
76. Fujimoto T, Kuwahara T, Eguchi T, Sakurai M, Komori T, Iwatsubo T. Parkinson's disease-associated mutant LRRK2 phosphorylates Rab7L1 and modifies trans-Golgi morphology. *Biochem Biophys Res Commun.* (2018) 495:1708–15. doi: 10.1016/j.bbrc.2017.12.024
77. Kuwahara T, Iwatsubo T. The emerging functions of LRRK2 and Rab GTPases in the endolysosomal system. *Front Neurosci.* (2020) 14:227. doi: 10.3389/fnins.2020.00227
78. Liu Z, Bryant N, Kumaran R, Beilina A, Abeliovich A, Cookson MR, et al. LRRK2 phosphorylates membrane-bound Rabs and is activated by GTP-bound Rab7L1 to promote recruitment to the trans-Golgi network. *Hum Mol Genet.* (2018) 27:385–95. doi: 10.1093/hmg/ddx410
79. Steger M, Diez F, Dhekne HS, Lis P, Nirujogi RS, Karayel O, et al. Systematic proteomic analysis of LRRK2-mediated Rab GTPase phosphorylation establishes a connection to ciliogenesis. *Elife.* (2017) 6:e31012. doi: 10.7554/eLife.31012.018
80. Steger M, Tonelli F, Ito G, Davies P, Trost M, Vetter M, et al. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. *Elife.* (2016) 5:e12813. doi: 10.7554/eLife.12813.023
81. Pfeffer SR. Rab GTPases: master regulators that establish the secretory and endocytic pathways. *Mol Biol Cell.* (2017) 28:712–5. doi: 10.1091/mbc.e16-10-0737
82. Eguchi T, Kuwahara T, Sakurai M, Komori T, Fujimoto T, Ito G, et al. LRRK2 and its substrate Rab GTPases are sequentially targeted onto stressed lysosomes and maintain their homeostasis. *Proc Natl Acad Sci USA.* (2018) 115:E9115–24. doi: 10.1073/pnas.1812196115
83. Kuwahara T, Funakawa K, Komori T, Sakurai M, Yoshii G, Eguchi T, et al. Roles of lysosomotropic agents on LRRK2 activation and Rab10 phosphorylation. *Neurobiol Dis.* (2020) 145:105081. doi: 10.1016/j.nbd.2020.105081
84. Bonet-Ponce L, Beilina A, Williamson CD, Lindberg E, Kluss JH, Saez-Atienzar S, et al. LRRK2 mediates tubulation and vesicle sorting from lysosomes. *Sci Adv.* (2020) 6:eabb2454. doi: 10.1126/sciadv.abb2454
85. Herbst S, Campbell P, Harvey J, Bernard EM, Papayannopoulos V, Wood NW, et al. LRRK2 activation controls the repair of damaged endomembranes in macrophages. *EMBO J.* (2020) 39:e104494. doi: 10.15252/embj.2020104494
86. Kuwahara T, Inoue K, D'Agati VD, Fujimoto T, Eguchi T, Saha S, et al. LRRK2 and RAB7L1 coordinately regulate axonal morphology and lysosome integrity in diverse cellular contexts. *Sci Rep.* (2016) 6:29945. doi: 10.1038/srep29945
87. Tong Y, Giaime E, Yamaguchi H, Ichimura T, Liu Y, Si H, et al. Loss of leucine-rich repeat kinase 2 causes age-dependent bi-phasic alterations of the autophagy pathway. *Mol Neurodegener.* (2012) 7:2. doi: 10.1186/1750-1326-7-2
88. Tong Y, Yamaguchi H, Giaime E, Boyle S, Kopan R, Kelleher, et al. Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of α-synuclein, and apoptotic cell death in aged mice. *Proc Natl Acad Sci USA.* (2010) 107:9879–84. doi: 10.1073/pnas.1004676107
89. Saez-Atienzar S, Bonet-Ponce L, Blesa JR, Romero FJ, Murphy MP, Jordan J, et al. The LRRK2 inhibitor GSK2578215A induces protective autophagy in SH-SY5Y cells: involvement of Drp-1-mediated mitochondrial fission and mitochondrial-derived ROS signaling. *Cell Death Dis.* (2014) 5:e1368. doi: 10.1038/cddis.2014.320
90. Schapansky J, Nardozzi JD, Felizia F, LaVoie MJ. Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. *Hum Mol Genet.* (2014) 23:4201–14. doi: 10.1093/hmg/ddu138
91. Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, et al. Interplay of LRRK2 with chaperone-mediated autophagy. *Nat Neurosci.* (2013) 16:394–406. doi: 10.1038/nn.3350
92. Mamais A, Manzoni C, Nazish I, Arber C, Sonustun B, Wray S, et al. Analysis of macroautophagy related proteins in G2019S LRRK2 Parkinson's disease brains with Lewy body pathology. *Brain Res.* (2018) 1701:75–84. doi: 10.1016/j.brainres.2018.07.023
93. Murphy KE, Gysbers AM, Abbott SK, Spiro AS, Furuta A, Cooper A, et al. Lysosomal-associated membrane protein 2 isoforms are differentially affected in early Parkinson's disease. *Mov Disord.* (2015) 30:1639–47. doi: 10.1002/mds.26141
94. Kim KS, Marcogliese PC, Yang J, Callaghan SM, Resende V, Abdel-Messih E, et al. Regulation of myeloid cell phagocytosis by LRRK2 via WAVE2 complex stabilization is altered in Parkinson's disease. *Proc Natl Acad Sci USA.* (2018) 115:E5164–73. doi: 10.1073/pnas.1718946115
95. Seaman MN. The retromer complex - endosomal protein recycling and beyond. *J Cell Sci.* (2012) 125:4693–702. doi: 10.1242/jcs.103440
96. MacLeod DA, Rhinn H, Kuwahara T, Zolin A, Di Paolo G, McCabe BD, et al. RAB7L1 interacts with LRRK2 to modify intraneuronal protein sorting and Parkinson's disease risk. *Neuron.* (2013) 77:425–39. doi: 10.1016/j.neuron.2012.11.033
97. Mir R, Tonelli F, Lis P, Macartney T, Polinski NK, Martinez TN, et al. The Parkinson's disease VPS35[D620N] mutation enhances LRRK2-mediated Rab protein phosphorylation in mouse and human. *Biochem J.* (2018) 475:1861–83. doi: 10.1042/BCJ20180248
98. Vilarino-Guell C, Wider C, Ross OA, Dachsel JC, Kachergus JM, Lincoln SJ, et al. VPS35 mutations in Parkinson disease. *Am J Hum Genet.* (2011) 89:162–7. doi: 10.1016/j.ajhg.2011.06.001
99. Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, Offman MN, et al. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am J Hum Genet.* (2011) 89:168–75. doi: 10.1016/j.ajhg.2011.06.008
100. Ando M, Funayama M, Li Y, Kashihara K, Murakami Y, Ishizu N, et al. VPS35 mutation in Japanese patients with typical Parkinson's disease. *Mov Disord.* (2012) 27:1413–7. doi: 10.1002/mds.25145
101. Kumar KR, Weissbach A, Heldmann M, Kasten M, Tunc S, Sue CM, et al. Frequency of the D620N mutation in VPS35 in Parkinson disease. *Arch Neurol.* (2012) 69:1360–4. doi: 10.1001/archneurol.2011.3367
102. Eleuteri S, Albanese A. VPS35-Based approach: a potential innovative treatment in Parkinson's disease. *Front Neurol.* (2019) 10:1272. doi: 10.3389/fneur.2019.01272
103. Seaman MN, McCaffery JM, Emr SD. A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. *J Cell Biol.* (1998) 142:665–81. doi: 10.1083/jcb.142.3.665
104. Williams ET, Chen X, Moore DJ. VPS35, the retromer complex and Parkinson's disease. *J Parkinsons Dis.* (2017) 7:219–33. doi: 10.3233/JPD-161020
105. Bonifacino JS, Hurley JH. Retromer. *Curr Opin Cell Biol.* (2008) 20:427–36. doi: 10.1016/j.ceb.2008.03.009

106. Seaman MN. Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer. *J Cell Biol.* (2004) 165:111–22. doi: 10.1083/jcb.200312034
107. van Weering JR, Sessions RB, Traer CJ, Kloer DP, Bhatia VK, Stamou D, et al. Molecular basis for SNX-BAR-mediated assembly of distinct endosomal sorting tubules. *EMBO J.* (2012) 31:4466–80. doi: 10.1038/emboj.2012.283
108. Wassmer T, Attar N, Bujny MV, Oakley J, Traer CJ, Cullen PJ. A loss-of-function screen reveals SNX5 and SNX6 as potential components of the mammalian retromer. *J Cell Sci.* (2007) 120:45–54. doi: 10.1242/jcs.03302
109. Nothwehr SF, Ha SA, Bruinsma P. Sorting of yeast membrane proteins into an endosome-to-Golgi pathway involves direct interaction of their cytosolic domains with Vps35p. *J Cell Biol.* (2000) 151:297–310. doi: 10.1083/jcb.151.2.297
110. Arighi CN, Hartnell LM, Aguilar RC, Haft CR, Bonifacino JS. Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. *J Cell Biol.* (2004) 165:123–33. doi: 10.1083/jcb.200312055
111. Follett J, Norwood SJ, Hamilton NA, Mohan M, Kovtun O, Tay S, et al. The Vps35 D620N mutation linked to Parkinson's disease disrupts the cargo sorting function of retromer. *Traffic.* (2014) 15:230–44. doi: 10.1111/tra.12136
112. McGough IJ, Steinberg F, Jia D, Barbuti PA, McMillan KJ, Heesom KJ, et al. Retromer binding to FAM21 and the WASH complex is perturbed by the Parkinson disease-linked VPS35(D620N) mutation. *Curr Biol.* (2014) 24:1670–6. doi: 10.1016/j.cub.2014.06.024
113. Tang FL, Erion JR, Tian Y, Liu W, Yin DM, Ye J, et al. VPS35 in dopamine neurons is required for endosome-to-golgi retrieval of Lamp2a, a receptor of chaperone-mediated autophagy that is critical for α-synuclein degradation and prevention of pathogenesis of Parkinson's disease. *J Neurosci.* (2015) 35:10613–28. doi: 10.1523/JNEUROSCI.0042-15.2015
114. Dhungel N, Eleuteri S, Li LB, Kramer NJ, Chartron JW, Spencer B, et al. Parkinson's disease genes VPS35 and EIF4G1 interact genetically and converge on α-synuclein. *Neuron.* (2015) 85:76–87. doi: 10.1016/j.neuron.2014.11.027
115. Alvarez-Erviti L, Rodriguez-Oroz MC, Cooper JM, Caballero C, Ferrer I, Obeso JA, et al. Chaperone-mediated autophagy markers in Parkinson disease brains. *Arch Neurol.* (2010) 67:1464–72. doi: 10.1001/archneurol.2010.198
116. Hampshire DJ, Roberts E, Crow Y, Bond J, Mubaidin A, Wriekat AL, et al. Kufor-Rakeb syndrome, pallido-pyramidal degeneration with supranuclear upgaze paresis and dementia, maps to 1p36. *J Med Genet.* (2001) 38:680–2. doi: 10.1136/jmg.38.10.680
117. Estiar MA, Leveille E, Spiegelman D, Dupre N, Trempe JF, Rouleau GA, et al. Clinical and genetic analysis of ATP13A2 in hereditary spastic paraplegia expands the phenotype. *Mol Genet Genomic Med.* (2020) 8:e1052. doi: 10.1002/mgg3.1052
118. Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ. Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. *Hum Mol Genet.* (2012) 21:2646–50. doi: 10.1093/hmg/dds089
119. Farias FH, Zeng R, Johnson GS, Wininger FA, Taylor JF, Schnabel RD, et al. A truncating mutation in ATP13A2 is responsible for adult-onset neuronal ceroid lipofuscinosis in Tibetan terriers. *Neurobiol Dis.* (2011) 42:468–74. doi: 10.1016/j.nbd.2011.02.009
120. van Veen S, Sorensen DM, Holemans T, Holen HW, Palmgren MG, Vangheluwe P. Cellular function and pathological role of ATP13A2 and related P-type transport ATPases in Parkinson's disease and other neurological disorders. *Front Mol Neurosci.* (2014) 7:48. doi: 10.3389/fnmol.2014.00048
121. van Veen S, Martin S, Van den Haute C, Benoy V, Lyons J, Vanhoutte R, et al. ATP13A2 deficiency disrupts lysosomal polyamine export. *Nature.* (2020) 578:419–24. doi: 10.1038/s41586-020-1968-7
122. Schmidt K, Wolfe DM, Stiller B, Pearce DA. Cd2+, Mn2+, Ni2+ and Se2+ toxicity to *Saccharomyces cerevisiae* lacking YPK9p the orthologue of human ATP13A2. *Biochem Biophys Res Commun.* (2009) 383:198–202. doi: 10.1016/j.bbrc.2009.03.151
123. Vrijsen S, Besora-Casals L, van Veen S, Zielich J, Van den Haute C, Hamouda NN, et al. ATP13A2-mediated endo-lysosomal polyamine export counters mitochondrial oxidative stress. *Proc Natl Acad Sci USA.* (2020) 117:31198–207. doi: 10.1073/pnas.1922342117
124. Dehay B, Martinez-Vicente M, Ramirez A, Perier C, Klein C, Vila M, et al. Lysosomal dysfunction in Parkinson disease: ATP13A2 gets into the groove. *Autophagy.* (2012) 8:1389–91. doi: 10.4161/auto.21011
125. Dehay B, Ramirez A, Martinez-Vicente M, Perier C, Canron MH, Doudnikoff E, et al. Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. *Proc Natl Acad Sci USA.* (2012) 109:9611–6. doi: 10.1073/pnas.1112368109
126. Demirsoy S, Martin S, Motamedi S, van Veen S, Holemans T, Van den Haute C, et al. ATP13A2/PARK9 regulates endo-/lysosomal cargo sorting and proteostasis through a novel PI(3, 5)P2-mediated scaffolding function. *Hum Mol Genet.* (2017) 26:1656–69. doi: 10.1093/hmg/ddx070
127. Bento CF, Ashkenazi A, Jimenez-Sanchez M, Rubinsztein DC. The Parkinson's disease-associated genes ATP13A2 and SYT11 regulate autophagy via a common pathway. *Nat Commun.* (2016) 7:11803. doi: 10.1038/ncomms11803
128. Matsui H, Sato F, Sato S, Koike M, Taruno Y, Saiki S, et al. ATP13A2 deficiency induces a decrease in cathepsin D activity, fingerprint-like inclusion body formation, and selective degeneration of dopaminergic neurons. *FEBS Lett.* (2013) 587:1316–25. doi: 10.1016/j.febslet.2013.02.046
129. Lopes da Fonseca T, Pinho R, Outeiro TF. A familial ATP13A2 mutation enhances α-synuclein aggregation and promotes cell death. *Hum Mol Genet.* (2016) 25:2959–71. doi: 10.1093/hmg/ddw147
130. Kong SM, Chan BK, Park JS, Hill KJ, Aitken JB, Cottle L, et al. Parkinson's disease-linked human PARK9/ATP13A2 maintains zinc homeostasis and promotes α-Synuclein externalization via exosomes. *Hum Mol Genet.* (2014) 23:2816–33. doi: 10.1093/hmg/ddu099
131. Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, et al. α-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet.* (2009) 41:308–15. doi: 10.1038/ng.300
132. Sato S, Koike M, Funayama M, Ezaki J, Fukuda T, Ueno T, et al. Lysosomal storage of subunit c of mitochondrial ATP synthase in brain-specific Atp13a2-deficient mice. *Am J Pathol.* (2016) 186:3074–82. doi: 10.1016/j.ajpath.2016.08.006
133. Dirr ER, Ekhator OR, Blackwood R, Holden JG, Masliah E, Schultheis PJ, et al. Exacerbation of sensorimotor dysfunction in mice deficient in Atp13a2 and overexpressing human wildtype α-synuclein. *Behav Brain Res.* (2018) 343:41–9. doi: 10.1016/j.bbr.2018.01.029
134. Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, et al. Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited α-synuclein accumulation and age-dependent sensorimotor deficits. *Hum Mol Genet.* (2013) 22:2067–82. doi: 10.1093/hmg/ddt057
135. Wang R, Tan J, Chen T, Han H, Tian R, Tan Y, et al. ATP13A2 facilitates HDAC6 recruitment to lysosome to promote autophagosome-lysosome fusion. *J Cell Biol.* (2019) 218:267–84. doi: 10.1083/jcb.201804165
136. Murphy KE, Cottle L, Gysbers AM, Cooper AA, Halliday GM. ATP13A2 (PARK9) protein levels are reduced in brain tissue of cases with Lewy bodies. *Acta Neuropathol Commun.* (2013) 1:11. doi: 10.1186/2051-5960-1-11
137. Lwin A, Orvisky E, Goker-Alpan O, LaMarca ME, Sidransky E. Glucocerebrosidase mutations in subjects with parkinsonism. *Mol Genet Metab.* (2004) 81:70–3. doi: 10.1016/j.ymgme.2003.11.004
138. O'Regan G, deSouza RM, Balestrino R, Schapira AH. Glucocerebrosidase mutations in Parkinson disease. *J Parkinsons Dis.* (2017) 7:411–22. doi: 10.3233/JPD-171092
139. Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurol.* (2012) 11:986–98. doi: 10.1016/S1474-4422(12)70190-4
140. Machaczka M, Rucinska M, Skotnicki AB, Jurczak W. Parkinson's syndrome preceding clinical manifestation of Gaucher's disease. *Am J Hematol.* (1999) 61:216–7. doi: 10.1002/(SICI)1096-8652(199907)61:3andlt;216::AID-AJH12andgt;3.0.CO;2-B
141. Neudorfer O, Giladi N, Elstein D, Abrahamov A, Turezkite T, Aghai E, et al. Occurrence of Parkinson's syndrome in type I Gaucher disease. *QJM.* (1996) 89:691–64. doi: 10.1093/qjmed/89.9.691
142. Iwaki H, Blauwendraat C, Leonard HL, Liu G, Maple-Grodem J, Corvol JC, et al. Genetic risk of Parkinson disease and progression: AN analysis of 13 longitudinal cohorts. *Neurol Genet.* (2019) 5:e348. doi: 10.1212/NXG.0000000000000348

143. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* (2009) 361:1651–61. doi: 10.1056/NEJMoa0901281
144. Zhang Y, Sun QY, Zhao YW, Shu L, Guo JF, Xu Q, et al. Effect of GBA mutations on phenotype of Parkinson's disease: a study on Chinese population and a meta-analysis. *Parkinsons Dis.* (2015) 2015:916971. doi: 10.1155/2015/916971
145. Cilia R, Tunesi S, Marotta G, Cereda E, Siri C, Tesei S, et al. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann Neurol.* (2016) 80:662–73. doi: 10.1002/ana.24777
146. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA Neurol.* (2013) 70:727–35. doi: 10.1001/jamaneurol.2013.1925
147. Liu G, Boot B, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, et al. Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann Neurol.* (2016) 80:674–85. doi: 10.1002/ana.24781
148. Winder-Rhodes SE, Evans JR, Ban M, Mason SL, Williams-Gray CH, Foltynie T, et al. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. *Brain.* (2013) 136:392–9. doi: 10.1093/brain/aws318
149. Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain.* (2015) 138:2648–58. doi: 10.1093/brain/awv179
150. Huh YE, Chiang MSR, Locascio JJ, Liao Z, Liu G, Choudhury K, et al. beta-Glucocerebrosidase activity in GBA-linked Parkinson disease: the type of mutation matters. *Neurology.* (2020) 95:e685–96. doi: 10.1212/WNL.0000000000009989
151. Butters TD. Gaucher disease. *Curr Opin Chem Biol.* (2007) 11:412–8. doi: 10.1016/j.cbpa.2007.05.035
152. Ron I, Horowitz M. ER retention and degradation as the molecular basis underlying Gaucher disease heterogeneity. *Hum Mol Genet.* (2005) 14:2387–98. doi: 10.1093/hmg/ddi240
153. Schmitz M, Alfalah M, Aerts JM, Naim HY, Zimmer KP. Impaired trafficking of mutants of lysosomal glucocerebrosidase in Gaucher's disease. *Int J Biochem Cell Biol.* (2005) 37:2310–20. doi: 10.1016/j.biocel.2005.05.008
154. Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology.* (2015) 84:880–7. doi: 10.1212/WNL.0000000000001315
155. Gegg ME, Burke D, Heales SJ, Cooper JM, Hardy J, Wood NW, et al. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. *Ann Neurol.* (2012) 72:455–63. doi: 10.1002/ana.23614
156. Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, et al. Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell.* (2011) 146:37–52. doi: 10.1016/j.cell.2011.06.001
157. Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, et al. Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson's disease. *Brain.* (2014) 137:834–48. doi: 10.1093/brain/awt367
158. Burbulla LF, Song P, Mazzulli JR, Zampese E, Wong YC, Jeon S, et al. Dopamine oxidation mediates mitochondrial and lysosomal dysfunction in Parkinson's disease. *Science.* (2017) 357:1255–61. doi: 10.1126/science.aam9080
159. Nguyen M, Krainc D. LRRK2 phosphorylation of auxilin mediates synaptic defects in dopaminergic neurons from patients with Parkinson's disease. *Proc Natl Acad Sci USA.* (2018) 115:5576–81. doi: 10.1073/pnas.1717590115
160. Balestrino R, Schapira AHV. Glucocerebrosidase and Parkinson disease: molecular, clinical, therapeutic implications. *Neuroscientist.* (2018) 24:540–59. doi: 10.1177/1073858417748875
161. Manning-Bog AB, Schule B, Langston JW. α-synuclein-glucocerebrosidase interactions in pharmacological Gaucher models: a biological link between Gaucher disease and parkinsonism. *Neurotoxicology.* (2009) 30:1127–32. doi: 10.1016/j.neuro.2009.06.009
162. Zunke F, Moise AC, Belur NR, Gelyana E, Stojkovska I, Dzaferbegovic H, et al. Reversible conformational conversion of α-Synuclein into toxic assemblies by glucosylceramide. *Neuron.* (2018) 97:92–107.e110. doi: 10.1016/j.neuron.2017.12.012
163. Fecchio C, Palazzi L, de Laureto PP. α-Synuclein and Polyunsaturated fatty acids: molecular basis of the interaction and implication in neurodegeneration. *Molecules.* (2018) 23:1531. doi: 10.3390/molecules23071531
164. Morabito G, Giannelli SG, Ordazzo G, Bido S, Castoldi V, Indrigo M, et al. AAV-PHP.B-Mediated global-scale expression in the mouse nervous system enables GBA1 gene therapy for wide protection from synucleinopathy. *Mol Ther.* (2017) 25:2727–42. doi: 10.1016/j.ymthe.2017.08.004
165. Mus L, Siani F, Giuliano C, Ghezzi C, Cerri S, Blandini F. Development and biochemical characterization of a mouse model of Parkinson's disease bearing defective glucocerebrosidase activity. *Neurobiol Dis.* (2019) 124:289–96. doi: 10.1016/j.nbd.2018.12.001
166. Soria FN, Engeln M, Martinez-Vicente M, Glangetas C, Lopez-Gonzalez MJ, Dovero S, et al. Glucocerebrosidase deficiency in dopaminergic neurons induces microglial activation without neurodegeneration. *Hum Mol Genet.* (2017) 26:2603–15. doi: 10.1093/hmg/ddx120
167. Atashrazm F, Hammond D, Perera G, Dobson-Stone C, Mueller N, Pickford R, et al. Reduced glucocerebrosidase activity in monocytes from patients with Parkinson's disease. *Sci Rep.* (2018) 8:15446. doi: 10.1038/s41598-018-33921-x
168. Duran R, Mencacci NE, Angeli AV, Shoai M, Deas E, Houlden H, et al. The glucocerobrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Mov Disord.* (2013) 28:232–6. doi: 10.1002/mds.25248
169. Spitz M, Pereira JS, Nicareta DH, Abreu Gde M, Bastos EF, Seixas TL, et al. Association of LRRK2 and GBA mutations in a Brazilian family with Parkinson's disease. *Parkinsonism Relat Disord.* (2015) 21:825–6. doi: 10.1016/j.parkreldis.2015.03.029
170. Yahalom G, Greenbaum L, Israeli-Korn S, Fay-Karmon T, Livneh V, Ruskey JA, et al. Carriers of both GBA and LRRK2 mutations, compared to carriers of either, in Parkinson's disease: Risk estimates and genotype-phenotype correlations. *Parkinsonism Relat Disord.* (2019) 62:179–84. doi: 10.1016/j.parkreldis.2018.12.014
171. Ysselstein D, Nguyen M, Young TJ, Severino A, Schwake M, Merchant K, et al. LRRK2 kinase activity regulates lysosomal glucocerebrosidase in neurons derived from Parkinson's disease patients. *Nat Commun.* (2019) 10:5570. doi: 10.1038/s41467-019-13413-w
172. Sanyal A, Novis HS, Gasser E, Lin S, LaVoie MJ. LRRK2 kinase inhibition rescues deficits in lysosome function due to heterozygous GBA1 expression in human iPSC-derived neurons. *Front Neurosci.* (2020) 14:442. doi: 10.3389/fnins.2020.00442
173. Lie PPY, Nixon RA. Lysosome trafficking and signaling in health and neurodegenerative diseases. *Neurobiol Dis.* (2019) 122:94–105. doi: 10.1016/j.nbd.2018.05.015
174. Ambrosi G, Ghezzi C, Zangaglia R, Levandis G, Pacchetti C, Blandini F. Ambroxol-induced rescue of defective glucocerebrosidase is associated with increased LIMP-2 and saposin C levels in GBA1 mutant Parkinson's disease cells. *Neurobiol Dis.* (2015) 82:235–42. doi: 10.1016/j.nbd.2015.06.008
175. Bendikov-Bar I, Maor G, Filocamo M, Horowitz M. Ambroxol as a pharmacological chaperone for mutant glucocerebrosidase. *Blood Cells Mol Dis.* (2013) 50:141–5. doi: 10.1016/j.bcmd.2012.10.007
176. McNeill A, Magalhaes J, Shen C, Chau KY, Hughes D, Mehta A, et al. Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. *Brain.* (2014) 137:1481–95. doi: 10.1093/brain/awu020
177. Migdalska-Richards A, Daly L, Bezard E, Schapira AH. Ambroxol effects in glucocerebrosidase and α-synuclein transgenic mice. *Ann Neurol.* (2016) 80:766–75. doi: 10.1002/ana.24790
178. Mishra A, Krishnamurthy S. Neurorestorative effects of sub-chronic administration of ambroxol in rodent model of Parkinson's disease. *Naunyn Schmiedebergs Arch Pharmacol.* (2020) 393:429–44. doi: 10.1007/s00210-019-01737-9
179. Richter F, Fleming SM, Watson M, Lemesre V, Pellegrino L, Ranes B, et al. A GCase chaperone improves motor function in a mouse model of synucleinopathy. *Neurotherapeutics.* (2014) 11:840–56. doi: 10.1007/s13311-014-0294-x
180. Sardi SP, Clarke J, Kinnecom C, Tamsett TJ, Li L, Stanek LM, et al. CNS expression of glucocerebrosidase corrects α-synuclein pathology and

memory in a mouse model of Gaucher-related synucleinopathy. *Proc Natl Acad Sci USA*. (2011) 108:12101–6. doi: 10.1073/pnas.1108197108
181. Sardi SP, Clarke J, Viel C, Chan M, Tamsett TJ, Treleaven CM, et al. Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies. *Proc Natl Acad Sci USA*. (2013) 110:3537–42. doi: 10.1073/pnas.1220464110
182. Sardi SP, Viel C, Clarke J, Treleaven CM, Richards AM, Park H, et al. Glucosylceramide synthase inhibition alleviates aberrations in synucleinopathy models. *Proc Natl Acad Sci USA*. (2017) 114:2699–704. doi: 10.1073/pnas.1616152114
183. Hernandez I, Luna G, Rauch JN, Reis SA, Giroux M, Karch CM, et al. A farnesyltransferase inhibitor activates lysosomes and reduces tau pathology in mice with tauopathy. *Sci Transl Med*. (2019) 11: eaat3005. doi: 10.1126/scitranslmed.aat3005
184. Mullard A. FDA outlines steps for individualized drug development. *Nat Rev Drug Discov*. (2021) 20:91. doi: 10.1038/d41573-021-00009-7
185. Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Bjorklund A. TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. *Proc Natl Acad Sci USA*. (2013) 110:E1817–26. doi: 10.1073/pnas.1305623110
186. Leeman DS, Hebestreit K, Ruetz T, Webb AE, McKay A, Pollina EA, et al. Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. *Science*. (2018) 359:1277–83. doi: 10.1126/science.aag3048
187. Johmura Y, Yamanaka T, Omori S, Wang TW, Sugiura Y, Matsumoto M, et al. Senolysis by glutaminolysis inhibition ameliorates various age-associated disorders. *Science*. (2021) 371:265–70. doi: 10.1126/science.abb5916
188. Martina JA, Chen Y, Gucek M, Puertollano R. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy*. (2012) 8:903–14. doi: 10.4161/auto.19653
189. Crews L, Spencer B, Desplats P, Patrick C, Paulino A, Rockenstein E, et al. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of α-synucleinopathy. *PLoS ONE*. (2010) 5:e9313. doi: 10.1371/journal.pone.0009313
190. Bai X, Wey MC, Fernandez E, Hart MJ, Gelfond J, Bokov AF, et al. Rapamycin improves motor function, reduces 4-hydroxynonenal adducted protein in brain, and attenuates synaptic injury in a mouse model of synucleinopathy. *Pathobiol Aging Age Relat Dis*. (2015) 5:28743. doi: 10.3402/pba.v5.28743
191. Blagosklonny MV. Rapamycin for longevity: opinion article. *Aging*. (2019) 11:8048–67. doi: 10.18632/aging.102355
192. Hoffmann AC, Minakaki G, Menges S, Salvi R, Savitskiy S, Kazman A, et al. Extracellular aggregated α-synuclein primarily triggers lysosomal dysfunction in neural cells prevented by trehalose. *Sci Rep*. (2019) 9:544. doi: 10.1038/s41598-018-35811-8
193. Lan DM, Liu FT, Zhao J, Chen Y, Wu JJ, Ding ZT, et al. Effect of trehalose on PC12 cells overexpressing wild-type or A53T mutant α-synuclein. *Neurochem Res*. (2012) 37:2025–32. doi: 10.1007/s11064-012-0823-0
194. Rodríguez-Navarro JA, Rodríguez L, Casarejos MJ, Solano RM, Gómez A, Perucho J, et al. Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. *Neurobiol Dis*. (2010) 39:423–38. doi: 10.1016/j.nbd.2010.05.014
195. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and α-synuclein. *J Biol Chem*. (2007) 282:5641–52. doi: 10.1074/jbc.M609532200
196. DeBosch BJ, Heitmeier MR, Mayer AL, Higgins CB, Crowley JR, Kraft TE, et al. Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. *Sci Signal*. (2016) 9:ra21. doi: 10.1126/scisignal.aac5472
197. Tanji K, Miki Y, Maruyama A, Mimura J, Matsumiya T, Mori F, et al. Trehalose intake induces chaperone molecules along with autophagy in a mouse model of Lewy body disease. *Biochem Biophys Res Commun*. (2015) 465:746–52. doi: 10.1016/j.bbrc.2015.08.076
198. He Q, Koprich JB, Wang Y, Yu WB, Xiao BG, Brotchie JM, et al. Treatment with trehalose prevents behavioral and neurochemical deficits produced in an AAV α-Synuclein rat model of Parkinson's disease. *Mol Neurobiol*. (2016) 53:2258–68. doi: 10.1007/s12035-015-9173-7
199. Yu HC, Lin CS, Tai WT, Liu CY, Shiau CW, Chen KF. Nilotinib induces autophagy in hepatocellular carcinoma through AMPK activation. *J Biol Chem*. (2013) 288:18249–59. doi: 10.1074/jbc.M112.446385
200. Hebron ML, Lonskaya I, Moussa CE. Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of α-synuclein in Parkinson's disease models. *Hum Mol Genet*. (2013) 22:3315–28. doi: 10.1093/hmg/ddt192
201. Xilouri M, Brekk OR, Landeck N, Pitychoutis PM, Papasilekas T, Papadopoulou-Daifoti Z, et al. Boosting chaperone-mediated autophagy *in vivo* mitigates α-synuclein-induced neurodegeneration. *Brain*. (2013) 136:2130–46. doi: 10.1093/brain/awt131
202. Anguiano J, Garner TP, Mahalingam M, Das BC, Gavathiotis E, Cuervo AM. Chemical modulation of chaperone-mediated autophagy by retinoic acid derivatives. *Nat Chem Biol*. (2013) 9:374–82. doi: 10.1038/nchembio.1230
203. Jegga AG, Schneider L, Ouyang X, Zhang J. Systems biology of the autophagy-lysosomal pathway. *Autophagy*. (2011) 7:477–89. doi: 10.4161/auto.7.5.14811
204. Su C, Yang X, Lou J. Geniposide reduces α-synuclein by blocking microRNA-21/lysosome-associated membrane protein 2A interaction in Parkinson disease models. *Brain Res*. (2016) 1644:98–106. doi: 10.1016/j.brainres.2016.05.011
205. Mullin S, Smith L, Lee K, D'Souza G, Woodgate P, Elflein J, et al. Ambroxol for the treatment of patients with Parkinson disease with and without glucocerebrosidase gene mutations: a nonrandomized, noncontrolled trial. *JAMA Neurol*. (2020) 77:427–34. doi: 10.1001/jamaneurol.2019.4611
206. Pagan FL, Wilmarth B, Torres-Yaghi Y, Hebron ML, Mulki S, Ferrante D, et al. Long-term safety and clinical effects of nilotinib in Parkinson's disease. *Mov Disord*. (2021) 36:740–9. doi: 10.1002/mds.28389
207. Simuni T, Fiske B, Merchant K, Coffey CS, Klingner E, Caspell-Garcia C, et al. Efficacy of nilotinib in patients with moderately advanced Parkinson disease: a randomized clinical trial. *JAMA Neurol*. (2021) 78:312–20. doi: 10.1001/jamaneurol.2020.4725
208. Tolosa E, Vila M, Klein C, Rascol O. LRRK2 in Parkinson disease: challenges of clinical trials. *Nat Rev Neurol*. (2020) 16:97–107. doi: 10.1038/s41582-019-0301-2

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REVIEW
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Genetic Defects and Pro-inflammatory Cytokines in Parkinson's Disease

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Parkinson's disease (PD) is a movement disorder attributed to the loss of dopaminergic (DA) neurons mainly in the substantia nigra pars compacta. Motor symptoms include resting tremor, rigidity, and bradykinesias, while non-motor symptoms include autonomic dysfunction, anxiety, and sleeping problems. Genetic mutations in a number of genes (e.g., *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7*) and the resultant abnormal activation of microglial cells are assumed to be the main reasons for the loss of DA neurons in PD with genetic causes. Additionally, immune cell infiltration and their participation in major histocompatibility complex I (MHCI) and/or MHCII-mediated processing and presentation of cytosolic or mitochondrial antigens activate the microglial cells and cause the massive generation of pro-inflammatory cytokines and chemokines, which are all critical for the propagation of brain inflammation and the neurodegeneration in PD with genetic and idiopathic causes. Despite knowing the involvement of several of such immune devices that trigger neuroinflammation and neurodegeneration in PD, the exact disease mechanism or the innovative biomarker that could detect disease severity in PD linked to *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7* defects is largely unknown. The current review has explored data from genetics, immunology, and *in vivo* and *ex vivo* functional studies that demonstrate that certain genetic defects might contribute to microglial cell activation and massive generation of a number of pro-inflammatory cytokines and chemokines, which ultimately drive the brain inflammation and lead to neurodegeneration in PD. Understanding the detailed involvement of a variety of immune mediators, their source, and the target could provide a better understanding of the disease process. This information might be helpful in clinical diagnosis, monitoring of disease progression, and early identification of affected individuals.

Keywords: neuroimmunology, immunogenetics, innate and adaptive immunity, glycosphingolipid, aggregated proteins, brain disease, neuroinflammation, mitochondrial disease

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative brain disorder that mainly happens due to progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNPC) and its impact on impairment of motor function that includes static tremor, bradykinesia, muscle stiffness, postural instability, balance difficulty, and walking problem (1, 2). Pro-inflammatory cytokines and chemokines have been linked to disease manifestations of Alzheimer's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis, prion disease, systemic lupus erythematosus, depression, migraine, and schizophrenia as reviewed in refs. (3–12). Microglial cells (MGCs) are residential macrophages (Mϕs) of the central nervous system (CNS), which are exquisitely sensitive to the pathophysiological insults and the resultant alteration in their morphology and phenotype to activated state (13). Such MGCs cause massive generation of pro-inflammatory cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (NO), which all contribute to the clearance of infectious agents (14). However, prolonged or excessive activation of MGCs results in pathological forms of inflammation that contribute to the progression of neurodegenerative and neoplastic diseases (15–17). Activated MGCs express major histocompatibility complex II (MHC class II), which is required for activation of naive $CD4^+$ T cells and the production of numerous pro-inflammatory cytokines and chemokines that modulate the differentiation of effector T cells (18).

Effector T cells, i.e., T helper 1 (Th1), Th2, Th17, T regulatory (Treg), and T follicular helper (Tfh) cells as well as their signature cytokines, i.e., interferon gamma (IFNγ; TH1), interleukin 4 (IL-4; TH2) (19, 20), IL-17 (TH17) (21, 22), transforming growth factor beta (TGFβ; Treg), and IL-6 (Tfh), drive tissue inflammation in several visceral and brain diseases (23–28). The T helper cell subsets can produce IL-10, a cytokine with broad immunoregulatory properties (29). Th1 cells produce IFNγ, IL-2, and tumor necrosis factor alpha (TNFα) to clear intracellular pathogens and evoke cell-mediated immunity, whereas Th2 cells produce IL-4, IL-5, and IL-13 to clear extracellular organisms and evoke strong allergic responses (19, 30–33). In contrast to Th1 and Th2 cell differentiation, which depend on their respective effector cytokines (IFNγ and IL-4), Th17 cell differentiation does not require IL-17 but has a critical need for TGFβ and IL-6 (34–36). Treg cells produce IL-10 and TGFβ to cause immune tolerance and inhibit IFNγ synthesis (37) as well as block T helper cell differentiation of naive T cells into effector T cells (38).

The MGCs' interaction to effector T cells and the resulting production of pro-inflammatory cytokines, chemokines, and the neurodegeneration have been observed in Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis (MS), and prion diseases (17, 39, 40). The SNPC of PD patients have shown $CD4^+$ T cells, $CD8^+$ T cells, human leukocyte antigen DR isotype (HLA-DR) expressing inflammatory subset of MGCs, and increased incidence of pro-inflammatory cytokines, i.e., IFNγ, TNF, IL-1β expressing glial cells (41–43). Additionally, the striatal dopaminergic (DA) regions and cerebrospinal fluid (CSF) of PD patients have shown elevated levels of IL-1β, IL-2, IL-6, TNF, and TGFβ1 (44, 45). Peripheral blood analyses of PD patients have shown marked increases of innate and adaptive immune cells that include monocytes (MOs), IFNγ, IL-4, and IL-17 producing memory and effector T cells as well as their association to severity of the disease (43, 46–51). Elevated serum levels of TNF (52, 53), IL-1β (52, 54, 55), and IL-6 (52–54) have been observed in PD patients as reviewed in Qin et al. (56). PD patients have also shown increased serum level of cytokine receptors such as TNF receptors (e.g., TNFRs) and their link to late disease onset (57, 58). MO differentiation into the tissue-specific MGCs, Mϕs, and dendritic cells (DCs) as well as the trafficking of $CD4^+$ and $CD8^+$ T cells to sites of inflammation requires growth factors, i.e., granulocyte colony-stimulating factor (GCSF), granulocyte Mϕ colony-stimulating factor (GMCSF), and the Mϕ colony-stimulating factor (MCSF), as well as the number of C-C motif ligand (CCL) and the C-X-C motif ligand (CXCL) chemokines (59–69). However, the exact mechanism by which such immune inflammation occurs in PD is unknown. It is speculated that abnormal brain or circulatory level of several proteins and enzymes has been associated with the development of neuroinflammation in PD. Indeed, several of such proteins have been associated with activation of residential MGCs and the infiltrated lymphocytes and their combined impact on the generation of pro-inflammatory cytokines (e.g., IFNβ, IFNγ, TNFα, IL-1β, IL-6, IL-18, and TGFβ1), which lead to the loss of DA neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 2,4,5-trihydroxyphenethylamine or 6-hydroxydopamin (6-OHDA)-induced mouse models of idiopathic PD (**Table 1A**). Additionally, human patients with idiopathic PD have also suggested elevated brain or circulatory level of proteins or enzymes linked to MGC activation, pro-inflammatory cytokine and chemokine (e.g., IFNβ, IFNγ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, CCL2, CXCL1) production, loss of DA neurons, and the development of motor symptoms (**Table 1B**). The current review is an update on the involvement of a variety of innate and adaptive immune mediators as well as their source and targets involved in the propagation of disease manifestations in mouse and human PD associated with *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7* defects. These results will likely provide much needed insights into the disease mechanism and will be useful for the identification of potential biomarkers at the level of distinguished cytokines and chemokines in different forms of PD.

LRRK2 GENE DEFECTS AND PRO-INFLAMMATORY IMMUNE MEDIATORS IN PD

The leucine-rich-repeat kinase 2 (*LRRK2*) gene encodes a large, multidomain LRRK2 protein comprised of a GTPase and a kinase domain (85). Although the precise physiological function of LRRK2 remains largely unknown, recent studies have indicated that LRRK2 is involved in cellular functions such as neurite outgrowth, cytoskeletal maintenance, vesicle

TABLE 1A | Cytokines and their source in the mouse model of idiopathic PD.

PD mouse model	Proteins/enzymes and their source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
MPTP- and 6-OHDA-induced disease	Striatum$^{\alpha-syn\ (P+)}$ Striatum$^{TH\ (P-)}$ Striatum$^{DA\ (P-)}$ SNPC$^{\alpha-syn\ (P+)}$ SNPR$^{\alpha-syn\ (P+)}$ Thalamus$^{\alpha-syn\ (P+)}$ DG$^{\alpha-syn\ (P+)}$ AON$^{\alpha-syn\ (P+)}$ OB$^{\alpha-syn\ (P+)}$ MC$^{\alpha-syn\ (P+)}$ SC$^{\alpha-syn\ (P+)}$ OC$^{\alpha-syn\ (P+)}$	IFNγ	SNPC^{P+} Sera^{P+} Striatum^{P+}	DA neuron death in SN, striatum, and NP	(70–72)
MPTP- and R-APO-induced disease	Striatum$^{TH\ (P-)}$ NP$^{NOSP\ (P+)}$ NP$^{OSPA170\ (P+)}$ MGCs$^{NADPHoxidase\ (P+)}$ ACs$^{Oxidase\ (P+)}$ MGCs$^{iNOS\ (P+)}$	TNFα	SNPC$^{P+andM+}$ Sera$^{P+andM+}$ Striatum^{P+} CP^{M+}	DA neuron death in SN, striatum, and NP	(71–76)
MPTP-induced disease	Striatum$^{TH\ (P-)}$ NP$^{NOS\ (P+)}$ NP$^{OSPA170\ (P+)}$ MGCs$^{NLRP3\ (P+)}$ MGCs$^{NADPHoxidase\ (P+)}$ ACs$^{myeloperoxidase\ (P+)}$ ACs$^{\circ xidase\ (P+)}$ MGCs$^{iNOS\ (P+)}$ SN-MGCs$^{NLRP3\ (P+)}$ SN-ACs$^{NLRP3\ (P+)}$ NCs$^{Caspase1\ (P+)}$	IL-1β	SNPC^{P+} Sera^{P+} Striatum^{P+} SN^{M+} CP^{M+} MB^{M+}	MGC activation, DA neuron death in SN, striatum, and NP	(71–78)
MPTP- and R-APO-induced disease	Striatum$^{TH\ (P-)}$ NP$^{NOS\ (P+)}$ NP$^{OSPA170\ (P+)}$	IL-6	CP^{M+}	DA neuron death in SN, striatum, and NP	(71–74)
MPTP-induced disease	SN-MGCs$^{NLRP3\ (P+)}$ SN-ACs$^{NLRP3\ (P+)}$ NCs$^{Caspase1\ (P+)}$	IL-18	MB^{M+}	MGC activation, DA neuron death in NP	(78)
MPTP-induced disease	Striatum$^{TH\ (P-)}$	TGFβ1	Striatum^{P+}	DA neuron death in NP	(72)

NCs, neurons; NP, nigrostriatal pathway; ACs, astrocytes; MGCs, microglial cells; α-syn, alpha-synuclein; TH, tyrosine hydroxylase; DA, dopamine; SN, substantia nigra; SNPC, substantia nigra pars compacta; SNPC, substantia nigra pars compacta; SNPR, substantia nigra par reticulata; DG, dentate gyrus; Hipp, hippocampus; AON, anterior olfactory nucleus; OB, olfactory bulb; MC, motor cortex; SC, sensory cortex; OC, orbital cortex; CP, caudate putamen; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; R-APO, R-apomorphine; NOS, nitric oxide synthase; OSPA170, oxidative stress-induced Protein A 170; M, mRNA expression; P, protein expression; +, increased level; –, decreased level; ∞, no change; ND, no data.

trafficking, autophagic protein degradation, and the regulation of signaling pathways, including the Wingless-INT (WNT), Fas-Fas ligand (FasL or CD95L or CD178)-associated protein with death domain (FADD), mitogen-activated protein kinase (MAPK), and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) (86–88).

The resting neuronal cells, i.e., neurons (NCs), MGCs, and astrocytes (ACs), expressed a low level of LRRK2 (89, 90). However, several of the pro-inflammatory mediators (e.g., IFNβ, IFNγ, TNFα, IL-6, and LPS) cause upregulation of LRRK2 in immune cells, i.e., monocytes (MOs), Mϕs, and T and B cells, and in neuronal cells, i.e., MGCs and NCs (88, 91–95). LRRK2 is critical for the propagation of Crohn's disease (96, 97), leprosy (98), and neuronal toxicity (99–102).

Indeed, *LRRK2* gene mutations have been linked to increased LRRK2 kinase substrate phosphorylation and the formation of intracellular alpha-synuclein (α-syn)-positive inclusions in Lewy bodies (LBs) and preferential loss of DA neurons and the development of motor symptoms, including tremor, rigidity, postural instability, and bradykinesia in late-onset familial and idiopathic PD (100, 103–119). The brain regions, blood, and cells of LRRK2-associated mouse models of PD have shown abnormal expression of LRRK2 kinase and their association with elevated brain and circulatory level of pro-inflammatory cytokines (e.g., IFNγ, TNFα, IL-1α, IL-1β, IL-6, IL-8, IL-10, and IL-12), chemokines (e.g., CCL2, CCL3, CCL4, CCL5, CXCL1, and CXCL10), and growth factors (e.g., GCSF and MCSF), as well as their link to the loss of NCs and the development of cognitive

TABLE 1B | Cytokines and their source in idiopathic human PD.

Human PD	Proteins/enzymes and their source	Pro-inflammatory cytokines and chemokines and their source		Brain defects	References
Idiopathic	$Sera^{PINK1\ (M/P-)}$ $Sera^{Parkin\ (M/P-)}$	IFN-β1	$Sera^{P+}$	Inflammation, motor defects, and loss of DA neurons	(79)
Idiopathic	$Sera^{TBARS\ (P+)}$ $Lymphocytes^{(P+)}$	IFN-γ	$Sera^{P+}$	Damage of DA neurons in nigrostriatal regions	(71)
Idiopathic	$Sera^{TBARS\ (P+)}$ $Lymphocytes^{(P+)}$ $Fibroblast^{COX-2\ (M+)}$	TNFα	$Sera^{P+}$ $Fibroblast^{P+}$ $Blood^{P+}$	Inflammation, damage of DA neurons in nigrostriatal regions	(56, 71, 80)
Idiopathic	$Sera^{TBARS\ (P+)}$ $Lymphocytes^{(P+)}$	IL-1β	$Sera^{P+}$ $Blood^{P+}$	Inflammation, damage of DA neurons in nigrostriatal regions	(56, 71, 81)
Idiopathic	$Sera^{TBARS\ (P+)}$ $Lymphocytes^{(P+)}$	IL-2	$Sera^{P+}$	Inflammation, damage of DA neurons in nigrostriatal regions	(71, 73, 82)
Idiopathic	$Sera^{TBARS\ (P+)}$ $Lymphocytes^{(P+)}$	IL-4	$Sera^{P+}$ $Blood^{P+}$	Inflammation, damage of DA neurons in nigrostriatal regions	(56, 71)
Idiopathic	$Sera^{TBARS\ (P+)}$ $Lymphocytes^{(P+)}$ $Fibroblast^{COX-2\ (M+)}$	IL-6	$Sera^{P+}$ $Fibroblast^{P+}$ $Blood^{P+}$ $Plasma^{P+}$	Inflammation, damage of DA neurons in nigrostriatal regions	(56, 71, 80, 83)
Idiopathic	$CRP^{(P+)}$	IL-10	$Blood^{P+}$	Inflammation, damage of DA neurons in nigrostriatal regions	(56)
Idiopathic	$Sera^{PINK1\ (M/P-)}$ $Sera^{Parkin\ (M/P-)}$	IL-12	$Sera^{P+}$	Inflammation, motor defects, and loss of DA neurons	(79)
Idiopathic	$Sera^{PINK1\ (M/P-)}$ $Sera^{Parkin\ (M/P)}$	IL-13	$Sera^{P+}$	Inflammation, motor defects, and loss of DA neurons	(79)
Idiopathic	$Sera^{PINK1\ (M/P-)}$ $Sera^{Parkin\ (M/P-)}$	CCL2/MCP1	$Sera^{P+}$	Inflammation, motor defects, and loss of DA neurons	(79)
Idiopathic	$Sera^{PINK1\ (M/P-)}$ $Sera^{Parkin\ (M/P-)}$	CXCL1/KC	$Sera^{P+}$	Inflammation, motor defects, and loss of DA neurons	(79)
Idiopathic	$Blood^{CRP\ (P+)}$ $CSF^{CRP\ (P+)}$	hs-CRP	$Sera^{P+}$ $Blood^{P+}$ $Plasma^{P+}$ CSF^{P+}	Inflammation, loss of DA neurons	(56, 84)

PINK1, PTEN-induced kinase 1; Parkin, 465-residue E3 ubiquitin ligase; TBARS, thiobarbituric acid reactive substances; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CXCL1, chemokine C-X-C motif ligand-1; CCL2, chemokine C-C motif ligand-2; CRP, C-reactive protein; hs-CRP, high sensitivity C-reactive protein; CSF, cerebrospinal fluid; COX-2, cyclooxygenase-2; DA, dopaminergic; M, mRNA expression; P, protein expression; +, higher/increased levels; –, decreased/lower levels; ∞, no change; ND, no data.

defects (**Table 2A**). The blood cells, sera, and CSF of LRRK2-associated human patients with PD have also shown abnormal expression of LRRK2 kinase and their link to elevated levels of pro-inflammatory cytokines and growth factors (e.g., IFNγ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, GCSF, PDGF, and VEGF), loss of NCs, and the development of cognitive defects in PD (**Table 2B**). These data suggest that LRKK2 defects and the resultant higher expression of LRRK2 kinases cause cellular activation and the higher generation of pro-inflammatory cytokines and chemokines (**Tables 2A**,**B**) that lead to DA neuron damage in LRRK2-associated PD (**Figure 1A**).

GBA1 GENE DEFECTS AND PRO-INFLAMMATORY IMMUNE MEDIATORS IN PD

The *GBA1* gene encodes the lysosomal enzyme, acid β-glucosidase (glucocerebrosidase, GCase). This later enzyme cleaves the β-D-glucosidic bond from the glycosphingolipid substrates (glucosylceramide; GC), yielding β-D-glucose and ceramide, and its deacylated product, glucosylsphingosine (GS), resulting in the formation of β-D-glucose and sphingosine (125, 126). The three types of Gaucher disease (GD), i.e., types 1, 2, and 3, have been characterized by recessive mutations in the *GBA1* gene. Pathogenic mutations in *GBA1* and the resultant GCase deficiency cause excess tissue accumulation of GC and chronic tissue inflammation in type 1 GD (59, 125, 127–133). We have identified immune complexes of GC-specific immunoglobulin G (IgG) antibodies in experimental and clinical Gaucher disease, which induce massive generation of complement C5a (C5a) and the activation of C5a receptor (e.g., C5aR1). Such C5a–C5aR1 activation is what tips the balance between GC formation and its degradation through the control of an enzyme termed as glucosylceramide synthase (GCS) that produces the GC and fuels inflammation in visceral tissues (e.g., blood, bone marrow, lung, liver, spleen, and lymph node) in type 1 experimental and clinical GD (131).

TABLE 2A | Cytokines and their source in the LRRK2 mouse model of PD.

PD mouse model	LRRK2 kinase level and its source	Pro-inflammatory cytokines, chemokines, and source		Brain defects	References
Heterozygous LRRK2 (R1441G) transgenic mice + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	IFNγ	SNPC^{P++} Striatum^{P+} Sera^{P+}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice LRRK2$^{+/+}$ + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	TNFα	MGCs$^{P/M(ND)}$ SNPC$^{P/M(ND)}$ Striatum^{P+} Sera^{P++}	Neuron death Increased cognitive impairment	(91, 95, 120–122)
Heterozygous LRRK2 (R1441G) transgenic mice + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	IL-1α	SNPC$^{P/M+}$ Striatum^{P++} Sera^{P-}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice LRRK2$^{+/+}$ + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	IL-1β	MGCs$^{P/M(ND)}$ Sera^{P+}	Neuron death Increased cognitive impairment	(91, 95, 120–122)
Heterozygous LRRK2 (R1441G) transgenic mice + LPS LRRK2$^{+/+}$ + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	IL-6	MGCs$^{P/M++}$ SNPC^{P++} Striatum^{P++} Sera^{P+}	Neuron death Increased cognitive impairment	(91, 95, 120–123)
Heterozygous LRRK2 (R1441G) transgenic mice	MGCs$^{P/M++}$	IL-8	MGCs$^{P/M++}$	Increased cognitive impairment	(1)
Heterozygous LRRK2 (R1441G) transgenic mice + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	IL-10	SNPC^{P+} Striatum^{P++} Sera^{P++}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice		IL-12	MGCs$^{P/M++}$	Increased cognitive impairment	(91, 122)
Heterozygous LRRK2 (R1441G) transgenic mice	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	CCL2/MCP1	SNPC^{P++} Striatum^{P++}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	CCL3/MIP1α	Striatum^{P++} S^{P++}	Neuron death	(91, 120)
Heterozygous LRRK2 (R1441G) transgenic mice	MGCs^{P++}	CCL4/MIP1β	MGCs$^{P/M++}$	Neuron death	(91)
Heterozygous LRRK2 (R1441G) transgenic mice + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	CCL5/RANTES	Sera^{P++}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	CXCL1/KC	SNPC^{P+} Striatum^{P+} Sera^{P++} MGCs$^{P/M++}$	Neuron death	(91, 120)
Heterozygous LRRK2 (R1441G) transgenic mice	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	CXCL10	SNPC^{P++} Striatum^{P+} Sera^{P-}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice + LPS	SNPC, MGCs$^{P/M++}$	GCSF	Sera^{P++}	Neuron death	(120)
Heterozygous LRRK2 (R1441G) transgenic mice + LPS	SNPC, MGCs$^{P/M++}$ PBMCs/B cells$^{P/M++}$	MCSF	Sera^{P++}	Neuron death	(120)

LRRK2, leucine-rich-repeat kinase 2; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CCL, chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; MCSF, macrophage colony-stimulating factor; GCSF, granulocyte colony-stimulating factor; SNPC, substantia nigra pars compacta; PBMCs, peripheral mononuclear cells; MGCs, microglial cells; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

Excess brain accumulation of GC has been linked to the formation of abnormal species of α-syn, microglial cell activation, generation of pro-inflammatory cytokines (e.g., TNFα, IL-1β, and IL-6), and the loss of neurons in patients with GD types 2 and 3 (134–139). Heterozygous mutations in the *GBA1* gene are implicated in dementia with LBs (DLB) in idiopathic PD (140, 141). Similarly, the heterozygous *GBA1* mutations have emerged as the major genetic risk for developing PD (133, 138, 142–159).

Brains of the GBA1 mouse model of PD have shown partial GCase deficiency and its impact on increased production of TNFα, IL-1β, TGFβ1, CCL2, CCL3, CCL5, VCAM-1, ICAM-1, and MCSF as well as their link to the neuronal cell death (**Table 3A**). Plasma, sera, CSF, and blood-derived MOs of PD patients with *GBA* mutations have shown partial GCase deficiency and its impact on the higher production of pro-inflammatory cytokines (e.g., IFNγ, TNFα, IL-1β, IL-2, IL-4, IL-6,

TABLE 2B | Cytokines and their source in the LRRK2-associated human PD.

Human PD	LRRK2 kinase level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
LRKK2 G2019S mutation carriers	$LRRK2^{M++}$	IFNγ	$PBMCs^{P-}$	Increased cognitive impairment	(7)
LRKK2 G2019S mutation carriers	MO^{P+} $T\ cells^{P++}$	TNFα	$T\ cells^{P+}$ $Sera^{P++}$	Neuron death Increased cognitive impairment	(91, 95, 121, 122, 124)
LRKK2 G2019S mutation carriers	MO^{P+} $T\ cells^{P++}$	IL-1β	MO^{P+} $Sera^{P++}$	Neuron death Increased cognitive impairment	(91, 95, 121, 122, 124)
LRKK2 G2019S mutation carriers	MO^{P+} $T\ cells^{P++}$	IL-2	MO^{P+} $T\ cells^{\pm}$	Neuron death	(124)
LRKK2 G2019S mutation carriers	MO^{P+} $T\ cells^{P++}$	IL-4	MO^{P+} $PBMCs^{P++}$	Neuron death	(120, 124)
LRKK2 G2019S mutation carriers	MO^{P+} $T\ cells^{P++}$	IL-6	MO^{P++} $PBMCs^{P++}$ $Sera^{P+}$ CSF^{P+}	Neuron death Increased cognitive impairment	(4, 120, 124)
LRKK2 G2019S mutation carriers	$LRRK2^{M++}$	IL-8	$Sera^{P++}$ CSF^{P++}	Increased cognitive impairment	(122)
LRKK2 G2019S mutation carriers	$LRRK2^{M++}$	IL-10	$PBMCs^{P++}$ $Sera^{P++}$	Increased cognitive impairment	(4)
LRKK2 G2019S mutation carriers	$LRRK2^{M++}$ MO^{P+} $T\ cells^{P++}$	IL-12	MO^{P++} $T\ cells^{P++}$ $Sera^{P++}$	Increased cognitive impairment	(91, 122, 124)
LRKK2 G2019S mutation carriers	MO^{P+} $T\ cells^{P++}$	IL-13	MO^{P++} $T\ cells^{ND}$	Increased cognitive impairment	(124)
LRKK2 G2019S mutation carrier	$LRRK2^{P++}$	GCSF	$Sera^{P++}$	Increased cognitive impairment	(4)
LRKK2 G2019S mutation carriers	$LRRK2^{P++}$	PDGF	$Sera^{P++}$	Increased cognitive impairment	(4)
LRKK2 G2019S mutation carriers	$LRRK2^{P++}$	VEGF	CSF^{P++}	Increased cognitive impairment	(91)

LRRK2, leucine-rich-repeat kinase 2; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CCL, chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; GCSF, granulocyte colony-stimulating factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; LRRK2, leucine-rich-repeat kinase 2; PBMCs, peripheral mononuclear cells; MO, monocytes; CSF, cerebrospinal fluid; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

IL-8, IL-13, CCL2, CCL3, CCL18, and SF), midbrain damage, and cognitive defects (**Table 3B**). These studies suggest that GBA defects and the resultant GCase deficiency cause excess tissue storage of glycosphingolipids and/or the formation of abnormal species of α-syn. These abnormal proteins and/or lipids trigger residential and infiltrated immune cell (e.g., MOs and MGCs) activation and massive brain generation of pro-inflammatory cytokines and chemokines (**Tables 3A**,**B**), which are all critical for the development of brain inflammation and neurodegeneration in GBA-associated PD (**Figure 1B**).

SNCA GENE DEFECTS AND PRO-INFLAMMATORY IMMUNE MEDIATORS IN PD

SNCA encodes the α-syn, which is an 18-kDa protein composed of 140 amino acids and expressed in presynaptic terminals of the neocortex, hippocampus, substantia nigra (SN), NCs, ACs, and oligodendrocytes as well as CSF, serum, plasma, and hematopoietic cells (166–173). The brain α-syn interacts with proteins and lipids and controls the synaptic vesicle recycling and neurotransmitter release (174–177). However, the SNCA defect and the resultant excess generation and/or formation of normal endogenous or aggregated Agg α-syn in cytoplasmic inclusions of NCs termed as LBs and Lewy neurites (LNs) lead to neuronal toxicity and neurodegeneration in early- and late-onset PD (166, 178–185). Strikingly, LBs and LNs of the idiopathic forms of PD have also shown excess of α-syn and the Agg α-syn without any *SNCA* mutation (183, 186–188). In contrast, overexpression of wild-type SNCA and the resultant higher production of WT α-syn show their link to neurotoxicity in *Drosophila melanogaster* (189) and rodent models (190). Normal and Agg α-syn have shown TLR2- or TLR4-mediated MGC activation and neuronal loss in PD and mouse models (70, 191–198). PD genome-wide association studies (GWAS) identified the risk variants in certain loci associated to disease risk such as HLA-DR locus, which encodes

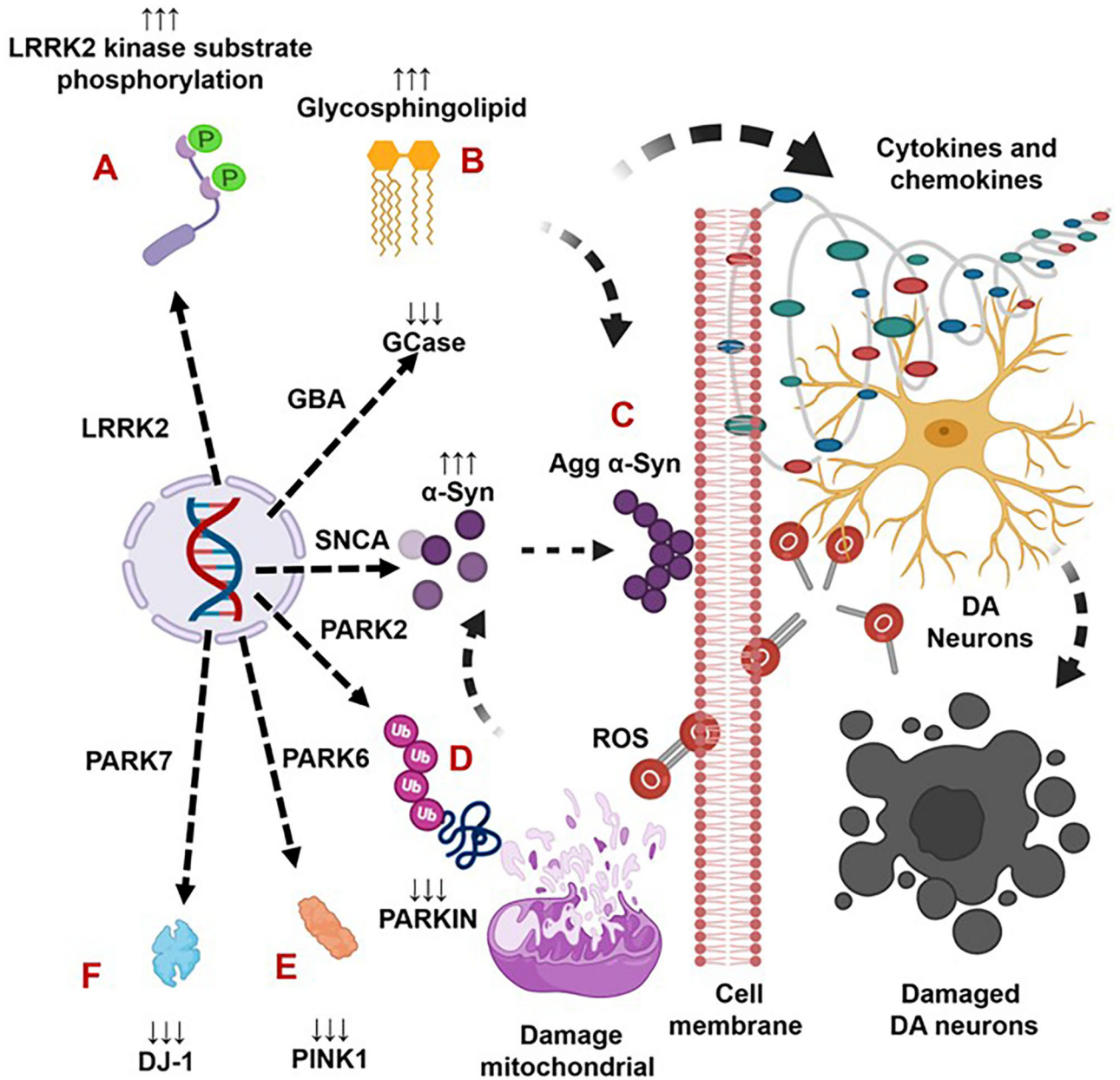

FIGURE 1 | The genetic mutation-induced inflammatory immune reactions develop neurodegeneration in Parkinson's disease. The LRRK2 defects cause over activation of LRRK2 kinases. This defect triggers the formation of aggregated alpha-synuclein (Agg α-syn) and increased generation of pro-inflammatory cytokines and chemokines that lead to the loss of dopaminergic (DA) neurons in LRRK2-associated PD **(A)**. The *GBA* mutations and the resultant deficiency of glucocerebrosidase (GCase) trigger the formation of glycosphingolipids and Agg α-syn, which trigger increased generation of pro-inflammatory cytokines and chemokines and lead to the loss of DA neurons in GBA-associated PD **(B)**. The SNCA defects and the resultant overproduction of normal/Agg α-syn activate the brain production of inflammatory cytokines and chemokines that cause death of DA neurons in SNCA-associated PD **(C)**. The PARK2, PARK6, and PARK7 defects and the subsequent deficiency of PARKIN, PINK, and DJ-1 proteins cause mitochondrial damage and the formation of Agg α-syn. These abnormalities trigger cellular activation and massive generation of ROS, pro-inflammatory cytokines, and chemokines that lead to the loss of DA neurons in PARK2-, PARK6-, and PARK7-associated PD **(D–F)**.

for the major histocompatibility complex I (MHC class II) known for triggering the antigen presentation to $CD4^+$ T cells (199–202). Two classical pathways of antigen presentation have been described for the presentation of endogenous antigens on MHC I molecules and the presentation of exogenous antigens, such as intracellular pathogens, on MHC class II molecules [reviewed by Blum et al. (203)]. The MHCII pathway is performed by specialized antigen-presenting cells, i.e., Mϕs, DCs, and DA neurons, which present peptides on MHCII molecules, ensuring its efficient recognition by $CD4^+$ T cells (204). In addition

TABLE 3A | Cytokines and their source in the mouse model of GBA1 PD.

PD mouse model	GCase level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	TNFα	Gray matter^{P++} Gray matter^{M++}	BBB permeabilization, neuronal death	(135, 160)
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	IL-1β	Gray matter^{M++}	Neuronal cell death, BBB permeabilization	(160)
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	TGFβ1	Gray matter^{M++}	BBB permeabilization, neuronal death	(160, 161)
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	CCL2/MCP1	Gray matter^{M++}	BBB permeabilization, neuronal death	(135, 160)
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	CCL3/MIP1α	Gray matter^{M++}	BBB permeabilization, neuronal death	(135, 160)]
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	CCL5/RANTES	Gray matter^{M++}	BBB permeabilization, neuronal death	(135, 160)
Neuronopathic$GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	VCAM-1	ECs^{M++}	BBB permeabilization, neuronal death	(135, 160)]
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	ICAM-1	ECs^{M++}	BBB permeabilization, neuronal death	(135, 160)
Neuronopathic $GBA^{+/-}$ GBA Het knock-in $GBA^{+/L444P}$	Brain: >25% GCase VMB $^{(P-)}$	MSCF	Gray matter^{M++}	BBB permeabilization, neuronal death	(135, 160)

GBA, acid β-glucosidase; GCase, glucocerebrosidase; GlcCer, glucosylceramide; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CCL, chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; RANTES, regulated upon activation normal T-cell expressed and presumably secreted; MCSF, macrophage colony-stimulating factor; GCSF, granulocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; MIP1, macrophage inflammatory proteins; PARC, pulmonary and activation-regulated chemokine; SCF, stem cell factor; VMB, ventral midbrain; ECs, endothelial cells; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

to the increased brain infiltration of effector T-cell subsets in PD patients (42, 43), MHCII-mediated presentation of α-syn to $CD4^+$ T cells has been linked to neuroinflammation in a mouse model and human PD (205–207). α-Syn peptide-stimulated T cells have shown development of activated subsets of helper and cytotoxic T cells and increased production of IFNγ, IL-2, and IL-5 (205). In addition, one of the peptide regions strongly binds to MHC encoded by HLA (DRB1*15:01, DRB5*01:01) linked to PD by GWAS (201, 208–210).

The sera, MGCs, and brain regions of the SNCA mouse model of PD have shown overexpression of different species of α-syn and pro-inflammatory cytokines (e.g., IFNγ, TNFα, IL-1α, IL-1β, IL-6, IL-10, TGFβ, CCL2, CCL3, CCL5, CXCL10, and ICAM-1) as well as their link to neuronal cell death and cognitive defects (**Table 4A**). The blood-derived immune cells, sera, and brain regions of PD patients with SNCA defect have also shown overexpression of α-syn and their association with cellular activation and increased generation of pro-inflammatory mediators (e.g., IFNγ, TNFα, IL-1β, IL-4, IL-5, IL-6, IL-18, and CCL2) as well as their link to neuronal cell damage (**Table 4B**). Hence, SNCA defects and the resultant increased making of normal and/or Agg α-syn promote the activation of peripheral immune cells and the brain MGCs. Such cells cause massive generation of NO, ROS, and pro-inflammatory cytokines and chemokines (**Tables 4A**,**B**), which are all critical for promoting brain inflammation and neurodegeneration in SNCA-associated PD (**Figure 1C**).

PARK2 GENE DEFECTS AND PRO-INFLAMMATORY IMMUNE MEDIATORS IN PD

The *PARK2* gene encodes cytosolic ubiquitin E3 ligase termed as parkin protein, which is critical for the targeting, breakdown, and recycling of damaged proteins as well as the regulation of mitophagy and survival of DA neurons (224). *PARK2* mutations cause a loss of parkin function that leads to the excess accumulation of dysfunctional mitochondria and the resultant massive generation of oxidative stress and death of DA neurons in autosomal recessive and idiopathic PD (225–235). $CD4^+$ and $CD8^+$ cell infiltration, MGC activation, increased generation of pro-inflammatory cytokines, and the loss of DA neurons have been observed in mouse model and human PD (43, 236).

Parkin plays a protective role during bacterial and viral infection and chemically induced oxidative and ER stress by altering the mitochondrial ROS and pro-inflammatory cytokine-mediated downstream signaling cascades (237–247). Biochemical and genetic studies reveal that parkin also acts in tandem with phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1), which is accountable for controlling the mitochondrial quality (248). Indeed, mutations in the genes that encode PINK1 and Parkin showed massive mitochondrial damage and the development of familial PD (229). It has been shown that autophagy, the recycling of self-components through lysosomal degradation, is involved in the presentation of endogenous antigens on both MHC class I

TABLE 3B | Cytokines and their source in the GBA-associated human PD.

Human PD	GCase level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
GBA-linked PD	$Plasma^{GCase(-)}$ $MOs^{GCase(-)}$	IFNγ	$Plasma^{P++}$	BBB leakage in the striatum and midbrain	(154, 162, 163)
	Plasma, CSF, nigrostriatal DA $regions^{GCase(-)}$ $MOs^{GCase(-)}$	TNFα	$Plasma^{P++}$	BBB leakage in the striatum and midbrain	(154, 162, 163)
GBA-linked PD	Plasma, CSF, nigrostriatal DA regions $MOs^{GCase(-)}$	IL-1β	$Plasma^{P++}$	BBB leakage in the striatum and midbrain	(154, 162, 163)
GBA-linked PD	Plasma, CSF, nigrostriatal DA $regions^{GCase(-)}$ $MOs^{GCase(-)}$	IL-2	$Plasma^{P++}$	BBB leakage in the striatum and midbrain	(154, 162, 163)
GBA-linked PD	CSF, nigrostriatal DA $regions^{GCase(ND)}$ $MOs^{GCase(-)}$	IL-4	$Plasma^{P-}$	BBB leakage in the striatum and midbrain	(154, 164)
GBA-linked PD	CSF, nigrostriatal DA $regions^{GCase(ND)}$ $MOs^{GCase(-)}$	IL-6	$Plasma^{P++}$	BBB leakage in the striatum and midbrain	(154, 165)
GBA-linked PD	Plasma >25% GCase $MOs^{GCase(-)}$	IL-8	$Plasma^{P++}$	Cognitive dysfunction	(154, 161)
GBA-linked PD	Plasma >25% GCase $MOs^{GCase(-)}$	IL-13	$Plasma^{P++}$	BBB leakage in the striatum and midbrain	(154, 162)
GBA-linked PD	Plasma: >25% GCase $MOs^{GCase(-)}$	CCL2/MCP-1	$Plasma^{P+}$	Cognitive dysfunction	(154, 161)
GBA-linked PD	Plasma: >25% GCase $MOs^{GCase(-)}$	CCL3/MIP1α	$Plasma^{P++}$	Cognitive dysfunction	(154, 161)
GBA-linked PD	Plasma: >25% GCase $MOs^{GCase(-)}$	CCL18/PARC	$Plasma^{P++}$	Cognitive dysfunction	(154, 161)
GBA-linked PD	Plasma: >25% GCase $MOs^{GCase(-)}$	SCF	$Plasma^{P-}$	Cognitive dysfunction	(154, 161)

GBA, acid β-glucosidase; GCase, glucocerebroside; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CCL, chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; VCAM-1, vascular cell adhesion molecule 1; MCP-1, monocyte chemoattractant protein-1; MIP1, macrophage inflammatory protein; PARC, pulmonary and activation-regulated chemokine; SCF, stem cell factor; CSF, cerebrospinal fluid; DA, dopaminergic; PBMCs, peripheral mononuclear cells; BMVECs, brain microvessel endothelial cells; BBB, blood–brain barrier; Mon, monocytes; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

and class II molecules (249, 250), highlighting that vacuolar content can also be presented on MHC class I/II molecules. The mitochondrial MHCI-mediated antigen processing and presentation to $CD8^+$ T cells have been valued for induction of neuroinflammation in mouse models and human PD (42, 43, 205, 251, 252). To understand the exact role of parkin and PINK1 in the development of brain inflammation in PD, Matheoud et al. (252) have discovered a pathway for mitochondrial antigen presentation, in which mitochondria-derived vesicles targeted endolysosomes for processing and presentation by MHC class I molecules. Using both *in vitro* and *in vivo* experiments, this study has demonstrated that parkin and PINK1 inhibit mitochondria-derived vesicle formation and mitochondrial antigen presentation, and therefore, in the absence of PINK1 or parkin, mitochondrial antigen presentation triggers DC and $CD8^+$ T-cell activation and increased generation of pro-inflammatory cytokines. These data suggest that PINK1 and/or parkin has a key role in the activation of innate and adaptive immune cells by repressing the presentation of mitochondrial antigens, which suggests the involvement of autoimmune reactions in PD (252). *PARK2* mutations and their link to α-syn inclusions and LB formation have also been observed in exceptional cases of PARK2-associated PD (253–255). The exact mechanism by which PARK2 defects propagate brain inflammation and neurodegeneration in PD is poorly defined.

The MGCs, Mϕs, and sera of the PARK2 mouse model displayed decreased expression of parkin and its link to the increased generation of pro-inflammatory cytokines and chemokines (e.g., IFNβ1, TNFα, IL-1β, IL-12, IL-13, IL-17, CCL2, and CXCL1), loss of DA neurons, and cognitive defects in PD (**Table 5A**). The sera, MGCs, Mϕs, and midbrain regions of PARK2-associated human PD also displayed decreased expression of parkin and its link to increased generation of pro-inflammatory cytokines (e.g., IFNβ1, TNFα, IL-1β, IL-6, IL-12, IL-13, CCL2, CCL4, and CXCL1), loss of DA neurons, and cognitive defects in PD (**Table 5B**). These findings suggest that PARK2 and the resultant deficiency of parkin are associated with mitochondrial damage and/or the formation of Agg α-syn. These defects cause cellular activation and massive generation

TABLE 4A | Cytokines and their source in the mouse model of SNCA PD.

PD mouse model	α-Syn and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
Local rAAV-A53T-α-syn injection in WT mice SN Aggregated α-syn-stimulated WT microglial cells MHCII/HLA-mediated activation of DC and $CD4^+$ T cells of D409/D409 murine model	$^{P/M++}$	IFNγ	Striatum^{P+} SNPC^{M-}	Neuron death	(205–207, 211–213)
Thy-1 α-syn overexpression murine model of PD A53T α-syn over-expressing SHSY5Y cells Astrocytoma cell line U373 engineered to express C-terminally truncated α-syn Local rAAV-A53T-α-syn injection in WT mice SN α-Syn-stimulated DM-A30P–A53T microglial cells Aggregated α-syn-stimulated WT microglial cells rα-syn-stimulated WT; A53T; A30P; E46K macrophages Monomeric α-syn-stimulated WT; A53T rat primary microglial cells WT, A53T α-syn overexpressing SHSY5Y microglial cell lines N-α-syn-stimulated WT microglial cells Syn-stimulated microglial cell lines (BV2) MHCII-mediated activation of DC and $CD4^+$ T cells of D409/D409 murine model α-Syn-injected (intra-SN) ABH Biozzi mice	SNPC^{P++} Striatum^{P++} Cortex^{P++} SNCA$^{P/M++}$ ND $^{P++}$ $^{P++}$ ND ND ND ND ND ND ND	TNFα	Striatum^{P++} SNPC^{P++} Cortex^{P++} Serum^{P++} MGCs$^{M/P++}$ Striatum^{P+} CC^{P+} Striatum^{M+} SNPC^{M+} CC$^{M/P+}$ SNPC^{M+} MGCs^{P++} SNPC^{M++}	Neuron death Increased cognitive impairment	(43, 191, 192, 197, 198, 206, 207, 211–222)
Thy-1 α-syn overexpression murine model of PD Local rAAV-A53T-α-syn injection in WT mice SN WT, A53T α-syn overexpressing SHSY5Y microglial cell lines	$^{P++}$ $^{P/M++}$ ND	IL-1α	MGCs^{M++} SNPC^{M++} SNPC$^{M/P++}$ MGCs$^{M/P++}$		(192, 213, 215)
Thy-1 α-syn overexpression murine model of PD A53T α-syn overexpressing SHSY5Y cells α-Syn-injected (intra-SN) ABH Biozzi mice Local rAAV-A53T-α-syn injection in WT mice SN α-syn-stimulated DM-A30P–A53T microglial cells α-Syn-stimulated WT microglial cells Syn-stimulated microglial cell lines (BV2) N-α-syn-stimulated WT microglial cells Monomeric α-syn-stimulated WT; A53T; A30P; E46K microglial cells Monomeric α-syn-stimulated WT; A53T rat microglial cells WT, A53T α-syn overexpressing SHSY5Y	$^{P/M++}$ Striatum^{P++} Cortex^{P++} $^{P/M++}$ $^{P/M++}$	IL-1β	Striatum^{P-} SNPC^{P-} Cortex^{P-} Serum^{P-} MGCs^{M++} SNPC$^{M/P++}$ MGCs$^{M/P++}$ Striatum^{P+} SNPC^{P++} MGCs^{M++} Striatum^{M+} SNPC^{M++} CC$^{M/P++}$ MGCs^{P+} MGCs^{P+}	Neuron death Increased cognitive impairment	(191, 192, 197, 211, 216, 218–220, 222, 223)
Thy-1 α-syn overexpression murine model of PD A53T alpha-synuclein overexpressing SHSY5Y cells Local rAAV-A53T-α-syn injection in WT mice SN α-syn I -stimulated DM-A30P–A53T microglial cells N-α-syn-stimulated WT microglial cells Aggregated α-syn-stimulated WT microglial cells Monomeric α-syn-stimulated WT; A53T; A30P; E46K microglial cells a-Syn-stimulated microglial cell lines (BV2) Transient transfection in microglial cell lines MHCII-mediated activation of DC and $CD4^+$ T cells of D409/D409 murine model Monomeric α-syn-stimulated WT; A53T; A30P	$^{P++}$ $^{P/M++}$ ND ND ND ND ND ND ND ND ND	IL-6	SNPC^{M++} SNPC$^{P/M++}$ Striatum^{M+} SNPC^{M+} MGCs^{P++} MGCs^{P++} MGCs^{P++}	Neuron death	(191, 192, 206, 207, 212, 215, 216, 218, 220, 221)
Monomeric α-syn stimulated WT; A53T; A30P; E46K microglial cells	$^{P/M++}$	IL-10	Striatum^{M+} SNPC^{M+} MGCs^{P++}	Neuron death	(38, 216, 222)
Monomeric α-syn-treated mice	$^{P++}$	TGFβ	SNPC^{M++}	Neuron death	(197, 213)
Aggregated α-syn-stimulated WT microglial cells Monomeric α-syn-stimulated WT; A53T; A30P; E46K microglial cells A53T alpha-synuclein overexpressing SHSY5Y cells	$^{P++}$	CCL2/MCP1	MGC^{P++}	Neuron death	(212, 218, 220)

(Continued)

TABLE 4A | Continued

PD mouse model	α-Syn and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
Monomeric α-syn-stimulated WT; A53T; A30P; E46K microglial cells	$MGCs^{P++}$	CCL3/MIP1α	$MGCs^{P++}$	Neuron death	(38)
Monomeric α-syn-stimulated WT; A53T; A30P; E46K microglial cells	$MGCs^{P++}$	CCL5/RANTES	$MGCs^{P++}$	Neuron death	(220)
Mutant α-syn overexpression murine model of PD	$^{P/M++}$	CX3CR1	$Striatum^{M+}$ $SNPC^{M+}$	Neuron death	(216)
A53T; A30P; E46K microglial cells	$^{P/M++}$	CXCL10	$MGCs^{M/P++}$	Neuron death	(222)
Local AAV α-syn overexpression murine model of PD	$^{P++}$	ICAM-1	$SNPC^{M+}$	Neuron death	(215)

SNCA, synuclein alpha; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CCL, chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; CX3CR1, chemokine C-X-R receptor; RANTES, regulated upon activation, normal T-cell expressed and presumably secreted; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; MIP1α, macrophage inflammatory proteins; SNPC, substantia nigra pars compacta; TC, T cells; MGCs, microglial cells; BG, basal ganglia; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

TABLE 4B | Cytokines and their source in the SNCA-associated human PD.

Human PD	α-Syn and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
PD patients' T cells	$^{P++}$	IFNγ	TC^{P++} $SNPC^{P+}$ BG^{P+}	Damaging of dopaminergic neurons	(205–207, 211, 212)
U373 cells overexpressing truncated α-synuclein: PD patients' brain	$^{P++}$	TNFα	$SNPC^{P+}$ $MGCs^{M+}$	Damaging of dopaminergic neurons	(122, 192, 197, 198, 206, 207, 211, 212, 214–219)
PD patients' brain Sera	$Brain^{P++}$ $Sera^{P++}$	IL-1β	$Sera^{P+}$ $MGCs^{M+}$ $PBMCs^{P++}$	Damaging of dopaminergic neurons	(61, 122, 191, 192, 197, 211, 216, 218–220, 223)
PD patients' T cells	$^{P++}$	IL-4	TC^{P++}	Damaging of dopaminergic neurons	(205)
PD patients' T cells	$^{P++}$	IL-5	TC^{P++}	Damaging of dopaminergic neurons	(205)
PD patients' brain Sera	$Brain^{P++}$ $Sera^{P++}$	IL-6	$SNPC^{P+}$ $MGCs^{M+}$ BG^{P+} $PBMCs^{P++}$	Damaging of dopaminergic neurons	(61, 122, 191, 192, 206, 207, 212, 215, 216, 218, 220, 221)
Patient sera	$Sera^{P++}$	IL-18	$PBMCs^{P++}$	Damaging of dopaminergic neurons	(61)
PD patients' brain	$Brain^{P++}$	CCL2/MCP1	$SNPC^{P+}$ $MGCs^{M+}$ BG^{P+}	Damaging of dopaminergic neurons	(212, 218, 220)

SNCA, synuclein alpha; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; MCP-1, monocyte chemoattractant protein-1; SNPC, substantia nigra pars compacta; TC, T cells; MGCs, microglial cells; BG, basal ganglia; ECs, endothelial cells; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

of pro-inflammatory cytokines and chemokines (**Tables 5A**,**B**), which lead to the loss of DA neurons in PARK2-associated PD (**Figure 1D**).

PARK6 GENE DEFECTS AND PRO-INFLAMMATORY IMMUNE MEDIATORS IN PD

The *PARK6* gene encodes PINK1, which is a universally expressed serine/threonine kinase with a mitochondrial targeting sequence that directs the import of PINK1 as well as the activation and recruitment of parkin into the mitochondria for clearance of damaged mitochondria (260–267). PINK1-deficient cells, including NCs, are more susceptible to various insults (268, 269). PINK1 and parkin control the degradation of dysfunctional mitochondria (270, 271). PARK6 defects and the resultant deficiency of PINK1 lead to mitochondrial dysfunctions and the development of autosomal recessive and early-onset PD (261, 272–274). *Pink1*-deficient *Drosophila* displayed mitochondrial damage associated with apoptotic muscle degeneration and DA neuron loss, whereas Parkin overexpression protected such

TABLE 5A | Cytokines and their source in the mouse model of PARK2 PD.

PD mouse model	Parkin level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
SED Parkin$^{-/-}$, Parkin$^{+/-}$	Sera$^{M-andP-}$ Sera^{P-}	IFNβ1	Sera^{P+} Sera^{P+}	Death of DA neurons in SNPC and motor defects	(79)
Parkin$^{-/-}$ and WT mice	Midbrain$^{M-andP-}$ Cortex$^{M-andP-}$ Mφs^{M-} MGCs^{M-}	TNFα	Midbrain^{M+} CortexND Mφs^{M+} MGCs^{M+}	Nigral cell degeneration and DA loss in SNPC	(256, 257)
Parkin$^{-/-}$ and WT mice	Mφs^{M-} MGCs^{M-}	IL-1β	Mφs^{M+} MiGCs^{M+}	Nigral cell degeneration and DA loss in SNPC Loss of fine motor skills	(257, 258)
SED Parkin$^{-/-}$, Parkin$^{+/-}$	Sera$^{M-andP-}$ Sera^{P-}	IL-12	Sera^{P+} Sera^{P+}	Death of DA neurons in SNPC and motor defects	(79)
SED Parkin$^{-/-}$, Parkin$^{+/-}$	Sera$^{M-andP-}$ Sera^{P-}	IL-13	Sera^{P+} Sera^{P+}	Death of DA neurons in SNPC and motor defects	(79)
SED Parkin$^{-/-}$, Parkin$^{+/-}$	Sera$^{M-andP-}$ Sera^{P-}	IL-17	Sera^{P+} Sera^{P+}	Death of DA neurons in SNPC and motor defects	(79)
SED Parkin$^{-/-}$, Parkin$^{+/-}$	Sera$^{M-andP-}$ Sera^{P-}	CCL2/MCP1	Sera^{P+} Sera^{P+}	Death of DA neurons in SNPC and motor defects	(79)
SED Parkin$^{-/-}$, Parkin$^{+/-}$	Sera$^{M-andP-}$ Sera^{P-}	CXCL1/KC	Sera^{P+} Sera^{P+}	Death of DA neurons in SNPC and motor defects	(79)

IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; CXCL, chemokine C-X-C motif ligand; CCL, chemokine C-C motif ligand; MGCs, microglial cells; Mφs, macrophages; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

TABLE 5B | Cytokines and their source in the PARK2-associated human PD.

Human PD	Parkin level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
Parkin$^{+/-}$ unaffected PD patients	Sera^{P-}	IFNβ1	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
PARK2/Parkin-associated PD	Mφs^{M-} MGCs $^{M-}$	TNFα	Mφs^{M+} MGCs^{M+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
PARK2/Parkin-associated PD	Mφs^{M-} MGCs^{M-}	IL-1β	Mφs^{M+} MGCs^{M+}	Motor deficits, loss of DA neurons in SNPC, inflammation-related nigral degeneration	(256, 257)
PD patients with biallelic PRKN/PINK1 mutations		IL-6	Sera^{P+}	Mitophagy dysfunction	(259)
Parkin$^{+/-}$ unaffected PD patients	Sera^{P-}	IL-12	Sera^{P+}	Mitophagy dysfunction and neuroinflammation	(259)
Parkin$^{+/-}$ unaffected PD patients	Sera^{P-}	IL-13	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
Parkin$^{+/-}$ unaffected PD patients	Sera^{P-}	CCL2/MCP1	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
Parkin$^{+/-}$ unaffected PD patients	Sera^{P-}	CCL4/MIP1β	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
Parkin$^{+/-}$ unaffected PD patients	Sera^{P-}	CXCL1/KC	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)

IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; CXCL, chemokine C-X-C motif ligand; CCL, chemokine C-C motif ligand; MGCs, microglial cells; Mφs, macrophages; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

PINK1-induced defects (248, 275, 276). Several studies have shown that PINK1, like parkin, modulates NF-κB activity and brain generation of pro-inflammatory cytokines (277). PINK1-deficient T cells have reduced protein kinase B (PKB or Akt) activity, which is critical for inducible regulatory T cells (iTreg) development (278). PINK1-deficient iTreg cells showed reduced capacity to suppress lymphocyte proliferation (278). Importantly, the autologous transfer of Treg cells to MPTP-treated mice attenuated MGC activation and provides neuroprotection (279).

Strikingly, Treg cells from PD patients also have impaired suppressor function (47). T-cell subset infiltration and their interaction with MGCs and DA neurons are critical for the development of neuroinflammation and neurodegeneration in MPTP-induced mouse model and human patients with PD (43, 47, 48, 280, 281). Gram-negative bacteria-induced intestinal infection in Pink1$^{-/-}$ mice showed mitochondrial antigen presentation to CD8^{+} T cells in the periphery and in the brain and their link to loss of DA axonal varicosities in the striatum and the motor impairment. These data suggest the relevance of the gut–brain axis that could develop brain inflammation and neurodegeneration in PD (282, 283).

The blood, brain regions, and cells of the mouse model of PARK6-associated PD have shown PINK1 deficiency and its impact on increased blood or brain generation of pro-inflammatory cytokines and chemokines (e.g., IFNγ, IFNβ1, TNFα, IL-1β, IL-2, IL-6, IL-10, IL-12, IL-13, IL-17, TGFβ, CCL2, CCL4, and CXCL1), loss of neuronal cells, and the development of cognitive defects in PD (**Table 6A**). Additionally, PARK6-associated PD patients have also shown PINK1 deficiency and its impact on increased generation of pro-inflammatory cytokines and chemokines (e.g., IFNβ1, IL-6, IL-12, IL-13, CCL2, CCL4, and CXCL1), loss of NCs, and the development of cognitive defects (**Table 6B**). These findings suggest that PARK6 and the resultant PINK1 defects trigger residential and infiltrated immune cell activation and increased production of pro-inflammatory cytokines and chemokines (**Tables 6A**,**B**), which ultimately lead to the loss of DA neurons in PARK6-associated PD (**Figure 1E**).

PARK7 GENE DEFECTS AND PRO-INFLAMMATORY IMMUNE MEDIATORS IN PD

PARK7 encodes a protein deglycase DJ-1, which belongs to the peptidase C56 family of proteins and ubiquitously expressed under physiological conditions (286). Like PINK1 and parkin, DJ-1 is required for controlling mitochondrial damage and production of oxidative stress (287–289). Several chemicals and physiological factors trigger the upregulation of DJ-1, which protects the oxidative and endoplasmic reticulum stress-induced damage of endothelial cells, Mϕs, fibroblast, NCs, and islet β cells (290–296), and therefore, DJ-1 deficiency has been associated with the development of several diseases (e.g., stroke, male infertility, cancers, diabetes, and neurodegenerative illnesses) (290, 297, 298). *Escherichia coli*- or *Pseudomonas aeruginosa*-mediated excess activation of MAPK signaling and the resultant induction of brain inflammation have been observed in DJ-1-deficient *Caenorhabditis elegans* (299). Mutations in PARK7 and the resultant deficiency or the oxidized form of DJ-1 protein cause autosomal recessive early-onset and idiopathic PD as reviewed in ref. (300).

Brain regions and their cells of the mouse model of PARK7-associated PD have shown DJ-1 deficiency and its effect on increased production of IFNγ, IL-1β, IL-1Ra, IL-6, IL-16, IL-17, CXCL11, and NGF as well as on the damage of ACs and DA neurons (**Table 7A**). Furthermore, abnormal cellular and brain region expression of DJ-1 has been associated with the formation of α-syn and Tau containing LBs, mitochondrial damage, increased production of ROS, and their link to the loss of NCs in PD patients with *PARK7* mutation (**Table 7B**). These data suggest that PARK7 and the resultant DJ-1 deficiency induced mitochondrial damage and/or the formation of Agg α-syn and Tau comprising LB. These abnormal proteins cause massive generation of pro-inflammatory cytokines and chemokines (**Tables 7A**,**B**), which ultimately lead to the death of DA neurons in PARK7-associated PD (**Figure 1F**).

CONCLUSION

The molecular mechanisms by which *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7* defects trigger neuroinflammation and neurodegeneration in PD are poorly defined and need more studies. However, the abnormal function of *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7* genes has been linked to alteration in innate and adaptive immune responses in cancer, stroke, diabetes, male infertility, Crohn's disease, and infectious diseases (59, 96–98, 125, 127–133, 237–245, 290, 297, 298, 306–309). Findings from mouse models, cell system, and human specimens have shown that the abnormal expressions of *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7* genes and their corresponding proteins or enzymes (e.g., LRRK2, GCase, α-syn, parkin, PINK1, and DJ-1) are linked to the activation of MGCs, ACs, and NCs and the massive production of growth factors (e.g., GCSF, GMCSF, MCSF) and CCL and CXCL chemokines (i.e., CCL2/MCP1, CCL3/MIP1α, CCL4/MIP1β, CCL5/RANTES, CXCL1, and CXCL10), which are all accountable for the development and trafficking of immunological cells from the peripheral blood and bone marrow to the sites of inflammation for the generation of pro-inflammatory cytokines that lead to tissue destruction (61–69). The CCL2/MCP1, CCL3/MIP1α, CCL4/MIP1β, CCL5/RANTES, CXCL1, and CXCL10 chemokines are specific chemoattractants for tissue recruitment of several inflammatory subsets of MOs, Mφs, DCs, and CD4^{+} and CD8^{+} T cells (59, 60). Certain inflammatory conditions cause accelerated migration of immunological cell precursors out of the bone marrow and into the circulation (310–312). A similar condition is thought to occur in PD due to genetic defects in *LRRK2*, *GBA*, *SNCA*, *PARK2*, *PARK6*, and *PARK7* genes and the resultant alteration in the expression of their corresponding proteins or enzymes, i.e., LRRK2, GCase, α-Syn, parkin, PINK1, and DJ-1, which leads to the establishment of a network of several of the innate and adaptive immune cells, i.e., MOs and memory and effector T cells (43, 46–51). Hence, it is possible that immune cell integration

TABLE 6A | Cytokines and their source in the mouse model of PARK6 PD.

PD mouse model	PINK1 level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
PINK1$^{-/-}$	Striatal varicosities^{P-}	IFNγ	Cytotoxic T cells^{P+}	Motor impairment and loss of DA neurons in striatum varicosities	(282)
SED PINK1$^{-/-}$ and $^{+/-}$	Sera^{P-}	IFNβ1	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
PINK1$^{-/-}$	Striatum$^{M-andP-}$ MGCs^{M-} Astrocytes^{M-} Cortex$^{M-and\ P-}$	TNFα	Striatum^{M+} MGCs^{M-} ACs^{M+} Cortex$^{M+and\ P+}$	Inflammation-induced DA death. Disruption of DA neuron dysfunction	(258, 284, 285)
PINK1$^{-/-}$	Striatum$^{M-andP-}$ MGCs^{M-} Astrocytes^{M-} Cortex$^{M-and\ P-}$	IL-1β	Striatum^{M+} MGCs^{M-} ACs^{M+} Cortex$^{M+and\ P+}$	Inflammation-induced DA neuronal death	(258, 284, 285)
PINK1$^{-/-}$	Striatal varicosities^{P-}	IL-2	Cytotoxic T cells^{P+}	Motor impairment and loss of DA neurons in striatum varicosities	(282)
PINK1$^{-/-}$	Striatum$^{M-andP-}$ Cortex$^{M-and\ P-}$	IL-6	Striatum^{P+} Cortex$^{M+and\ P+}$	Inflammation-induced DA neuronal death. Disruption of DA neuron dysfunction	(258, 277, 284, 285)
PINK1$^{-/-}$	Striatum$^{M-andP-}$ MGCs^{M-} Cortex$^{M-and\ P-}$	IL-10	Striatum^{P+} MGCs^{M-} Cortex$^{M+and\ P+}$	Inflammation-induced DA neuronal death. Disruption of DA neuron dysfunction	(258, 277, 284, 285)
PINK1$^{-/-}$ and $^{+/-}$	Striatum$^{M-andP-}$ Sera^{P-}	IL-12	Striatum^{P+} Sera^{P+}	Inflammation-induced DA neuronal death. Disruption of DA neuron dysfunction	(79, 258, 277)
SED PINK1$^{-/-}$ and $^{+/-}$	Sera^{P-}	IL-13	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
SED PINK1$^{-/-}$ and $^{+/-}$	Sera^{P-}	IL-17	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
PINK1$^{-/-}$	Microglia^{M-} Astrocytes^{M-}	TGFβ	MGCs^{M-} ACs^{M+}	Inflammation-induced DA death	(284)
SED PINK1$^{-/-}$ and $^{+/-}$	Sera^{P-}	CCL2/MCP1	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
SED PINK1$^{-/-}$ and $^{+/-}$	Sera^{P-}	CCL4/MIP1β	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)
SED PINK1$^{-/-}$ and $^{+/-}$	Sera^{P-}	CXCL1/KC	Sera^{P+}	Inflammation, motor defects, and loss of DA neurons in SNPC	(79)

PINK1; PTEN-induced kinase 1; IFN, interferon; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin; α, alpha; β, beta; γ, gamma; CXCL, chemokine C-X-C motif ligand; CCL, chemokine C-C motif ligand; MGCs, microglial cells; Mφs, macrophages; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

TABLE 6B | Cytokines and their source in the PARK6-associated human PD.

Human PD	PINK1 level and its source	Pro-inflammatory cytokines/chemokines	Cytokines, chemokines, and their source	Brain defects	References
PARK6/PINK1-associated PD	Sera^{P-}	IFNβ1	Sera^{P+}	Loss of DA neurons and motor defects	(79)
PARK6/PINK1-associated PD	Sera^{P-}	IL-6	Sera^{P+}	Cortical injuries and neuronal death	(259)
PARK6/PINK1-associated PD	Sera^{P-}	IL-12	Sera^{P+}	Cortical injuries and neuronal death	(79)
PARK6/PINK1-associated PD	Sera^{P-}	IL-13	Sera^{P+}	Loss of DA neurons and motor defects	(79)
PARK6/PINK1-associated PD	Sera^{P-}	CCL2/MCP1	Sera^{P+}	Loss of DA neurons and motor defects	(79)
PARK6/PINK1-associated PD	Sera^{P-}	CCL4/MIP1β	Sera^{P+}	Loss of DA neurons and motor defects	(79)
PARK6/PINK1-associated PD	Sera^{P-}	CXCL1/KC	Sera^{P+}	Loss of DA neurons and motor defects	(79)

PINK1, PTEN-induced kinase 1; IFN, interferon; IL, interleukin; β, beta; CXCL, chemokine C-X-C motif ligand; CCL, chemokine C-C motif ligand; DA, dopaminergic; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

TABLE 7A | Cytokines and their source in the mouse model of PARK7 PD.

PD mouse model	DJ-1 level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
DJ-1–/–	$SN^{M-\text{ and }P-}$	IFNγ	SN^{P+}	Loss of DA neurons in the nigrostriatal pathway and striatal dopamine	(301)
DJ-1–/–, DJ-1 knockdown (shRNA)	$SN^{M-\text{ and }P-}$ $MGCs^{M-}$	IL-1β	$SN^{M-\text{ and }P-}$ $MGCs^{P+}$	Inflammation induced DA neuronal death. Loss of DA neurons in the nigrostriatal pathway and striatal dopamine	(258, 301–303)
DJ-1–/–	$SN^{M-\text{ and }P-}$	IL-1Ra	SN^{P+}	Loss of DA neurons in the nigrostriatal pathway and striatal dopamine	(301)
DJ-1–/–, DJ-1 knockdown (shRNA)	$MGCs^{M-}$ $ACs^{M-\text{and }P-}$	IL-6	$MGCs^{P+}$ ACs^{P+}	Increased DA neurotoxicity. Deregulation of astrocytic neuroinflammatory damage	(302–304)
DJ-1–/–	$SN^{M-\text{ and }P-}$	IL-16	SN^{P+}	Loss of DA neurons in the nigrostriatal pathway and striatal dopamine	(301)
DJ-1–/–	$SN^{M-\text{ and }P-}$	IL-17	SN^{P+}	Loss of DA neurons in the nigrostriatal pathway and striatal dopamine	(301)
DJ-1–/–	$SN^{M-\text{ and }P-}$	CXCL11	SN^{P+}	Loss of DA neurons in the nigrostriatal pathway and striatal dopamine	(301)
DJ-1–/–	$ACs^{M-\text{ and }P-}$	NGF	ACs^{P+}	Deregulation of astrocytic neuroinflammatory damage	(304)

DJ-1, protein deglycase-1; IFN, interferon; IL, interleukin; ILRa, interleukin receptor antagonist; α, alpha; β, beta; γ, gamma; CXCL, chemokine C-X-C motif ligand; CCL, chemokine C-C motif ligand; NGF, nerve growth factor; SN, substantia nigra; MGCs, microglial cells; ACs, astrocytes; shRNA, short hairpin ribonucleic acid; DA, dopaminergic; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

TABLE 7B | Cytokines and their source in the PARK7-associated human PD.

Human PD	DJ-1 level and its source	Pro-inflammatory cytokines, chemokines, and their source		Brain defects	References
PARK7/DJ-1-associated PD	Alpha synuclein in $SNPC^{P-}$ DJ-1 in HEK293 cells$^{M-\text{and}P-}$ DJ-1 in substantia nigra^{P+} Oxidized DJ-1 in Lewy bodies^{P+} Oxidized DJ-1 in astrocytes^{P+} DJ-1 and Tau protein in neurofibrillary tangles^{P+} Postmortem full brain$^{M-\text{and }P-}$	ND	ND	Loss of DA neurons in SNPC, Lewy body formation, motor defects, muscle wasting NO-induced DA neuronal	(300, 305)

Of special note, no definite or concrete data have been found about cytokine levels in PARK7-human associated PD.
DJ-1, protein deglycase-1; SNPC, substantia nigra pars compacta; HEK293, human embryonic kidney-293; NO, nitric oxide synthase; DA, dopaminergic; M, mRNA expression; P, protein expression; ++, higher increases; +, moderate increases; –, decreased level; ∞, no change; ND, no data.

and the resultant generation of pro-inflammatory cytokines at the periphery alter the blood–brain barrier integrity. This situation permits the recruitment of immune cells, to the specific region of the brain where infiltrated (e.g., MOs, DCs, $CD4^+$ T cells, and $CD8^+$ T cells) and residential immune cells (e.g., MGCs) meet and amplify their activation, and the resultant massive generation of pro-inflammatory cytokines (e.g., IFNγ, TNFα, IL-1β, IL-6, IL-8, IL-12, and IL-17), which are all lethal to DA neurons, and this condition develops neurodegeneration in PD.

AUTHOR CONTRIBUTIONS

AFM and SLH prepared and designed the tables. RR designed the figures and assisted in the writing and critical review of the text. MKP conceptualized, designed, wrote, reviewed, edited, and approved the submitted version of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry*. (2008) 79:368. doi: 10.1136/jnnp.2007.131045
2. Bonnet AM, Jutras MF, Czernecki V, Corvol JC, Vidailhet M. Nonmotor symptoms in Parkinson's disease in 2012: relevant clinical aspects. *Parkinson Dis*. (2012) 2012:198316. doi: 10.1155/2012/198316
3. Subhramanyam CS, Wang C, Hu Q, Dheen ST. Microglia-mediated neuroinflammation in neurodegenerative diseases. *Semin Cell Dev Biol*. (2019) 94:112–20. doi: 10.1016/j.semcdb.2019.05.004
4. Talaat RM, Mohamed SF, Bassyouni IH, Raouf AA. Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: correlation with disease activity. *Cytokine*. (2015) 72:146–53. doi: 10.1016/j.cyto.2014.12.027
5. Taipa R, das Neves SP, Sousa AL, Fernandes J, Pinto C, Correia AP, et al. Proinflammatory and anti-inflammatory cytokines in the CSF of patients with Alzheimer's disease and their correlation with cognitive decline. *Neurobiol Aging*. (2019) 76:125–32. doi: 10.1016/j.neurobiolaging.2018.12.019
6. Mrugacz M, Ostrowska L, Bryl A, Szulc A, Zelazowska-Rutkowska B, Mrugacz G. Pro-inflammatory cytokines associated with clinical severity of dry eye disease of patients with depression. *Adv Med Sci*. (2017) 62:338–44. doi: 10.1016/j.advms.2017.03.003
7. He W, Long T, Pan Q, Zhang S, Zhang Y, Zhang D, et al. Microglial NLRP3 inflammasome activation mediates IL-1β release and contributes to central sensitization in a recurrent nitroglycerin-induced migraine model. *J Neuroinflamm*. (2019) 16:78. doi: 10.1186/s12974-019-1459-7
8. Sirivichayakul S, Kanchanatawan B, Thika S, Carvalho AF, Maes M. A new schizophrenia model: immune activation is associated with the induction of different neurotoxic products which together determine memory impairments and schizophrenia symptom dimensions. *CNS Neurol Disord Drug Targets*. (2019) 18:124–40. doi: 10.2174/1871527317666181119115532
9. Decourt B, Lahiri DK, Sabbagh MN. Targeting tumor necrosis factor alpha for Alzheimer's disease. *Curr Alzheimer Res*. (2017) 14:412–25. doi: 10.2174/1567205013666160930110551
10. Zhang L, Zheng H, Wu R, Kosten TR, Zhang XY, Zhao J. The effect of minocycline on amelioration of cognitive deficits and pro-inflammatory cytokines levels in patients with schizophrenia. *Schizophrenia Res*. (2019) 212:92–8. doi: 10.1016/j.schres.2019.08.005
11. Moaaz M, Youssry S, Elfatatry A, El Rahman MA. Th17/Treg cells imbalance and their related cytokines (IL-17, IL-10 and TGF-β) in children with autism spectrum disorder. *J Neuroimmunol*. (2019) 337:577071. doi: 10.1016/j.jneuroim.2019.577071
12. Hickman S, Izzy S, Sen P, Morsett L, El Khoury J. Microglia in neurodegeneration. *Nat Neurosci*. (2018) 21:1359–69. doi: 10.1038/s41593-018-0242-x
13. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci*. (2005) 8:752–8. doi: 10.1038/nn1472
14. Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. *Nat Rev Immunol*. (2011) 11:775–87. doi: 10.1038/nri3086
15. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. (2010) 140:918–34. doi: 10.1016/j.cell.2010.02.016
16. Cameron B, Landreth GE. Inflammation, microglia, alzheimer's disease. *Neurobiol Dis*. (2010) 37:503–9. doi: 10.1016/j.nbd.2009.10.006
17. Perry VH, Nicoll JAR, Holmes C. Microglia in neurodegenerative disease. *Nat Rev Neurol*. (2010) 6:193–201. doi: 10.1038/nrneurol.2010.17
18. O'Keefe GM, Nguyen VT, Benveniste EN. Regulation and function of class II major histocompatibility complex, CD40, and B7 expression in macrophages and microglia: implications in neurological diseases. *J Neurovirol*. (2002) 8:496–512. doi: 10.1080/13550280290100941
19. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol*. (1989) 7:145–73. doi: 10.1146/annurev.iy.07.040189.001045
20. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies *in vivo*. *Immunity*. (2001) 15:985–95. doi: 10.1016/S1074-7613(01)00243-6
21. Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. (2006) 126:1121–33. doi: 10.1016/j.cell.2006.07.035
22. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity*. (2004) 21:467–76. doi: 10.1016/j.immuni.2004.08.018
23. Elsaesser H, Sauer K, Brooks DG. IL-21 is required to control chronic viral infection. *Science*. (2009) 324:1569–72. doi: 10.1126/science.1174182
24. Harker JA, Lewis GM, Mack L, Zuniga EI. Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. *Science*. (2011) 334:825–9. doi: 10.1126/science.1208421
25. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science*. (2010) 327:1098–102. doi: 10.1126/science.1178334
26. Machhi J, Kevadiya BD, Muhammad IK, Herskovitz J, Olson KE, Mosley RL, et al. Harnessing regulatory T cell neuroprotective activities for treatment of neurodegenerative disorders. *Mol Neurodegener*. (2020) 15:32. doi: 10.1186/s13024-020-00375-7
27. Sommer A, Winner B, Prots I. The Trojan horse - neuroinflammatory impact of T cells in neurodegenerative diseases. *Mol Neurodegener*. (2017) 12:78. doi: 10.1186/s13024-017-0222-8
28. Solleiro-Villavicencio H, Rivas-Arancibia S. Effect of chronic oxidative stress on neuroinflammatory response mediated by CD4+T cells in neurodegenerative diseases. *Front Cell Neurosci*. (2018) 12:114. doi: 10.3389/fncel.2018.00114
29. Jankovic D, Kugler DG, Sher A. IL-10 production by CD4+ effector T cells: a mechanism for self-regulation. *Mucosal Immunol*. (2010) 3:239–46. doi: 10.1038/mi.2010.8
30. O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity*. (1998) 8:275–83. doi: 10.1016/S1074-7613(00)80533-6
31. Sher A, Coffman RL. Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annu Rev Immunol*. (1992) 10:385–409. doi: 10.1146/annurev.iy.10.040192.002125
32. Romagnani S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol*. (1994) 12:227–57. doi: 10.1146/annurev.iy.12.040194.001303
33. Bottomly K. A functional dichotomy in CD4+ T lymphocytes. *Immunol Today*. (1988) 9:268–74. doi: 10.1016/0167-5699(88)91308-4
34. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med*. (2003) 198:1951–7. doi: 10.1084/jem.20030896
35. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med*. (2005) 201:233–40. doi: 10.1084/jem.20041257
36. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. (2006) 441:235–8. doi: 10.1038/nature04753
37. Sojka DK, Fowell DJ. Regulatory T cells inhibit acute IFN-gamma synthesis without blocking T-helper cell type 1 (Th1) differentiation via a compartmentalized requirement for IL-10. *Proc Natl Acad Sci USA*. (2011) 108:18336–41. doi: 10.1073/pnas.1110566108
38. Amarnath S, Mangus CW, Wang JC, Wei F, He A, Kapoor V, et al. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. *Sci Transl Med*. (2011) 3:111ra120. doi: 10.1126/scitranslmed.3003130
39. Ellwardt E, Walsh JT, Kipnis J, Zipp F. Understanding the role of T cells in CNS homeostasis. *Trends Immunol*. (2016) 37:154–65. doi: 10.1016/j.it.2015.12.008
40. Korn T, Kallies A. T cell responses in the central nervous system. *Nat Rev Immunol*. (2017) 17:179–94. doi: 10.1038/nri.2016.144
41. Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debré P, et al. FcεRII/CD23 is expressed in Parkinson's disease and induces, *in vitro*, production of nitric oxide and tumor necrosis factor-α in glial cells. *J Neurosci*. (1999) 19:3440. doi: 10.1523/JNEUROSCI.19-09-03440.1999

42. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology.* (1988) 38:1285–91. doi: 10.1212/WNL.38.8.1285
43. Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Investig.* (2009) 119:182–92. doi: 10.1172/JCI36470
44. Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, et al. Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients. *Neurosci Lett.* (1994) 180:147–50. doi: 10.1016/0304-3940(94)90508-8
45. Chen X, Hu Y, Cao Z, Liu Q, Cheng Y. Cerebrospinal fluid inflammatory cytokine aberrations in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis: a systematic review and meta-analysis. *Front Immunol.* (2018) 9:2122. doi: 10.3389/fimmu.2018.02122
46. Grozdanov V, Bliederhaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, et al. Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. *Acta Neuropathol.* (2014) 128:651–63. doi: 10.1007/s00401-014-1345-4
47. Saunders JAH, Estes KA, Kosloski LM, Allen HE, Dempsey KM, Torres-Russotto DR, et al. CD4+ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson's disease. *J Neuroimmune Pharmacol.* (2012) 7:927–38. doi: 10.1007/s11481-012-9402-z
48. Baba Y, Kuroiwa A, Uitti RJ, Wszolek ZK, Yamada T. Alterations of T-lymphocyte populations in Parkinson disease. *Parkinsonism Related Disord.* (2005) 11:493–8. doi: 10.1016/j.parkreldis.2005.07.005
49. Chen X, Liu Z, Cao B-B, Qiu Y-H, Peng Y-P. TGF-β1 neuroprotection via inhibition of microglial activation in a rat model of Parkinson's disease. *J Neuroimmune Pharmacol.* (2017) 12:433–46. doi: 10.1007/s11481-017-9732-y
50. Yang L, Guo C, Zhu J, Feng Y, Chen W, Feng Z, et al. Increased levels of pro-inflammatory and anti-inflammatory cellular responses in parkinson's disease patients: search for a disease indicator. *Med Sci Monit.* (2017) 23:2972–8. doi: 10.12659/MSM.904240
51. Kustrimovic N, Rasini E, Legnaro M, Bombelli R, Aleksic I, Blandini F, et al. Dopaminergic receptors on CD4+ T naive and memory lymphocytes correlate with motor impairment in patients with Parkinson's disease. *Sci Rep.* (2016) 6:33738. doi: 10.1038/srep33738
52. Bu X-L, Wang X, Xiang Y, Shen L-L, Wang Q-H, Liu Y-H, et al. The association between infectious burden and Parkinson's disease: a case-control study. *Parkinsonism Related Disord.* (2015) 21:877–81. doi: 10.1016/j.parkreldis.2015.05.015
53. Williams-Gray CH, Wijeyekoon R, Yarnall AJ, Lawson RA, Breen DP, Evans JR, et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Movement Disord.* (2016) 31:995–1003. doi: 10.1002/mds.26563
54. Dursun E, Gezen-Ak D, Hanagasi H, Bilgiç B, Lohmann E, Ertan S, et al. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *J Neuroimmunol.* (2015) 283:50–7. doi: 10.1016/j.jneuroim.2015.04.014
55. Hu Y, Yu S-Y, Zuo L-J, Cao C-J, Wang F, Chen Z-J, et al. Parkinson disease with REM sleep behavior disorder. *Neurology.* (2015) 84:888. doi: 10.1212/WNL.0000000000001308
56. Qin X-Y, Zhang S-P, Cao C, Loh YP, Cheng Y. Aberrations in peripheral inflammatory cytokine levels in Parkinson disease: a systematic review and meta-analysis. *JAMA Neurol.* (2016) 73:1316–24. doi: 10.1001/jamaneurol.2016.2742
57. McCoy MK, Martinez TN, Ruhn KA, Szymkowski DE, Smith CG, Botterman BR, et al. Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease. *J Neurosci.* (2006) 26:9365. doi: 10.1523/JNEUROSCI.1504-06.2006
58. Scalzo P, Kümmer A, Cardoso F, Teixeira AL. Serum levels of interleukin-6 are elevated in patients with Parkinson's disease and correlate with physical performance. *Neurosci Lett.* (2010) 468:56–8. doi: 10.1016/j.neulet.2009.10.062
59. Pandey MK, Grabowski GA. Immunological cells and functions in Gaucher disease. *Crit Rev Oncog.* (2013) 18:197–220. doi: 10.1615/CritRevOncog.2013004503
60. Pandey MK, Grabowski GA. Cytology of Gaucher disease in advances in Gaucher disease: basic and clinical perspectives. *Future Med.* (2013) 78-93. doi: 10.2217/ebo.12.209
61. Ripa RS. Granulocyte-colony stimulating factor therapy to induce neovascularization in ischemic heart disease. *Dan Med J.* (2012) 59:B4411.
62. Zohlnhofer D, Ott I, Mehilli J, Schomig K, Michalk F, Ibrahim T, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. *JAMA.* (2006) 295:1003. doi: 10.1001/jama.296.16.1968-b
63. Zhang H, Bai H, Yi Z, He X, Mo S. Effect of stem cell factor and granulocyte-macrophage colony-stimulating factor-induced bone marrow stem cell mobilization on recovery from acute tubular necrosis in rats. *Ren Fail.* (2012) 34:350–7. doi: 10.3109/0886022X.2011.647340
64. Rapoport AP, Abboud CN, DiPersio JF. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF): receptor biology, signal transduction, neutrophil activation. *Blood Rev.* (1992) 6:43–57. doi: 10.1016/0268-960X(92)90007-D
65. Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood.* (1994) 84:1737–46. doi: 10.1182/blood.V84.6.1737.1737
66. Avigan D, Wu Z, Gong J, Joyce R, Levine J, Elias A, et al. Selective *in vivo* mobilization with granulocyte macrophage colony-stimulating factor (GM-CSF)/granulocyte-CSF as compared to G-CSF alone of dendritic cell progenitors from peripheral blood progenitor cells in patients with advanced breast cancer undergoing autologous transplantation. *Clin Cancer Res.* (1999) 5:2735–41.
67. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med.* (2006) 354:610–21. doi: 10.1056/NEJMra052723
68. Mantovani A, Bonecchi R, Locati M. Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat Rev Immunol.* (2006) 6:907–18. doi: 10.1038/nri1964
69. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, dendritic cells. *Science.* (2010) 327:656–61. doi: 10.1126/science.1178331
70. Chesselet M-F, Richter F, Zhu C, Magen I, Watson MB, Subramaniam SR. A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("line 61") mice. *Neurotherapeutics.* (2012) 9:297–314. doi: 10.1007/s13311-012-0104-2
71. Brodacki B, Staszewski J, Toczyłowska B, Kozłowska E, Drela N, Chalimoniuk M, et al. Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFalpha, and INFgamma concentrations are elevated in patients with atypical and idiopathic parkinsonism. *Neurosci Lett.* (2008) 441:158–62. doi: 10.1016/j.neulet.2008.06.040
72. Ciesielska A, Joniec I, Kurkowska-Jastrzebska I, Przybyłkowski A, Gromadzka G, Członkowska A, et al. Influence of age and gender on cytokine expression in a murine model of Parkinson's disease. *Neuroimmunomodulation.* (2007) 14:255–65. doi: 10.1159/000113432
73. Mandel S, Grünblatt E, Youdim M. cDNA microarray to study gene expression of dopaminergic neurodegeneration and neuroprotection in MPTP and 6-hydroxydopamine models: implications for idiopathic Parkinson's disease. *Neuroimmunomodulation.* (2000) 117–24. doi: 10.1007/978-3-7091-6301-6_7
74. Lofrumento DD, Saponaro C, Cianciulli A, De Nuccio F, Mitolo V, Nicolardi G, et al. MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain. *Neuroimmunomodulation.* (2011) 18:79–88. doi: 10.1159/000320027
75. Chung YC, Bok E, Huh SH, Park J-Y, Yoon S-H, Kim SR, et al. Cannabinoid receptor type 1 protects nigrostriatal dopaminergic neurons against MPTP neurotoxicity by inhibiting microglial activation. *J Immunol.* (2011) 187:6508. doi: 10.4049/jimmunol.1102435

76. Chung YC, Kim SR, Jin BK. Paroxetine prevents loss of nigrostriatal dopaminergic neurons by inhibiting brain inflammation and oxidative stress in an experimental model of Parkinson's disease. *J Immunol.* (2010) 185:1230–7. doi: 10.4049/jimmunol.1000208
77. Lee E, Hwang I, Park S, Hong S, Hwang B, Cho Y, et al. MPTP-driven NLRP3 inflammasome activation in microglia plays a central role in dopaminergic neurodegeneration. *Cell Death Different.* (2019) 26:213–28. doi: 10.1038/s41418-018-0124-5
78. Javed H, Thangavel R, Selvakumar GP, Dubova I, Schwartz N, Ahmed ME, et al. NLRP3 inflammasome and glia maturation factor coordinately regulate neuroinflammation and neuronal loss in MPTP mouse model of Parkinson's disease. *Int Immunopharmacol.* (2020) 83:106441. doi: 10.1016/j.intimp.2020.106441
79. Sliter DA, Martinez J, Hao L, Chen X, Sun N, Fischer TD, et al. Parkin and PINK1 mitigate STING-induced inflammation. *Nature.* (2018) 561:258–62. doi: 10.1038/s41586-018-0448-9
80. Lopez de Maturana R, Aguila JC, Sousa A, Vazquez N, Del Rio P, Aiastui A, et al. Leucine-rich repeat kinase 2 modulates cyclooxygenase 2 and the inflammatory response in idiopathic and genetic Parkinson's disease. *Neurobiol Aging.* (2014) 35:1116–24. doi: 10.1016/j.neurobiolaging.2013.11.018
81. McGeer PL, Yasojima K, McGeer EG. Association of interleukin-1β polymorphisms with idiopathic Parkinson's disease. *Neurosci Lett.* (2002) 326:67–9. doi: 10.1016/S0304-3940(02)00300-2
82. Klüter H, Vieregge P, Stolze H, Kirchner H. Defective production of interleukin-2 in patients with idiopathic Parkinson's disease. *J Neurol Sci.* (1995) 133:134–9. doi: 10.1016/0022-510X(95)00180-A
83. Dufek M, Rektorova I, Thon V, Lokaj J, Rektor I. Interleukin-6 may contribute to mortality in Parkinson's disease patients: a 4-year prospective study. *Parkinson's Dis.* (2015) 2015:898192. doi: 10.1155/2015/898192
84. Qiu X, Xiao Y, Wu J, Gan L, Huang Y, Wang J. C-reactive protein and risk of Parkinson's disease: a systematic review and meta-analysis. *Front Neurol.* (2019) 10:384. doi: 10.3389/fneur.2019.00384
85. Rudenko IN, Chia R, Cookson MR. Is inhibition of kinase activity the only therapeutic strategy for LRRK2-associated Parkinson's disease? *BMC Med.* (2012) 10:20. doi: 10.1186/1741-7015-10-20
86. Rideout HJ, Stefanis L. The neurobiology of LRRK2 and its role in the pathogenesis of Parkinson's disease. *Neurochem Res.* (2014) 39:576–92. doi: 10.1007/s11064-013-1073-5
87. Wallings R, Manzoni C, Bandopadhyay R. Cellular processes associated with LRRK2 function and dysfunction. *FEBS J.* (2015) 282:2806–26. doi: 10.1111/febs.13305
88. Gardet A, Benita Y, Li C, Sands BE, Ballester I, Stevens C, et al. LRRK2 is involved in the IFN-gamma response and host response to pathogens. *J Immunol.* (2010) 185:5577–85. doi: 10.4049/jimmunol.1000548
89. Schapansky J, Nardozzi JD, Felizia F, LaVoie MJ. Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. *Hum Mol Genet.* (2014) 23:4201–14. doi: 10.1093/hmg/ddu138
90. Miklossy J, Arai T, Guo JP, Klegeris A, Yu S, McGeer EG, et al. LRRK2 expression in normal and pathologic human brain and in human cell lines. *J Neuropathol Exp Neurol.* (2006) 65:953–63. doi: 10.1097/01.jnen.0000235121.98052.54
91. Gillardon F, Schmid R, Draheim H. Parkinson's disease-linked leucine-rich repeat kinase 2(R1441G) mutation increases proinflammatory cytokine release from activated primary microglial cells and resultant neurotoxicity. *Neuroscience.* (2012) 208:41–8. doi: 10.1016/j.neuroscience.2012.02.001
92. Kuss M, Adamopoulou E, Kahle PJ. Interferon-γ induces leucine-rich repeat kinase LRRK2 via extracellular signal-regulated kinase ERK5 in macrophages. *J Neurochem.* (2014) 129:980–7. doi: 10.1111/jnc.12668
93. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer.* (1980) 26:171–6. doi: 10.1002/ijc.2910260208
94. Thévenet J, Pescini Gobert R, van Huijsduijnen RH, Wiessner C, Sagot YJ. Regulation of LRRK2 expression points to a functional role in human monocyte maturation. *PLoS ONE.* (2011) 6:e21519. doi: 10.1371/journal.pone.0021519
95. Moehle MS, Webber PJ, Tse T, Sukar N, Standaert DG, DeSilva TM, et al. LRRK2 inhibition attenuates microglial inflammatory responses. *J Neurosci.* (2012) 32:1602–11. doi: 10.1523/JNEUROSCI.5601-11.2012
96. Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet.* (2010) 42:1118–25. doi: 10.1038/ng.717
97. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet.* (2008) 40:955–62. doi: 10.1038/ng.175
98. Zhang FR, Huang W, Chen SM, Sun LD, Liu H, Li Y, et al. Genomewide association study of leprosy. *N Engl J Med.* (2009) 361:2609–18. doi: 10.1056/NEJMoa0903753
99. Zhang D, Lin J, Han J. Receptor-interacting protein (RIP) kinase family. *Cell Mol Immunol.* (2010) 7:243–9. doi: 10.1038/cmi.2010.10
100. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci USA.* (2005) 102:16842. doi: 10.1073/pnas.0507360102
101. Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, et al. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol Dis.* (2006) 23:329–41. doi: 10.1016/j.nbd.2006.04.001
102. Luzón-Toro B, Rubio de la Torre E, Delgado A, Pérez-Tur J, Hilfiker S. Mechanistic insight into the dominant mode of the Parkinson's disease-associated G2019S LRRK2 mutation. *Hum Mol Genet.* (2007) 16:2031–9. doi: 10.1093/hmg/ddm151
103. Steger M, Tonelli F, Ito G, Davies P, Trost M, Vetter M, et al. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. *eLife.* (2016) 5:1–28. doi: 10.7554/eLife.12813
104. Greggio E. Role of LRRK2 kinase activity in the pathogenesis of Parkinson's disease. *Biochem Soc Trans.* (2012) 40:1058–62. doi: 10.1042/BST20120054
105. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* (2008) 7:583–90. doi: 10.1016/S1474-4422(08)70117-0
106. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet.* (2009) 41:1303–7. doi: 10.1038/ng.485
107. Haugarvoll K, Rademakers R, Kachergus JM, Nuytemans K, Ross OA, Gibson JM, et al. Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. *Neurology.* (2008) 70:1456–60. doi: 10.1212/01.wnl.0000304044.22253.03
108. Haugarvoll K, Wszolek ZK. Clinical features of LRRK2 parkinsonism. *Parkinsonism Relat Disord.* (2009) 3(15 Suppl):S205–8. doi: 10.1016/S1353-8020(09)70815-6
109. Simón-Sánchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet.* (2009) 41:1308–12. doi: 10.1038/ng.487
110. Wider C, Dickson DW, Wszolek ZK. Leucine-rich repeat kinase 2 gene-associated disease: redefining genotype-phenotype correlation. *Neurodegener Dis.* (2010) 7:175–9. doi: 10.1159/000289232
111. Sheng Z, Zhang S, Bustos D, Kleinheinz T, Le Pichon CE, Dominguez SL, et al. Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. *Sci Transl Med.* (2012) 4:164ra161. doi: 10.1126/scitranslmed.3004485
112. Jaleel M, Nichols RJ, Deak M, David Campbell G, Gillardon F, Knebel A, et al. LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem J.* (2007) 405:307–17. doi: 10.1042/BJ20070209
113. Lee BD, Shin JH, VanKampen J, Petrucelli L, West AB, Ko HS, et al. Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. *Nat Med.* (2010) 16:998–1000. doi: 10.1038/nm.2199
114. Daher JP, Volpicelli-Daley LA, Blackburn JP, Moehle MS, West AB. Abrogation of α-synuclein-mediated dopaminergic neurodegeneration in LRRK2-deficient rats. *Proc Natl Acad Sci USA.* (2014) 111:9289–94. doi: 10.1073/pnas.1403215111

115. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron.* (2004) 44:601–7. doi: 10.1016/j.neuron.2004.11.005
116. Paisán-Ruíz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* (2004) 44:595–600. doi: 10.1016/j.neuron.2004.10.023
117. Dächsel JC, Farrer MJ. LRRK2 and Parkinson disease. *Arch Neurol.* (2010) 67:542–7. doi: 10.1001/archneurol.2010.79
118. Singleton AB, Farrer MJ, Bonifati V. The genetics of Parkinson's disease: progress and therapeutic implications. *Movement Disord.* (2013) 28:14–23. doi: 10.1002/mds.25249
119. Lesage S, Dürr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N Engl J Med.* (2006) 354:422–3. doi: 10.1056/NEJMc055540
120. Kozina E, Sadasivan S, Jiao Y, Dou Y, Ma Z, Tan H, et al. Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration *in vivo*. *Brain.* (2018) 141:1753–69. doi: 10.1093/brain/awy077
121. Russo I, Berti G, Plotegher N, Bernardo G, Filograna R, Bubacco L, et al. Leucine-rich repeat kinase 2 positively regulates inflammation and down-regulates NF-κB p50 signaling in cultured microglia cells. *J Neuroinflamm.* (2015) 12:230. doi: 10.1186/s12974-015-0449-7
122. Dzamko N, Rowe DB, Halliday GM. Increased peripheral inflammation in asymptomatic leucine-rich repeat kinase 2 mutation carriers. *Movement Disord.* (2016) 31:889–97. doi: 10.1002/mds.26529
123. Kim B, Yang M-S, Choi D, Kim J-H, Kim H-S, Seol W, et al. Impaired inflammatory responses in murine Lrrk2-knockdown brain microglia. *PLoS ONE.* (2012) 7: e34693. doi: 10.1371/journal.pone.0034693
124. Cook DA, Kannarkat GT, Cintron AF, Butkovich LM, Fraser KB, Chang J, et al. LRRK2 levels in immune cells are increased in Parkinson's disease. *NPJ Parkinson's Dis.* (2017) 3:11. doi: 10.1038/s41531-017-0010-8
125. Grabowski GA, Petsko GA, Kolodny EH. Gaucher disease. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, editors. *The Online Metabolic and Molecular Bases of Inherited Disease*. New York, NY: Mc Graw Hill (2010) 1–13.
126. Brady RO, Kanfer J, Shapiro D. The metabolism of glucocerebrosides. Purification I, and properties of a glucocerebroside-cleaving enzyme from spleen tissue. *J Biol Chem.* (1965) 240:39–43. doi: 10.1016/S0021-9258(18)97611-8
127. Xu YH, Quinn B, Witte D, Grabowski GA. Viable mouse models of acid beta-glucosidase deficiency: the defect in Gaucher disease. *Am J Pathol.* (2003) 163:2093–101. doi: 10.1016/S0002-9440(10)63566-3
128. Pandey MK, Rani R, Zhang W, Setchell K, Grabowski GA. Immunological cell type characterization and Th1-Th17 cytokine production in a mouse model of Gaucher disease. *Mol Genet Metab.* (2012) 106:310–22. doi: 10.1016/j.ymgme.2012.04.020
129. Pandey MK, Grabowski GA. Cytology of Gaucher disease. In: Grabowski GA, editor. *Advances in Gaucher Disease: Basic and Clinical Perspectives*. London: Future Medicine Ltd. (2013). p. 78–93.
130. Pandey MK, Tinch SL, Jabre NA, Grabowski GA. Gaucher disease: glucosylceramide-mediated TLR4-MyD88 induction causes enhanced CXCL-13 secretion and increased B cell trafficking in a mouse model. *Mol Genet Metab.* (2014) 111:S84. doi: 10.1016/j.ymgme.2013.12.199
131. Pandey MK, Burrow TA, Rani R, Martin LJ, Witte D, Setchell KD, et al. Complement drives glucosylceramide accumulation and tissue inflammation in Gaucher disease. *Nature.* (2017) 543:108–12. doi: 10.1038/nature21368
132. Pandey MK, Grabowski GA, Kohl J. An unexpected player in Gaucher disease: the multiple roles of complement in disease development. *Semin Immunol.* (2018) 37:30–42. doi: 10.1016/j.smim.2018.02.006
133. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *New Engl J Med.* (2009) 361:1651–61. doi: 10.1056/NEJMoa0901281
134. Wong K, Sidransky E, Verma A, Mixon T, Sandberg GD, Wakefield LK, et al. Neuropathology provides clues to the pathophysiology of Gaucher disease. *Mol Genet Metab.* (2004) 82:192–207. doi: 10.1016/j.ymgme.2004.04.011
135. Vitner EB, Farfel-Becker T, Eilam R, Biton I, Futerman AH. Contribution of brain inflammation to neuronal cell death in neuronopathic forms of Gaucher's disease. *Brain.* (2012) 135:1724–35. doi: 10.1093/brain/aws095
136. Vitner EB, Dekel H, Zigdon H, Shachar T, Farfel-Becker T, Eilam R, et al. Altered expression and distribution of cathepsins in neuronopathic forms of Gaucher disease and in other sphingolipidoses. *Human Mol Genet.* (2010) 19:3583–90. doi: 10.1093/hmg/ddq273
137. Dasgupta N, Xu YH, Li R, Peng Y, Pandey MK, Tinch SL, et al. Neuronopathic Gaucher disease: dysregulated mRNAs and miRNAs in brain pathogenesis and effects of pharmacologic chaperone treatment in a mouse model. *Human Mol Genet.* (2015) 24:7031–48. doi: 10.1093/hmg/ddv404
138. Gegg ME, Burke D, Heales SJR, Cooper JM, Hardy J, Wood NW, et al. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. *Ann Neurol.* (2012) 72:455–63. doi: 10.1002/ana.23614
139. Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, et al. Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson's disease. *Brain.* (2014) 137:834–48. doi: 10.1093/brain/awt367
140. Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, et al. GBA mutations increase risk for Lewy body disease with and without Alzheimer disease pathology. *Neurology.* (2012) 79:1944–50. doi: 10.1212/WNL.0b013e3182735e9a
141. Moors TE, Paciotti S, Ingrassia A, Quadri M, Breedveld G, Tasegian A, et al. Characterization of brain lysosomal activities in GBA-related and sporadic Parkinson's disease and dementia with lewy bodies. *Mol Neurobiol.* (2019) 56:1344–55. doi: 10.1007/s12035-018-1090-0
142. Halperin A, Elstein D, Zimran A. Increased incidence of Parkinson disease among relatives of patients with Gaucher disease. *Blood Cells Mol Dis.* (2006) 36:426–8. doi: 10.1016/j.bcmd.2006.02.004
143. Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E. Parkinsonism among Gaucher disease carriers. *J Med Genet.* (2004) 41:937. doi: 10.1136/jmg.2004.024455
144. Rosenbloom B, Balwani M, Bronstein JM, Kolodny E, Sathe S, Gwosdow AR, et al. The incidence of Parkinsonism in patients with type 1 Gaucher disease: data from the ICGG Gaucher registry. *Blood Cells Mol Dis.* (2011) 46:95–102. doi: 10.1016/j.bcmd.2010.10.006
145. Lopez G, Kim J, Wiggs E, Cintron D, Groden C, Tayebi N, et al. Clinical course and prognosis in patients with Gaucher disease and parkinsonism. *Neurol Genet.* (2016) 2:e57. doi: 10.1212/NXG.0000000000000057
146. Zhang Y, Shu L, Zhou X, Pan H, Xu Q, Guo J, et al. A meta-analysis of *GBA*-related clinical symptoms in Parkinson's disease. *Parkinson's Dis.* (2018) 2018:3136415. doi: 10.1155/2018/3136415
147. Velez-Pardo C, Lorenzo-Betancor O, Jimenez-Del-Rio M, Moreno S, Lopera F, Cornejo-Olivas M, et al. The distribution and risk effect of GBA variants in a large cohort of PD patients from Colombia and Peru. *Parkinsonism Related Disord.* (2019) 63:204–8. doi: 10.1016/j.parkreldis.2019.01.030
148. Cilia R, Tunesi S, Marotta G, Cereda E, Siri C, Tesei S, et al. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann Neurol.* (2016) 80:662–73. doi: 10.1002/ana.24777
149. Thaler A, Gurevich T, Bar Shira A, Gana Weisz M, Ash E, Shiner T, et al. A "dose" effect of mutations in the GBA gene on Parkinson's disease phenotype. *Parkinsonism Related Disord.* (2017) 36:47–51. doi: 10.1016/j.parkreldis.2016.12.014
150. Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, et al. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain.* (2009) 132:1783–94. doi: 10.1093/brain/awp044
151. Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology.* (2015) 84:880–7. doi: 10.1212/WNL.0000000000001315
152. Jesús S, Huertas I, Bernal-Bernal I, Bonilla-Toribio M, Cáceres-Redondo MT, Vargas-González L, et al. GBA variants influence motor and non-motor features of Parkinson's disease. *PLoS ONE.* (2016) 11:e0167749. doi: 10.1371/journal.pone.0167749
153. Malek N, Weil RS, Bresner C, Lawton MA, Grosset KA, Tan M, et al. Features of GBA-associated Parkinson's disease at presentation in the UK Tracking Parkinson's study. *J Neurol Neurosurg Psychiatry.* (2018) 89:702–9. doi: 10.1136/jnnp-2017-317348

154. Atashrazm F, Hammond D, Perera G, Dobson-Stone C, Mueller N, Pickford R, et al. Reduced glucocerebrosidase activity in monocytes from patients with Parkinson's disease. *Sci Rep.* (2018) 8:15446. doi: 10.1038/s41598-018-33921-x
155. Westbroek W, Gustafson AM, Sidransky E. Exploring the link between glucocerebrosidase mutations and parkinsonism. *Trends Mol Med.* (2011) 17:485–93. doi: 10.1016/j.molmed.2011.05.003
156. Clarke E, Jantrachotechatchawan C, Buhidma Y, Broadstock M, Yu L, Howlett D, et al. Age-related neurochemical and behavioural changes in D409V/WT GBA1 mouse: relevance to lewy body dementia. *Neurochem Int.* (2019) 129:104502. doi: 10.1016/j.neuint.2019.104502
157. Alcalay RN, Dinur T, Quinn T, Sakanaka K, Levy O, Waters C, et al. Comparison of parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes. *JAMA Neurol.* (2014) 71:752–7. doi: 10.1001/jamaneurol.2014.313
158. Hugo Fernandes JR, Elizabeth Hartfield M, Helen Christian C, Emmanoulidou E, Zheng Y, Booth H, et al. ER stress and autophagic perturbations lead to elevated extracellular α-synuclein in GBA-N370S Parkinson's iPSC-derived dopamine neurons. *Stem Cell Rep.* (2016) 6:342–56. doi: 10.1016/j.stemcr.2016.01.013
159. Schöndorf DC, Aureli M, McAllister FE, Hindley CJ, Mayer F, Schmid B, et al. iPSC-derived neurons from GBA1-associated Parkinson's disease patients show autophagic defects and impaired calcium homeostasis. *Nat Commun.* (2014) 5:4028. doi: 10.1038/ncomms5028
160. Yun SP, Kim D, Kim S, Kim S, Karuppagounder SS, Kwon S-H, et al. alpha-Synuclein accumulation and GBA deficiency due to L444P GBA mutation contributes to MPTP-induced parkinsonism. *Mol Neurodegen.* (2018) 13:1 doi: 10.1186/s13024-017-0233-5
161. Chahine LM, Qiang J, Ashbridge E, Minger J, Yearout D, Horn S, et al. Clinical and biochemical differences in patients having Parkinson disease with vs without GBA mutations. *JAMA Neurol.* (2013) 70:852–8. doi: 10.1001/jamaneurol.2013.1274
162. Miliukhina IV, Usenko TS, Senkevich KA, Nikolaev MA, Timofeeva AA, Agapova EA, et al. Plasma cytokines profile in patients with Parkinson's disease associated with mutations in GBA gene. *Bull Exp Biol Med.* (2020) 168:423–6. doi: 10.1007/s10517-020-04723-x
163. Nagatsu T, Mogi M, Ichinose H, Togari A. Changes in cytokines and neurotrophins in Parkinson's disease. *J Neural Transm Suppl.* (2000) 277–90. doi: 10.1007/978-3-7091-6301-6_19
164. Álvarez-Luquín DD, Arce-Sillas A, Leyva-Hernández J, et al. Regulatory impairment in untreated Parkinson's disease is not restricted to Tregs: other regulatory populations are also involved. *J Neuroinflammation.* (2019) 16:212. doi: 10.1186/s12974-019-1606-1
165. Hofmann KW, Schuh AFS, Saute J, Townsend R, Fricke D, Leke R, et al. Interleukin-6 Serum Levels in Patients with Parkinson's Disease. *Neurochem Res.* (2009) 34:1401–4. doi: 10.1007/s11064-009-9921-
166. Miller DW, Hague SM, Clarimon J, Baptista M, Gwinn-Hardy K, Cookson MR, et al. Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. *Neurology.* (2004) 62:1835–8. doi: 10.1212/01.WNL.0000127517.33208.F4
167. Scherzer CR, Grass JA, Liao Z, Pepivani I, Zheng B, Eklund AC, et al. GATA transcription factors directly regulate the Parkinson's disease-linked gene alpha-synuclein. *Proc Natl Acad Sci USA.* (2008) 105:10907–12. doi: 10.1073/pnas.0802437105
168. El-Agnaf OM, Salem SA, Paleologou KE, Cooper LJ, Fullwood NJ, Gibson MJ, et al. Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB J.* (2003) 17:1945–7. doi: 10.1096/fj.03-0098fje
169. Borghi R, Marchese R, Negro A, Marinelli L, Forloni G, Zaccheo D, et al. Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects. *Neurosci Lett.* (2000) 287:65–7. doi: 10.1016/S0304-3940(00)01153-8
170. Barbour R, Kling K, Anderson JP, Banducci K, Cole T, Diep L, et al. Red blood cells are the major source of alpha-synuclein in blood. *Neurodegener Dis.* (2008) 5:55–9. doi: 10.1159/000112832
171. Kim S, Seo JH, Suh YH. Alpha-synuclein Parkinson's disease, and Alzheimer's disease. *Parkinsonism Relat Disord.* (2004) 1(10 Suppl.): S9–13. doi: 10.1016/j.parkreldis.2003.11.005
172. Richter-Landsberg C, Gorath M, Trojanowski JQ, Lee VM. alpha-synuclein is developmentally expressed in cultured rat brain oligodendrocytes. *J Neurosci Res.* (2000) 62:9–14. doi: 10.1002/1097-4547(20001001)62:1<9::AID-JNR2>3.0.CO;2-U
173. Mori F, Tanji K, Yoshimoto M, Takahashi H, Wakabayashi K. Demonstration of alpha-synuclein immunoreactivity in neuronal and glial cytoplasm in normal human brain tissue using proteinase K and formic acid pretreatment. *Exp Neurol.* (2002) 176:98–104. doi: 10.1006/exnr.2002.7929
174. Chandra S, Chen X, Rizo J, Jahn R, Südhof TC. A broken alpha -helix in folded alpha -synuclein. *J Biol Chem.* (2003) 278:15313–8. doi: 10.1074/jbc.M213128200
175. Atias M, Tevet Y, Sun J, Stavsky A, Tal S, Kahn J, et al. Synapsins regulate α-synuclein functions. *Proc Natl Acad Sci USA.* (2019) 116:11116. doi: 10.1073/pnas.1903054116
176. Sun J, Wang L, Bao H, Premi S, Das U, Chapman ER, et al. Functional cooperation of α-synuclein and VAMP2 in synaptic vesicle recycling. *Proc Natl Acad Sci USA.* (2019) 116:11113. doi: 10.1073/pnas.1903049116
177. Vargas KJ, Makani S, Davis T, Westphal CH, Castillo PE, Chandra SS. Synucleins regulate the kinetics of synaptic vesicle endocytosis. *J Neurosci.* (2014) 34:9364. doi: 10.1523/JNEUROSCI.4787-13.2014
178. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science.* (1997) 276:2045.
179. Zarranz JJ, Alegre J, Gómez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of α-synuclein causes parkinson and Lewy body dementia. *Ann Neurol.* (2004) 55:164–73. doi: 10.1002/ana.10795
180. Farrer M, Kachergus J, Forno L, Lincoln S, Wang D-S, Hulihan M, et al. Comparison of kindreds with parkinsonism and α-synuclein genomic multiplications. *Ann Neurol.* (2004) 55:174–9. doi: 10.1002/ana.10846
181. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet.* (2004) 364:1167–9. doi: 10.1016/S0140-6736(04)17103-1
182. Ibáñez P, Bonnet AM, Débarges B, Lohmann E, Tison F, Agid Y, et al. Causal relation between α-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet.* (2004) 364:1169–71. doi: 10.1016/S0140-6736(04)17104-3
183. Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jakes R, Goedert M. α-synuclein in Lewy bodies. *Nature.* (1997) 388:839–40. doi: 10.1038/42166
184. Braak H, Tredici KD, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
185. Olanow CW, Brundin P. Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? *Movement Disord.* (2013) 28:31–40. doi: 10.1002/mds.25373
186. Golbe LI. Alpha-synuclein and Parkinson's disease. *Movement Disord.* (1999) 14:6–9. doi: 10.1002/1531-8257(199901)14:1<6::AID-MDS1004>3.0.CO;2-L
187. Johnson J, Hague SM, Hanson M, Gibson A, Wilson KE, Evans EW, et al. SNCA multiplication is not a common cause of Parkinson disease or dementia with Lewy bodies. *Neurology.* (2004) 63:554. doi: 10.1212/01.WNL.0000133401.09043.44
188. Cookson MR. The biochemistry of Parkinson's disease. *Annu Rev Biochem.* (2005) 74:29–52. doi: 10.1146/annurev.biochem.74.082803.133400
189. Feany MB, Bender WW. A Drosophila model of Parkinson's disease. *Nature.* (2000) 404:394–8. doi: 10.1038/35006074
190. Maries E, Dass B, Collier TJ, Kordower JH, Steece-Collier K. The role of α-synuclein in Parkinson's disease: insights from animal models. *Nat Rev Neurosci.* (2003) 4:727–38. doi: 10.1038/nrn1199
191. Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ. Synuclein activates microglia in a model of Parkinson's disease. *Neurobiol Aging.* (2008) 29:1690–701. doi: 10.1016/j.neurobiolaging.2007.04.006
192. Alvarez-Erviti L, Couch Y, Richardson J, Cooper JM, Wood MJA. Alpha-synuclein release by neurons activates the inflammatory response in a microglial cell line. *Neurosci Res.* (2011) 69:337–42. doi: 10.1016/j.neures.2010.12.020

193. Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, et al. Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies. *PLoS ONE.* (2013) 8:e55375. doi: 10.1371/journal.pone.0055375
194. Kim C, Ho D-H, Suk J-E, You S, Michael S, Kang J, et al. Neuron-released oligomeric α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. *Nat Commun.* (2013) 4:1562. doi: 10.1038/ncomms2534
195. Roodveldt C, Labrador-Garrido A, Gonzalez-Rey E, Lachaud CC, Guilliams T, Fernandez-Montesinos R, et al. Preconditioning of microglia by α-synuclein strongly affects the response induced by toll-like receptor (TLR) stimulation. *PLoS ONE.* (2013) 8:e79160. doi: 10.1371/journal.pone.0079160
196. Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, et al. Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. *Glia.* (2013) 61:349–60. doi: 10.1002/glia.22437
197. Couch Y, Alvarez-Erviti L, Sibson NR, Wood MJA, Anthony DC. The acute inflammatory response to intranigral α-synuclein differs significantly from intranigral lipopolysaccharide and is exacerbated by peripheral inflammation. *J Neuroinflamm.* (2011) 8:166. doi: 10.1186/1742-2094-8-166
198. Watson MB, Richter F, Lee SK, Gabby L, Wu J, Masliah E, et al. Regionally-specific microglial activation in young mice over-expressing human wildtype alpha-synuclein. *Exp Neurol.* (2012) 237:318–34. doi: 10.1016/j.expneurol.2012.06.025
199. Ferreira M, Massano J. An updated review of Parkinson's disease genetics and clinicopathological correlations. *Acta Neurol Scand.* (2017) 135:273–84. doi: 10.1111/ane.12616
200. Holmans P, Moskvina V, Jones L, Sharma M, Vedernikov A, Buchel F, et al. A pathway-based analysis provides additional support for an immune-related genetic susceptibility to Parkinson's disease. *Hum Mol Genet.* (2013) 22:1039–49. doi: 10.1093/hmg/ddt554
201. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet.* (2010) 42:781–5. doi: 10.1038/ng.642
202. Witoelar A, Jansen IE, Wang Y, Desikan RS, Gibbs JR, Blauwendraat C, et al. Genome-wide pleiotropy between Parkinson disease and autoimmune diseases. *JAMA Neurol.* (2017) 74:780–92. doi: 10.1001/jamaneurol.2017.0469
203. Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. *Annu Rev Immunol.* (2013) 31:443–73. doi: 10.1146/annurev-immunol-032712-095910
204. Neefjes J, Jongsma MLM, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol.* (2011) 11:823–36. doi: 10.1038/nri3084
205. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature.* (2017) 546:656–61. doi: 10.1038/nature22815
206. Pandey MK, Gross C, Caballero M, Mckay MA, Tiwari D, Schroeder LM, et al. Immune cells encounter with α-synuclein fuels neurodegeneration in Parkinson's disease. *J Immunol.* (2016) 196:51–5.
207. Magnusen D, McKay M, Magnusen A, Pandey M. Dendritic cells, CD4+ T cells, and α synuclein triangle fuels neuroinflammation in Parkinson's disease (P3.053). *Neurology.* (2018) 90(15 Supplement).
208. William Wissemann T, Erin Hill-Burns M, Cyrus Zabetian P, Stewart Factor A, Patsopoulos N, Hoglund B, et al. Association of Parkinson disease with structural and regulatory variants in the HLA region. *Am J Human Genetics.* (2013) 93:984–93. doi: 10.1016/j.ajhg.2013.10.009
209. Hill-Burns EM, Wissemann WT, Hamza TH, Factor SA, Zabetian CP, Payami H. Identification of a novel Parkinson's disease locus via stratified genome-wide association study. *BMC Genomics.* (2014) 15:118. doi: 10.1186/1471-2164-15-118
210. Kannarkat GT, Cook DA, Lee JK, Chang J, Chung J, Sandy E, et al. Common genetic variant association with altered HLA expression, synergy with pyrethroid exposure, and risk for Parkinson's disease: an observational and case–control study. *NPJ Parkinson's Dis.* (2015) 1:15002. doi: 10.1038/npjparkd.2015.2
211. Chung CY, Koprich JB, Siddiqi H, Isacson O. Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV α-synucleinopathy. *J Neurosci.* (2009) 29:3365. doi: 10.1523/JNEUROSCI.5427-08.2009
212. Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, et al. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem.* (2008) 104:1504–25. doi: 10.1111/j.1471-4159.2007.05087.x
213. Sznejder-Pachołek A, Joniec-Maciejak I, Wawer A, Ciesielska A, Mirowska-Guzel D. The effect of α-synuclein on gliosis and IL-1α, TNFα, IFNγ, TGFβ expression in murine brain. *Pharmacol Rep.* (2017) 69:242–51. doi: 10.1016/j.pharep.2016.11.003
214. Stefanova N, Schanda K, Klimaschewski L, Poewe W, Wenning GK, Reindl M. Tumor necrosis factor-α-induced cell death in U373 cells overexpressing α-synuclein. *J Neurosci Res.* (2003) 73:334–40. doi: 10.1002/jnr.10662
215. Theodore S, Cao S, McLean PJ, Standaert DG. Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *J Neuropathol Exp Neurol.* (2008) 67:1149–58. doi: 10.1097/NEN.0b013e31818e5e99
216. Su X, Federoff HJ, Maguire-Zeiss KA. Mutant α-synuclein overexpression mediates early proinflammatory activity. *Neurotoxicity Res.* (2009) 16:238–54. doi: 10.1007/s12640-009-9053-x
217. Lee S-b, Park SM, Ahn KJ, Chung KC, Paik SR, Kim J. Identification of the amino acid sequence motif of α-synuclein responsible for macrophage activation. *Biochem Biophys Res Commun.* (2009) 381:39–43. doi: 10.1016/j.bbrc.2009.02.002
218. Lee E-J, Woo M-S, Moon P-G, Baek M-C, Choi I-Y, Kim W-K, et al. α-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. *J Immunol.* (2010) 185:615. doi: 10.4049/jimmunol.0903480
219. Beraud D, Twomey M, Bloom B, Mittereder A, Neitzke K, Ton V, et al. α-synuclein alters toll-like receptor expression. *Front Neurosci.* (2011) 5:80. doi: 10.3389/fnins.2011.00080
220. Roodveldt C, Labrador-Garrido A, Gonzalez-Rey E, Fernandez-Montesinos R, Caro M, Lachaud CC, et al. Glial innate immunity generated by non-aggregated alpha-synuclein in mouse: differences between wild-type and Parkinson's disease-linked mutants. *PLoS ONE.* (2010) 5:e13481. doi: 10.1371/journal.pone.0013481
221. Rojanathammanee L, Murphy EJ, Combs CK. Expression of mutant alpha-synuclein modulates microglial phenotype *in vitro*. *J Neuroinflamm.* (2011) 8:44. doi: 10.1186/1742-2094-8-44
222. Hoenen C, Gustin A, Birck C, Kirchmeyer M, Beaume N, Felten P, et al. Alpha-synuclein proteins promote pro-inflammatory cascades in microglia: stronger effects of the A53T mutant. *PLoS ONE.* (2016) 11:e0162717. doi: 10.1371/journal.pone.0162717
223. Chatterjee K, Roy A, Banerjee R, Choudhury S, Mondal B, Halder S, et al. Inflammasome and α-synuclein in Parkinson's disease: a cross-sectional study. *J Neuroimmunol.* (2020) 338:577089. doi: 10.1016/j.jneuroim.2019.577089
224. Lim KL, Ng XH, Grace LG, Yao TP. Mitochondrial dynamics and Parkinson's disease: focus on parkin. *Antioxid Redox Signal.* (2012) 16:935–49. doi: 10.1089/ars.2011.4105
225. Dawson TM, Dawson VL. The role of parkin in familial and sporadic Parkinson's disease. *Movement Disord.* (2010) 25:S32–9. doi: 10.1002/mds.22798
226. Ishikawa A, Takahashi H. Clinical and neuropathological aspects of autosomal recessive juvenile parkinsonism. *J Neurol.* (1998) 245:P4–9. doi: 10.1007/PL00007745
227. Periquet M, Lücking CB, Vaughan JR, Bonifati V, Dürr A, De Michele G, et al. Origin of the mutations in the parkin gene in Europe: exon rearrangements are independent recurrent events, whereas point mutations may result from founder effects. *Am J Human Genet.* (2001) 68:617–26. doi: 10.1086/318791
228. Kilarski LL, Pearson JP, Newsway V, Majounie E, Knipe MD, Misbahuddin A, et al. Systematic review and UK-based study of PARK2 (parkin), PINK1, PARK7 (DJ-1) and LRRK2 in early-onset Parkinson's disease. *Movement Disord.* (2012) 27:1522–9. doi: 10.1002/mds.25132

229. Pickrell AM, Youle RJ. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron.* (2015) 85:257–73. doi: 10.1016/j.neuron.2014.12.007
230. Mori H, Kondo T, Yokochi M, Matsumine H, Nakagawa-Hattori Y, Miyake T, et al. Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. *Neurology.* (1998) 51:890. doi: 10.1212/WNL.51.3.890
231. van de Warrenburg BPC, Lammens M, Lücking CB, Denèfle P, Wesseling P, Booij J, et al. Clinical and pathologic abnormalities in a family with parkinsonism and parkin gene mutations. *Neurology.* (2001) 56:555. doi: 10.1212/WNL.56.4.555
232. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature.* (1998) 392:605–8. doi: 10.1038/33416
233. Klein C, Lohmann K. Parkinson disease(s). *Neurology.* (2009) 72:106. doi: 10.1212/01.wnl.0000333666.65522.8d
234. Klein C, Schlossmacher MG. Parkinson disease, 10 years after its genetic revolution: multiple clues to a complex disorder. *Neurology.* (2007) 69:2093. doi: 10.1212/01.wnl.0000271880.27321.a7
235. Pilcher H. Parkin implicated in sporadic Parkinson's disease. *Lancet Neurol.* (2005) 4:798. doi: 10.1016/S1474-4422(05)70237-4
236. González H, Contreras F, Prado C, Elgueta D, Franz D, Bernales S, et al. Dopamine receptor D3 expressed on CD4+ T cells favors neurodegeneration of dopaminergic neurons during Parkinson's disease. *J Immunol.* (2013) 190:5048–56. doi: 10.4049/jimmunol.1203121
237. de Léséleuc L, Orlova M, Cobat A, Girard M, Huong NT, Ba NN, et al. PARK2 mediates interleukin 6 and monocyte chemoattractant protein 1 production by human macrophages. *PLoS Negl Trop Dis.* (2013) 7:e2015. doi: 10.1371/journal.pntd.0002015
238. Chopra R, Kalaiarasan P, Ali S, Srivastava AK, Aggarwal S, Garg VK, et al. PARK2 and proinflammatory/anti-inflammatory cytokine gene interactions contribute to the susceptibility to leprosy: a case–control study of North Indian population. *BMJ Open.* (2014) 4:e004239. doi: 10.1136/bmjopen-2013-004239
239. Mira MT, Alcaïs A, Van Thuc N, Moraes MO, Di Flumeri C, Hong Thai V, et al. Susceptibility to leprosy is associated with PARK2 and PACRG. *Nature.* (2004) 427:636–40. doi: 10.1038/nature02326
240. Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS, et al. The ubiquitin ligase parkin mediates resistance to intracellular pathogens. *Nature.* (2013) 501:512–6. doi: 10.1038/nature12566
241. Greene JC, Whitworth AJ, Andrews LA, Parker TJ, Pallanck LJ. Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. *Human Mol Genet.* (2005) 14:799–811. doi: 10.1093/hmg/ddi074
242. Dupré-Crochet S, Erard M, Nüβe O. ROS production in phagocytes: why, when, and where? *J Leukocyte Biol.* (2013) 94:657–70. doi: 10.1189/jlb.1012544
243. Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. *J Exp Med.* (2011) 208:417–20. doi: 10.1084/jem.20110367
244. Pelletier M, Lepow TS, Billingham LK, Murphy MP, Siegel RM. New tricks from an old dog: mitochondrial redox signaling in cellular inflammation. *Semin Immunol.* (2012) 24:384–92. doi: 10.1016/j.smim.2013.01.002
245. Li J, Ma C, Long F, Yang D, Liu X, Hu Y, et al. Parkin impairs antiviral immunity by suppressing the mitochondrial reactive oxygen species-Nlrp3 axis and antiviral inflammation. *iScience.* (2019) 16:468–84. doi: 10.1016/j.isci.2019.06.008
246. Henn IH, Bouman L, Schlehe JS, Schlierf A, Schramm JE, Wegener E, et al. Parkin mediates neuroprotection through activation of IκB kinase/nuclear factor-κB signaling. *J Neurosci.* (2007) 27:1868. doi: 10.1523/JNEUROSCI.5537-06.2007
247. Anne Müller-Rischart K, Pilsl A, Beaudette P, Patra M, Hadian K, Funke M, et al. The E3 ligase parkin maintains mitochondrial integrity by increasing linear ubiquitination of NEMO. *Mol Cell.* (2013) 49:908–21. doi: 10.1016/j.molcel.2013.01.036
248. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature.* (2006) 441:1157–61. doi: 10.1038/nature04788
249. English L, Chemali M, Duron J, Rondeau C, Laplante A, Gingras D, et al. Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. *Nat Immunol.* (2009) 10:480–7. doi: 10.1038/ni.1720
250. Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, et al. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science.* (2005) 307:593. doi: 10.1126/science.1104904
251. Forno LS, DeLanney LE, Irwin I, Di Monte D, Langston JW. Astrocytes and Parkinson's disease. *Prog Brain Res.* (1992) 94:429–36. doi: 10.1016/S0079-6123(08)61770-7
252. Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, et al. Parkinson's disease-related proteins PINK1 and Parkin repress mitochondrial antigen presentation. *Cell.* (2016) 166:314–27. doi: 10.1016/j.cell.2016.05.039
253. Farrer M, Chan P, Chen R, Tan L, Lincoln S, Hernandez D, et al. Lewy bodies and parkinsonism in families with parkin mutations. *Ann Neurol.* (2001) 50:293–300. doi: 10.1002/ana.1132
254. Sasaki S, Shirata A, Yamane K, Iwata M. Parkin-positive autosomal recessive juvenile parkinsonism with α-synuclein-positive inclusions. *Neurology.* (2004) 63:678. doi: 10.1212/01.WNL.0000134657.25904.0B
255. Pramstaller PP, Schlossmacher MG, Jacques TS, Scaravilli F, Eskelson C, Pepivani I, et al. Lewy body Parkinson's disease in a large pedigree with 77 Parkin mutation carriers. *Ann Neurol.* (2005) 58:411–22. doi: 10.1002/ana.20587
256. Frank-Cannon TC, Tran T, Ruhn KA, Martinez TN, Hong J, Marvin M, et al. Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. *J Neurosci.* (2008) 28:10825. doi: 10.1523/JNEUROSCI.3001-08.2008
257. Tran TA, Nguyen AD, Chang J, Goldberg MS, Lee JK, Tansey MG. Lipopolysaccharide and tumor necrosis factor regulate Parkin expression via nuclear factor-kappa B. *PLoS ONE.* (2011) 6:e23660. doi: 10.1371/journal.pone.0023660
258. Stojkovska I, Wagner BM, Morrison BE. Parkinson's disease and enhanced inflammatory response. *Exp Biol Med.* (2015) 240:1387–95. doi: 10.1177/1535370215576313
259. Borsche M, König IR, Delcambre S, Petrucci S, Balck A, Brüggemann N, et al. Mitochondrial damage-associated inflammation highlights biomarkers in PRKN/PINK1 parkinsonism. *Brain.* (2020) 143:3041–51. doi: 10.1093/brain/awaa246
260. Bonifati V, Rohé CF, Breedveld GJ, Fabrizio E, De Mari M, Tassorelli C, et al. Early-onset parkinsonism associated with PINK1 mutations. *Neurology.* (2005) 65:87. doi: 10.1212/01.wnl.0000167546.39375.82
261. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MMK, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science.* (2004) 304:1158. doi: 10.1126/science.1096284
262. Silvestri L, Caputo V, Bellacchio E, Atorino L, Dallapiccola B, Valente EM, et al. Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Human Mol Genet.* (2005) 14:3477–92. doi: 10.1093/hmg/ddi377
263. Kim Y, Park J, Kim S, Song S, Kwon S-K, Lee S-H, et al. PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. *Biochem Biophys Res Commun.* (2008) 377:975–80. doi: 10.1016/j.bbrc.2008.10.104
264. Narendra DP, Jin SM, Tanaka A, Suen D-F, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol.* (2010) 8:e1000298. doi: 10.1371/journal.pbio.1000298
265. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RLA, Kim J, et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci USA.* (2010) 107:378. doi: 10.1073/pnas.0911187107
266. Pridgeon JW, Olzmann JA, Chin L-S, Li L. PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol.* (2007) 5:e172. doi: 10.1371/journal.pbio.0050172
267. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol.* (2010) 189:211–21. doi: 10.1083/jcb.200910140

268. Deng H, Jankovic J, Guo Y, Xie W, Le W. Small interfering RNA targeting the PINK1 induces apoptosis in dopaminergic cells SH-SY5Y. *Biochem Biophys Res Commun*. (2005) 337:1133–8. doi: 10.1016/j.bbrc.2005.09.178
269. Youn J, Lee C, Oh E, Park J, Kim JS, Kim H-T, et al. Genetic variants of PARK genes in Korean patients with early-onset Parkinson's disease. *Neurobiol Aging*. (2019) 75:224.e9–15. doi: 10.1016/j.neurobiolaging.2018.10.030
270. Büeler H. Mitochondrial dynamics, cell death and the pathogenesis of Parkinson's disease. *Apoptosis*. (2010) 15:1336–53. doi: 10.1007/s10495-010-0465-0
271. Dagda RK, Chu CT. Mitochondrial quality control: insights on how Parkinson's disease related genes PINK1, parkin, and Omi/HtrA2 interact to maintain mitochondrial homeostasis. *J Bioenerget Biomembr*. (2009) 41:473–9. doi: 10.1007/s10863-009-9255-1
272. Valente EM, Salvi S, Ialongo T, Marongiu R, Elia AE, Caputo V, et al. PINK1 mutations are associated with sporadic early-onset parkinsonism. *Ann Neurol*. (2004) 56:336–41. doi: 10.1002/ana.20256
273. Gustavsson EK, Trinh J, McKenzie M, Bortnick S, Petersen MS, Farrer MJ, et al. Genetic identification in early onset parkinsonism among Norwegian patients. *Movement Disord Clin Prac*. (2017) 4:499–508. doi: 10.1002/mdc3.12501
274. Kawajiri S, Saiki S, Sato S, Hattori N. Genetic mutations and functions of PINK1. *Trends Pharmacol Sci*. (2011) 32:573–80. doi: 10.1016/j.tips.2011.06.001
275. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*. (2006) 441:1162–6. doi: 10.1038/nature04779
276. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang J-W, et al. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. *Proc Natl Acad Sci USA*. (2006) 103:10793. doi: 10.1073/pnas.0602493103
277. Akundi RS, Huang Z, Eason J, Pandya JD, Zhi L, Cass WA, et al. Increased mitochondrial calcium sensitivity and abnormal expression of innate immunity genes precede dopaminergic defects in Pink1-deficient mice. *PLoS ONE*. (2011) 6:e16038. doi: 10.1371/journal.pone.0016038
278. Ellis GI, Zhi L, Akundi R, Büeler H, Marti F. Mitochondrial and cytosolic roles of PINK1 shape induced regulatory T-cell development and function. *Euro J Immunol*. (2013) 43:3355–60. doi: 10.1002/eji.201343571
279. Reynolds AD, Stone DK, Hutter JAL, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal Dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol*. (2010) 184:2261. doi: 10.4049/jimmunol.0901852
280. Fiszer U, Mix E, Fredrikson S, Kostulas V, Olsson T, Link H. γδ+ T cells are increased in patients with Parkinson's disease. *J Neurol Sci*. (1994) 121:39–45. doi: 10.1016/0022-510X(94)90154-6
281. Stevens CH, Rowe D, Morel-Kopp M-C, Orr C, Russell T, Ranola M, et al. Reduced T helper and B lymphocytes in Parkinson's disease. *J Neuroimmunol*. (2012) 252:95–9. doi: 10.1016/j.jneuroim.2012.07.015
282. Matheoud D, Cannon T, Voisin A, Penttinen AM, Ramet L, Fahmy AM, et al. Intestinal infection triggers Parkinson's disease-like symptoms in Pink1(-/-) mice. *Nature*. (2019) 571:565–9. doi: 10.1038/s41586-019-1405-y
283. Houser MC, Tansey MG. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinson's Dis*. (2017) 3:3. doi: 10.1038/s41531-016-0002-0
284. Sun L, Shen R, Agnihotri SK, Chen Y, Huang Z, Büeler H. Lack of PINK1 alters glia innate immune responses and enhances inflammation-induced, nitric oxide-mediated neuron death. *Sci Rep*. (2018) 8:383. doi: 10.1038/s41598-017-18786-w
285. Kim J, Byun J-W, Choi I, Kim B, Jeong H-K, Jou I, et al. PINK1 deficiency enhances inflammatory cytokine release from acutely prepared brain slices. *Exp Neurobiol*. (2013) 22:38–44. doi: 10.5607/en.2013.22.1.38
286. Honbou K, Suzuki NN, Horiuchi M, Niki T, Taira T, Ariga H, et al. The crystal structure of DJ-1, a protein related to male fertility and Parkinson's disease. *J Biol Chem*. (2003) 278:31380–4. doi: 10.1074/jbc.M305878200
287. Wilson MA. The role of cysteine oxidation in DJ-1 function and dysfunction. *Antioxidants Redox Signal*. (2011) 15:111–22. doi: 10.1089/ars.2010.3481
288. Kahle PJ, Waak J, Gasser T. DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders. *Free Radical Biol Med*. (2009) 47:1354–61. doi: 10.1016/j.freeradbiomed.2009.08.003
289. Girotto S, Cendron L, Bisaglia M, Tessari I, Mammi S, Zanotti G, et al. DJ-1 is a copper chaperone acting on SOD1 activation. *J Biol Chem*. (2014) 289:10887–99. doi: 10.1074/jbc.M113.535112
290. Jain D, Jain R, Eberhard D, Eglinger J, Bugliani M, Piemonti L, et al. Age- and diet-dependent requirement of DJ-1 for glucose homeostasis in mice with implications for human type 2 diabetes. *J Mol Cell Biol*. (2012) 4:221–30. doi: 10.1093/jmcb/mjs025
291. Mitsumoto A, Nakagawa Y, Takeuchi A, Okawa K, Iwamatsu A, Takanezawa Y. Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat. *Free Radical Res*. (2001) 35:301–10. doi: 10.1080/10715760100300831
292. Mitsumoto A, Nakagawa Y. DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. *Free Radical Res*. (2001) 35:885–93. doi: 10.1080/10715760100301381
293. Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, Mizusawa H. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, proteasome inhibition. *Biochem Biophys Res Commun*. (2003) 312:1342–8. doi: 10.1016/j.bbrc.2003.11.056
294. Taira T, Saito Y, Niki T, Iguchi-Ariga SMM, Takahashi K, Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep*. (2004) 5:213–8. doi: 10.1038/sj.embor.7400074
295. Shinbo Y, Niki T, Taira T, Ooe H, Takahashi-Niki K, Maita C, et al. Proper SUMO-1 conjugation is essential to DJ-1 to exert its full activities. *Cell Death Different*. (2006) 13:96–108. doi: 10.1038/sj.cdd.4401704
296. Cao J, Lou S, Ying M, Yang B. DJ-1 as a human oncogene and potential therapeutic target. *Biochem Pharmacol*. (2015) 93:241–50. doi: 10.1016/j.bcp.2014.11.012
297. Yanagisawa D, Kitamura Y, Inden M, Takata K, Taniguchi T, Morikawa S, et al. DJ-1 protects against neurodegeneration caused by focal cerebral ischemia and reperfusion in rats. *J Cereb Blood Flow Metab*. (2008) 28:563–78. doi: 10.1038/sj.jcbfm.9600553
298. An C-N, Jiang H, Wang Q, Yuan R-P, Liu J-M, Shi W-L, et al. Down-regulation of DJ-1 protein in the ejaculated spermatozoa from Chinese asthenozoospermia patients. *Fertility Sterility*. (2011) 96:19–23.e2. doi: 10.1016/j.fertnstert.2011.04.048
299. Cornejo Castro EM, Waak J, Weber SS, Fiesel FC, Oberhettinger P, Schütz M, et al. Parkinson's disease-associated DJ-1 modulates innate immunity signaling in *Caenorhabditis elegans*. *J Neural Transm*. (2010) 117:599–604. doi: 10.1007/s00702-010-0397-4
300. Repici M, Giorgini F. DJ-1 in Parkinson's disease: clinical insights and therapeutic perspectives. *J Clin Med*. (2019) 8:1377. doi: 10.3390/jcm8091377
301. Chien CH, Lee MJ, Liou HC, Liou HH, Fu WM. Microglia-derived cytokines/chemokines are involved in the enhancement of LPS-induced loss of nigrostriatal dopaminergic neurons in DJ-1 knockout mice. *PLoS ONE*. (2016) 11:e0151569. doi: 10.1371/journal.pone.0151569
302. Nash Y, Schmukler E, Trudler D, Pinkas-Kramarski R, Frenkel D. DJ-1 deficiency impairs autophagy and reduces alpha-synuclein phagocytosis by microglia. *J Neurochem*. (2017) 143:584–94. doi: 10.1111/jnc.14222
303. Trudler D, Weinreb O, Mandel SA, Youdim MBH, Frenkel D. DJ-1 deficiency triggers microglia sensitivity to dopamine toward a pro-inflammatory phenotype that is attenuated by rasagiline. *J Neurochem*. (2014) 129:434–47. doi: 10.1111/jnc.12633
304. Waak J, Weber SS, Waldenmaier A, Görner K, Alunni-Fabbroni M, Schell H, et al. Regulation of astrocyte inflammatory responses by the Parkinson's disease-associated gene DJ−1. *FASEB J*. (2009) 23:2478–89. doi: 10.1096/fj.08-125153
305. Taipa R, Pereira C, Reis I, Alonso I, Bastos-Lima A, Melo-Pires M, et al. DJ-1 linked parkinsonism (PARK7) is associated with Lewy body pathology. *Brain*. (2016) 139:1680–7. doi: 10.1093/brain/aww080
306. Sturchio A, Dwivedi AK, Vizcarra JA, Chirra M, Keeling EG, Mata IF, et al. Genetic parkinsonisms and cancer: a systematic review and

meta-analysis. *Rev Neurosci.* (2020) 32:159–67. doi: 10.1515/revneuro-2020-0083

307. Poulogiannis G, McIntyre RE, Dimitriadi M, Apps JR, Wilson CH, Ichimura K, et al. PARK2 deletions occur frequently in sporadic colorectal cancer and accelerate adenoma development in Apc mutant mice. *Proc Natl Acad Sci USA*. (2010) 107:15145–50. doi: 10.1073/pnas.1009941107
308. Veeriah S, Taylor BS, Meng S, Fang F, Yilmaz E, Vivanco I, et al. Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. *Nat Genet.* (2010) 42:77–82. doi: 10.1038/ng.491
309. Lee S, She J, Deng B, Kim J, de Andrade M, Na J, et al. Multiple-level validation identifies PARK2 in the development of lung cancer and chronic obstructive pulmonary disease. *Oncotarget.* (2016) 7:44211–23. doi: 10.18632/oncotarget.9954
310. Ueda Y, Yang K, Foster SJ, Kondo M, Kelsoe G. Inflammation controls B lymphopoiesis by regulating chemokine CXCL12 expression. *J Exp Med.* (2004) 199:47–58. doi: 10.1084/jem.20031104
311. Nagaoka H, Gonzalez-Aseguinolaza G, Tsuji M, Nussenzweig MC. Immunization and infection change the number of recombination activating gene (RAG)-expressing B cells in the periphery by altering immature lymphocyte production. *J Exp Med.* (2000) 191:2113–20. doi: 10.1084/jem.191.12.2113
312. Nadazdin O, Abrahamian G, Boskovic S, Smith RN, Schoenfeld DA, Madsen JC, et al. Stem cell mobilization and collection for induction of mixed chimerism and renal allograft tolerance in cynomolgus monkeys. *J Surg Res.* (2011) 168:294–300. doi: 10.1016/j.jss.2010.02.027

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Exploring the Genotype–Phenotype Correlation in *GBA*-Parkinson Disease: Clinical Aspects, Biomarkers, and Potential Modifiers

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Variants in the glucocerebrosidase (*GBA*) gene are the most common genetic risk factor for Parkinson disease (PD). These include pathogenic variants causing Gaucher disease (GD) (divided into "severe," "mild," or "complex"—resulting from recombinant alleles—based on the phenotypic effects in GD) and "risk" variants, which are not associated with GD but nevertheless confer increased risk of PD. As a group, *GBA*-PD patients have more severe motor and nonmotor symptoms, faster disease progression, and reduced survival compared with noncarriers. However, different *GBA* variants impact variably on clinical phenotype. In the heterozygous state, "complex" and "severe" variants are associated with a more aggressive and rapidly progressive disease. Conversely, "mild" and "risk" variants portend a more benign course. Homozygous or compound heterozygous carriers usually display severe phenotypes, akin to heterozygous "complex" or "severe" variants carriers. This article reviews genotype–phenotype correlations in *GBA*-PD, focusing on clinical and nonclinical aspects (neuroimaging and biochemical markers), and explores other disease modifiers that deserve consideration in the characterization of these patients.

Keywords: Parkinson's, *GBA*, glucocerebrosidase, genotype-phenotype, biomarker

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INTRODUCTION

Biallelic pathogenic variants in the glucocerebrosidase (*GBA*) gene (OMIM 606463) leading to deficient activity of the lysosomal enzyme gene product GCase (EC 3.2.1.45) cause the recessively inherited multisystem disorder Gaucher disease (GD) (1). The standard classification of *GBA* variants is based on their phenotypic effect in GD, with "complex" rearrangements and "severe" variants causing neuronopathic GD, and "mild" variants causing non-neuronopathic GD (2). Patients with non-neuronopathic GD but especially heterozygous carriers of pathogenic *GBA* variants have an increased risk of developing Parkinson disease (PD) (3). Increased risk for PD has also been associated with a number of variants (p.E326K, p.T369M) not clearly pathogenic for GD (4, 5). In parallel with this increasing genotypic heterogeneity, detailed assessments of several cohorts of *GBA*-PD have suggested phenotypic differences associated with distinct genotypes.

Herein, we examine the clinical syndrome of *GBA*-PD compared to *GBA*-negative (noncarriers) PD and critically discuss genotype–phenotype correlation within the *GBA*-PD population from both clinical and biomarker perspectives. Finally, we present other potential disease modifiers that may impact on clinical phenotypes of *GBA*-PD.

METHODS

We performed a literature search for English-written publications on PD patients with *GBA* mutations using the National Center for Biotechnology Information's PubMed database (https://www.ncbi.nlm.nih.gov/pubmed) using the following search terms: "parkinson" AND "*GBA* OR 1q22" in the title and "gene OR genetic OR mutation OR mutated." We selected the articles relevant to our review and included additional articles from their reference lists. *GBA* variants were defined as follows:

- "mild" and "severe" variants are determined as per the GD classification of variant severity
- "risk variants" are those that increase PD risk but are not pathogenic for GD
- "complex" variants result from conversions, fusions, and insertions of parts of the pseudogene *GBAP1* into *GBA*.

GENOTYPE–PHENOTYPE CORRELATION WITH CLINICAL PROFILE IN *GBA*-PARKINSON DISEASE

Epidemiological, Demographic, and Prognostic Features

The frequency of *GBA* carriers is population-specific and ranges from 10% to 31% in Ashkenazi Jewish (AJ) to 3% to 12% in non-AJ North American (with European background) populations (6). As an example of ethnic heterogeneity of *GBA* variants, the p.N370S variant is very common in AJ populations with European origin and non-AJ European/West Asian populations, whereas it is not generally detected in East Asian populations (7–9). The penetrance of *GBA* variants in PD is low, age-specific, and controversial across studies, with an estimated 9.1% of carriers developing PD over their lifetime (10). At age 60 and 80 years, PD risks of ∼5 and 9%−12% respectively are reported among GD patients, which is significantly greater than those of noncarriers (0.7 and 2.1%, respectively) but similar to the 1.5–14% and 8–19% respective prevalence among *GBA* heterozygote carriers (11–15), suggesting that PD risk is not further increased by carrying a second *GBA* mutant allele (11). Among familial PD cohorts, much higher penetrance is reported (13.7% at 60 years and 29.7% at 80 years) (16), though this is likely a contribution from other genetic factors in these cohorts. When considering the impact of different variants on penetrance, the odds ratio (OR) of developing PD was found to be much higher in people carrying severe rather than mild variants (10.3–13.6 vs. 2.2) (17, 18). However, no such differences in penetrance were reported between mild and severe variants in familial PD cases (16), again suggesting the influence of additional genetic factors in such cohorts. The most frequent risk variant associated with *GBA*-PD, p.E326K, has been associated with a PD OR of 1.60–2 in European PD populations and up to 5.5 in AJ patients (19–21), suggesting a similar if not higher risk compared with mild variants.

Compared with noncarriers, *GBA*-PD patients usually present symptoms earlier on (6, 22–26). PD patients with biallelic *GBA* variants (either homozygous or compound heterozygous), hereafter referred to as GD-PD, also have an earlier age at onset compared with heterozygous carriers (11, 27), indicating a possible "dose" effect of *GBA* influencing age at onset. When *GBA* variants were stratified, the majority of studies consistently reported earlier age at onset in severe variant carriers compared with mild or risk (17, 22, 28) and in patients with null or complex alleles relative to those with missense mutations (29). Rarely, no differences in age at onset were found (30, 31).

In terms of disease progression, carriers of any *GBA* variant reach progression milestones earlier compared with noncarriers (23, 32). In one study evaluating the impact of different variants on survival, severe and mild variants (considered together) were associated with a 2-fold greater risk of death with mean time to mortality approximately 1 year earlier compared with noncarriers, whereas risk variants showed similar mortality rates and time to mortality compared with noncarriers (32). In another longitudinal study conducted on PD patients of AJ ancestry, though, no significant effect of either mild or severe variants on survival was found (33). When survival of patients with mild and severe variants was directly compared, no differences were reported; nevertheless, only severe variants were associated with a greater risk of death relative to noncarriers (34). Overall, these results could suggest that only severe mutations might confer poor survival rates.

Motor and Nonmotor Features

Compared with noncarriers, the usual presentation of *GBA*-PD is that of an akinetic-rigid syndrome, with early development of motor fluctuations and dyskinesia (22, 29, 35). Stratifying by *GBA* variants, severe variants have been associated with more severe and rapidly progressive motor phenotype, and shorter time to development of axial symptoms such as postural instability, as opposed to mild or risk variants (22, 32). At the other end of the spectrum, risk variants are more likely to associate with benign phenotypes and occurrence of motor fluctuations later in the disease course (22).

People with *GBA*-PD suffer from a higher burden of nonmotor symptoms both in the prodromal phase and during manifest disease. One study reported higher scores of the nonmotor symptoms scale in *GBA*-PD patients compared with noncarrier PD patients (12). Similar results were confirmed by another study showing that PD patients with severe variants or GD-PD patients had higher nonmotor symptoms questionnaire scores compared with PD patients carrying mild variants or noncarriers (31).

Among the nonmotor features associated with PD, hyposmia, constipation, and REM sleep behavior disorder (RBD) are the most important prodromal risk factors for PD, and hereafter, we will discuss them individually. Olfactory function has been consistently shown to be worse and deteriorate over time in asymptomatic *GBA* carriers and GD patients compared with noncarriers (36–38). Interestingly, one study reported that more severe hyposmia at baseline could predict the development of parkinsonism in these individuals (39). Poorer hyposmia has also been reported in PD patients carrying pathogenic (severe/mild) variants vs. noncarriers (40), as well as in asymptomatic carriers

of severe vs. mild variants (31). Moreover, GD-PD patients showed more severe loss of olfaction compared with *GBA* heterozygous and noncarrier PD patients (27), suggesting that possibly not only type but also "dose" of *GBA* variants may affect olfactory function. Regarding constipation, only few reports have investigated its occurrence separately from other autonomic features in *GBA*-PD, finding that constipation may present more frequently relative to noncarriers (41, 42). Data regarding RBD are controversial. On the one hand, RBD has been reported to occur more frequently in *GBA*-PD and GD-PD compared with noncarrier PD patients (28, 42) and in PD patients carrying severe variants compared with patients carrying mild variants (31). On the other hand, no differences were reported in cohorts of *GBA* carriers or GD patients in comparison to noncarrier healthy controls (36). In a longitudinal evaluation study, *GBA* carriers and GD patients showed a worsening of RBD symptoms over time (39); however, whether a deterioration of RBD symptoms in these subjects leads to the development of PD is not known.

After the diagnosis of PD, *GBA*-PD patients show an increased risk of cognitive decline (22, 23, 25, 26, 41, 43, 44), including those receiving deep brain stimulation surgery (45, 46). Specific cognitive domains seem to be more affected, particularly in visual short-term memory (47). When stratified by variant, in one study, PD carriers of severe or biallelic variants showed worse cognitive function compared with noncarriers, whereas mild or risk variants did not (30). However, other studies found that risk variants (p.E326K) were associated with similar cognitive deterioration (26, 48), or faster progression to dementia compared with pathogenic *GBA* variants (49, 50), as opposed to what is expected on the basis of the impact on GCase activity.

Increased frequency of psychiatric symptoms, such as hallucinations, delusions, and impulsive–compulsive behavior (ICB), has also been reported in *GBA*-PD patients vs. noncarriers (22, 41, 44, 51). The risk of psychiatric disturbances seems to be genotype specific. Severe or complex variant carriers were more affected than mild variant carriers (22, 31), and risk variant carriers showed the mildest phenotype (22).

Regarding autonomic function, this has been reported to be more affected in *GBA*-PD (22, 41, 42, 44), but no association of type or "dose" of *GBA* variants with autonomic phenotypes has been reported to date (22, 27).

In summary, within *GBA*-PD patients, motor symptoms, psychiatric disturbances, and possibly hyposmia are more severe and might show genotype–phenotype correlations. The genotype–phenotype association for cognitive and autonomic function is less clear, although these features are clearly more severely affected. Whether constipation and RBD are overrepresented features in *GBA*-PD or asymptomatic *GBA* carriers, and a genotype–phenotype correlation exists for these symptoms, has not yet been elucidated. The more rapid decline in motor and nonmotor features in *GBA*-PD and the influence of specific *GBA* variants in these patients should be considered in the context of personalized treatment strategies. For instance, clinicians should be particularly cautious in the use of medications increasing the risk of falls, or worsening autonomic function in *GBA*-PD patients, and should recommend to these patients to start physiotherapy and cognitive engagement strategies early in the disease course (52).

GENOTYPE–PHENOTYPE CORRELATION WITH NONCLINICAL BIOMARKERS IN *GBA*-PARKINSON DISEASE

Neuroimaging

Presynaptic Dopamine Terminal Function

The degree of dopaminergic dysfunction in *GBA*-PD has been evaluated in few studies. Cilia et al. (34) showed that compared with noncarriers, PD patients carrying a severe (but not mild) variant had a significant dopamine transporter (DAT) deficit. When mild and severe variant carriers were directly compared, individuals with severe variants showed more pronounced deficit, mainly in the striatum contralateral to the most affected side (34). One study evaluating a small cohort of PD patients carrying risk variants (p.E326K and p.T369M) vs. noncarriers reported a reduced [^{18}F]FDopa uptake in the bilateral caudate nuclei, anteromedial putamen ipsilateral, and nucleus accumbens contralateral to the most affected site in carriers (53), but no comparison was made with patients carrying other variants. Surprisingly, in a cohort of early PD patients mostly carrying mild *GBA* variants (89% p.N370S), patients showed higher specific binding ratio (SBR) in the contralateral caudate and putamen when compared with noncarriers (54), and higher SBR values in caudate, putamen, and striatum were also reported in non-manifesting p.N370S carriers relative to healthy controls (55). The proposed mechanisms underlying this observation might be either of a compensatory upregulation of tracer uptake in the early stage of the disease (associated with slower decline rate in DAT signal) or the result of disruption of dopamine release prior to dopaminergic terminal loss (54, 55). Longitudinal assessments evaluating DAT deficit progression in *GBA* carriers bearing different variants will elucidate the implicated mechanisms.

Brain Metabolism

Metabolic networks have been investigated in *GBA*-PD patients using [^{18}F]-FDG PET, suggesting greater disease activity compared with noncarriers, as shown by increased PD-related pattern (PDRP) and a trend toward increased PD-related cognitive pattern (PDCP) levels (56). Recently, Greuel et al. (53) reported a similar pattern in a small cohort of PD patients carrying risk variants (p.E326K and p.T369M), with both higher PDRP and PDCP levels and significant [^{18}F]-FDG PET hypoactivity in the parietal lobe. These findings reflect the higher cognitive burden seen in *GBA*-PD and suggest that even risk variants such as p.E326K and p.T369M might be associated with a severe cognitive decline.

Substantia Nigra Hyperechogenicity

In a cross-sectional study using transcranial sonography, both asymptomatic *GBA* heterozygous carriers and GD patients showed an enlarged hyperechogenic area of the substantia nigra compared with healthy controls, but longitudinal studies are needed to determine the predictive value of these findings (57).

In manifest PD, transcranial sonography could not discriminate between *GBA*-PD and noncarriers, although a higher percentage of *GBA*-PD patients showed interrupted brain stem raphe, a marker of serotonergic system impairment (41).

Brain Atrophy

Segmentation of cortical and subcortical structures can provide information about regional atrophy. By comparing *GBA*-PD and *GBA* asymptomatic carriers vs. noncarrier PD and healthy controls, lower structural volumes and widespread cortical thinning were found among patients with PD compared with asymptomatic participants, but none of these differences were related to the genetic status (58). Given the more severe clinical profile associated with *GBA*-PD, one would have expected a more diffuse impairment in these individuals and possibly even in the asymptomatic carriers. Therefore, the applicability of this tool remains uncertain.

Biochemical Markers

Alpha-Synuclein

Total α-synuclein has been evaluated in the cerebrospinal fluid (CSF) of *GBA*-PD patients, showing lower levels compared with noncarriers (59). After genotypic stratification, severe variants displayed the lowest levels, and mild variants had lower levels compared with risk variants, suggesting a genotype–phenotype association (28). Interestingly, a similar correlation between genotype and CSF α-synuclein has been found in cohorts of patients with Lewy body dementia carrying *GBA* variants (60).

Plasma oligomeric α-synuclein levels are considered one of the major factors in neurodegeneration in PD (61). *GBA*-PD patients showed increased levels compared with noncarriers, with a trend toward higher levels in those carrying severe/mild variants followed by risk variants (62). The possible association of plasma oligomeric α-synuclein and severity of *GBA* variants reinforces the hypothesis that decreased GCase enzymatic activity plays a central role in PD pathogenesis.

The presence of phospho-α-synuclein pathology in skin biopsies has been evaluated in one study of 10 *GBA*-PD patients (six p.N370S, three p.E326K, one p.L444P). Six out of 10 demonstrated phospho-α-synuclein deposition, mainly in autonomic but also somatosensory fibers (63). These findings resemble what is seen in PD noncarriers, suggesting that skin biopsies might be used to investigate α-synuclein pathology *in vivo*, but they might not be useful to discriminate among different *GBA* genotypes.

Metabolic Fingerprints: Glucocerebrosidase Activity, Lysosphingolipids, and Others

Enzymatic activity of GCase seems to be a promising biomarker in *GBA*-PD, showing a genotype–phenotype association. Lower GCase enzymatic activity measured in dried blood spots has been reported in *GBA*-PD patients compared with noncarriers (64), and after genotypic stratification for *GBA* variants, increasing severity was associated with decreasing residual GCase activity (22, 62, 65) and longitudinally with a steeper decline of enzymatic activity (65). When measured in CSF, GCase activity was again significantly reduced in *GBA*-PD (66). Investigating how single *GBA* variants affect CSF GCase levels and whether they correlate with levels measured in dried blood spots might be of particular interest.

Lipid dysregulation has been proposed as one of the pathogenic mechanisms underlying *GBA*-PD (67). Elevation of different lipids, such as ceramide, total monohexosylceramide (glucosylceramide + galactosylceramide), sphingomyelin, and sphingosine (glucosylsphingosine + galactosylsphingosine), has been reported in *GBA*-PD vs. noncarriers (68). Galactosylsphingosine and glucosylsphingosine tended to be higher in patients carrying severe/mild variants compared with risk variants (69); however, their elevation was not correlated with either GCase activity measured in dried blood spots or plasma α-synuclein levels (69), arguing against a causal relationship between GCase deficiency and substrate accumulation.

To date, one study has evaluated the metabolomic profile of *GBA*-PD patients using gas chromatography/mass spectrometry. Using untargeted approach, elevated levels of several amino acids including asparagine, ornithine, glutamine, glycine, and polyol pathway metabolites were found in plasma of *GBA*-PD patients carrying risk variants compared with noncarriers (53). Interestingly, the two groups were substantially identical in terms of clinical features, suggesting that assessing the metabolomic profile might be a good biomarker to differentiate patients in early stages.

Inflammatory Mediators

Biomarkers of systemic inflammation have been investigated in few studies in *GBA*-PD, with contrasting results. In one study, higher levels of interleukin-8 differentiated *GBA*-PD from noncarriers and were associated with poorer cognitive function (43). The same study also reported elevation of other cytokines, such as monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1α in *GBA*-PD, whereas another study reported reduced levels of MCP-1 (70). The discrepancy between these results might be due to small sample sizes, different methodologies, and perhaps lack of stratification by variant type.

Overall, multiple biomarkers have been proposed so far in *GBA*-PD. Dopaminergic imaging and metabolic imaging seem promising candidates to elucidate possible genotype–phenotype correlations. Reduced total CSF α-synuclein, increased plasma oligomeric α-synuclein, and reduced GCase activity have shown genotype–phenotype associations that would require further confirmation in future studies. Whether lipidic and metabolic profiles are influenced by genotype remains elusive. Furthermore, the application of validated biomarkers to the prodrome and progression of PD in general remains a controversial area (71).

The most important clinical and nonclinical data about genotype–phenotype correlations in *GBA*-PD are summarized in **Table 1**.

POTENTIAL DISEASE MODIFIERS IN *GBA*-PARKINSON DISEASE

Aside from direct genotype–phenotype correlations within *GBA*-PD, several other genetic and environmental factors may influence both disease penetrance and clinical features. These are

TABLE 1 | Summary of main studies reporting genotype–phenotype correlations in *GBA*-Parkinson Disease.

Study Ref	Patient Groups (n. of patients)	Clinical features			Nonclinical biomarkers
		AAO, mortality	Motor features	Nonmotor features	
Lerche et al. (28)	M (16) vs. S (21) vs. R (43)	Younger AAO (S vs. R)	No difference	Highest history of dementia (S)	Low CSF total α-synuclein (S < M < R)
Huh et al. (65)	M (15) vs. S* (6) vs. R (26)	NA	NA	NA	Reduced GCase activity (S < M < R)
Petrucci et al. (22)	M (32) vs. S (39) vs. R (24) vs. C (16)	Younger AAO (S vs. M+C+R)	Lower AKR onset (R vs. M+S+C)	Lower risk of ICB (R) Lower risk of dementia and delusions (M)	Reduced GCase activity (C < S < M < R)
Cilia et al. (34)	M (67) vs. S (56)	Trend toward younger AAO (S) Higher risk of death compared to noncarriers (S)	No difference	Higher risk of dementia (S)	Reduced parieto-occipital blood perfusion (S) More pronounced nigrostriatal terminal reduction (S)
Liu et al. (30)	M (28) vs. S (26) vs. R (127) vs. D (14)	No difference in AAO	No difference	Higher risk of cognitive decline (S, D)	NA
Stoker et al. (32)	M+S (17) vs. R (31)	Higher risk of mortality compared with noncarriers (M+S)	Increased risk of postural instability (M+S)	Higher risk of dementia (M+S)	NA
Malek et al. (40)	M+S (48) vs. R (117); early disease stage (duration from diagnosis less than 1.5 years)	Younger AAO compared with noncarriers (M+S)	More severe symptoms compared with noncarriers (M+S)	More frequent hyposmia compared with noncarriers (M+S) No difference in cognitive function compared with noncarriers (M+S, R)	NA
Pchelina et al. (69)	M+S (11) vs. R (12)	NA	NA	NA	Trend toward higher levels of hexosylsphingosines (M+S > R)
Pchelina et al. (62)	M+S (11) vs. R (11)	NA	NA	NA	Trend toward reduced GCase activity (M+S < R) Trend toward higher plasma oligomeric levels of α-synuclein (M+S > R)
Thaler et al. (31)	M (139) vs. S (48) vs. D (16)	No difference in AAO	More severe symptoms (S, D vs. M)	More severe hyposmia, nonmotor symptoms, depression (S) Higher frequency of RBD (S, D vs. M)	NA
Gan-Or et al. (18)	M+S (71) vs. D (6)	Younger AAO (D vs. M+S)	No difference	NA	NA

*AAO, age at onset; AKR, akinetic-rigid; C, complex variant (defined as two or more variants in cis as the result of conversion, fusion, insertion of parts of GBAP1 into GBA); CSF, cerebrospinal fluid; D, dual (more than one GBA variant); GBA, glucocerebrosidase gene; GCase, glucocerebrosidase enzyme; ICB, impulsive–compulsive behavior; M, mild variant; NA, not applicable; PD, Parkinson disease; R, risk variant; RBD, REM sleep behavior disorder; S, severe variant; *, in this study, severe GBA-PD patients also include homozygous and compound heterozygous carriers of any GBA variant.*

important to consider and control for when evaluating *GBA*-PD cohorts to avoid erroneous causal attribution of observed symptoms to *GBA* genotype alone.

Among genetic factors, common single-nucleotide polymorphisms (SNPs) within the *GBA* locus have been proposed as potential modifiers of *GBA*-PD age at onset and motor progression (72, 73). Beyond *GBA*, the Alzheimer disease Bridging Integrator 1 (*BIN1*) locus (OMIM 601248), which is involved in synaptic vesicle endocytosis in the central nervous system, has also been proposed as a modifier of age at onset in *GBA*-PD, with the rs13403026 SNP being associated with older age at onset in both mild and severe *GBA* carriers (74). Another candidate is the Metaxin 1 (*MTX1*) gene (OMIM 600605), which is located close to *GBA* and encodes a mitochondrial protein. Homozygous c.184A/A genotype in the *MTX1* gene is associated with earlier age at onset in *GBA*-PD (75). Using a genome-wide association study, specific variants in close proximity to α-synuclein (*SNCA*; OMIM 163890) and cathepsin B (*CTSB*; OMIM 116810) genes (rs356219 and rs1293298) were found to associate with earlier age at onset in *GBA* carriers (76). Interestingly, the G/G *SNCA* rs356219 genotype was also associated with a more aggressive phenotype in a small cohort of *GBA*-PD patients (77). These findings suggest a possible synergistic effect of *GBA* and *SNCA* variants and deserve further evaluation in stratified groups of *GBA* carriers.

Although the mechanisms by which GCase influences PD pathogenesis are still debated (78), any factor influencing lysosomal GCase activity might potentially be disease modifying.

For instance, it has been demonstrated that α-synuclein itself can induce aberrant maturation and endoplasmic reticulum/Golgi apparatus trafficking of GCase and therefore reduce the mature form of GCase and its lysosomal activity (79). More recently, it has been suggested that mutant leucine-rich repeat kinase (*LRRK2*; OMIM 609007) products may act as a negative regulator of GCase activity. GCase activity was shown to be reduced in human dopaminergic neurons carrying different *LRRK2* mutations, and the treatment of dopaminergic neurons from patients with either *LRRK2* or *GBA* variants with LRRK2 kinase inhibitor could increase GCase activity and rescue neurons from PD-related damage (80).

Among non-genetic risk factors, a recent study conducted on a large cohort of asymptomatic *GBA* carriers has evaluated the role of metabolic syndrome, a well-known risk factor for PD (81), as a possible disease determinant. The authors did not find any association between metabolic syndrome and risk of PD; however, hypertriglyceridemia and prediabetes were possibly overrepresented in those destined to later develop PD, regardless of *GBA* genotypes (82). In one study evaluating multiple environmental factors linked to PD, a more frequent exposure to pesticides was reported in *GBA*-PD patients vs. noncarriers, whereas no difference in smoking or coffee drinking was found (83). These preliminary data need further validation but may suggest that certain components of metabolic syndrome such as insulin resistance, or previous exposure to pesticides, should be carefully considered as potential disease determinants in *GBA* carriers.

CONCLUSIONS

Increasing numbers of *GBA* variants have been associated with an elevated risk of PD, but the standard classification of *GBA* variants incompletely reflects the complex and rapidly evolving genetic landscape of *GBA*-PD. Moreover, data from cohorts of *GBA*-PD patients suggest that carriers of different variants display specific clinical profiles, with complex or severe variants associated with a more aggressive and rapidly progressive PD phenotype and mild or risk variants with a more benign phenotype.

Stratifying *GBA* carriers in both the prodromal and manifest phase of PD is of paramount importance, first, to address questions about prognosis, advanced treatment response, and counseling and, second, to recognize early the presence of subclinical/clinical symptoms that might help more precise selection of individuals for clinical trials. Multimodal evaluations including metabolic imaging and assessment of GCase activity, α-synuclein levels, and lipid and metabolic profile may shed light on inter-genotype differences, discover new biomarkers for clinical and research setting, and unveil novel mechanisms underlying *GBA*-PD pathogenesis.

AUTHOR CONTRIBUTIONS

EM conceived the manuscript and wrote the first draft. AS conceived and reviewed the manuscript. Both authors contributed to the article and approved the submitted version.

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REFERENCES

1. Stirnemann JN, Belmatoug F, Camou C, Serratrice R, Froissart C,, Caillaud T, et al. *A* Review of gaucher disease pathophysiology, clinical presentation and treatments. *Int J Mol Sci.* (2017) 18:441. doi: 10.3390/ijms18020441
2. Beutler E, Gelbart T, Scott CR. Hematologically important mutations: Gaucher disease. *Blood Cells Mol Dis.* (2005) 35:355–64. doi: 10.1016/j.bcmd.2005.07.005
3. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* (2009) 361:1651–61. doi: 10.1056/NEJMoa0901281
4. Duran R, Mencacci NE, Angeli AV, Shoai M, Deas E, Houlden H, et al. The glucocerobrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Mov Disord.* (2013) 28:232–6. doi: 10.1002/mds.25248
5. Mallett V, Ross JP, Alcalay RN, Ambalavanan A, Sidransky E, Dion PA, et al. GBA p.T369M substitution in Parkinson disease: POLYMORPHISM or association? A meta-analysis. *Neurol Genet.* (2016) 2:e104. doi: 10.1212/NXG.0000000000000104
6. Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, et al. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain.* (2009) 132(Pt 7):1783–94. doi: 10.1093/brain/awp044
7. Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Hum Mutat.* (2008) 29:567–83. doi: 10.1002/humu.20676
8. Pulkes T, Choubtum L, Chitphuk S, Thakkinstian A, Pongpakdee S, Kulkantrakorn K, et al. Glucocerebrosidase mutations in Thai patients with Parkinson's disease. *Parkinsonism Relat Disord.* (2014) 20:986–91. doi: 10.1016/j.parkreldis.2014.06.007
9. Zhang Y, Shu L, Sun Q, Zhou X, Pan H, Guo J, et al. Integrated genetic analysis of racial differences of common GBA variants in Parkinson's disease: a meta-analysis. *Front Mol Neurosci.* (2018) 11:43. doi: 10.3389/fnmol.2018.00043
10. Riboldi GM, Di Fonzo AB. GBA, Gaucher Disease, and Parkinson's Disease: from genetic to clinic to new therapeutic approaches. *Cells.* (2019) 8:364. doi: 10.3390/cells8040364
11. Alcalay RN, Dinur T, Quinn T, Sakanaka K, Levy O, Waters C, et al. Comparison of Parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes. *JAMA Neurol.* (2014) 71:752–7. doi: 10.1001/jamaneurol.2014.313
12. McNeill A, Duran R, Hughes DA, Mehta A, *Schapira* AH. A clinical and family history study of Parkinson's disease in heterozygous glucocerebrosidase mutation carriers. *J Neurol Neurosurg Psychiatry.* (2012) 83:853–4. doi: 10.1136/jnnp-2012-302402
13. Rana HQ, Balwani M, Bier L, Alcalay RN. Age-specific Parkinson disease risk in GBA mutation carriers: information for genetic counseling. *Genet Med.* (2013) 15:146–9. doi: 10.1038/gim.2012.107
14. Rosenbloom B, Balwani M, Bronstein JM, Kolodny E, Sathe S, Gwosdow AR, et al. The incidence of Parkinsonism in patients with type 1 Gaucher disease: data from the ICGG Gaucher Registry. *Blood Cells Mol Dis.* (2011) 46:95–102. doi: 10.1016/j.bcmd.2010.10.006

15. Balestrino R, Tunesi S, Tesei S, Lopiano L, Zecchinelli AL, Goldwurm S. Penetrance of glucocerebrosidase (GBA) mutations in Parkinson's disease: a kin cohort study. *Mov Disord.* (2020) 35: 2111–4. doi: 10.1002/mds.28200
16. Anheim M, Elbaz A, Lesage S, Durr A, Condroyer C, Viallet F, et al. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. *Neurology.* (2012) 78:417–20. doi: 10.1212/WNL.0b013e318245f476
17. Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology.* (2015) 84:880–7. doi: 10.1212/WNL.0000000000001315
18. Gan-Or Z, Giladi N, Rozovski U, Shifrin C, Rosner S, Gurevich T, et al. Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset. *Neurology.* (2008) 70:2277–83. doi: 10.1212/01.wnl.0000304039.11891.29
19. Ran C, Brodin L, Forsgren L, Westerlund M, Ramezani M, Gellhaar S, et al. Strong association between glucocerebrosidase mutations and Parkinson's disease in Sweden. *Neurobiol Aging.* (2016) 45:212 e5–212 e11. doi: 10.1016/j.neurobiolaging.2016.04.022
20. Ruskey JA, Greenbaum L, Ronciere L, Alam A, Spiegelman D, Liong C, et al. Increased yield of full GBA sequencing in Ashkenazi Jews with Parkinson's disease. *Eur J Med Genet.* (2019) 62:65–9. doi: 10.1016/j.ejmg.2018.05.005
21. Blauwendraat C, Bras JM, Nalls MA, Lewis PA, Hernandez DG, Singleton AB, et al. International Parkinson's disease genomics. Coding variation in GBA explains the majority of the SYT11-GBA Parkinson's disease GWAS locus. *Mov Disord.* (2018) 33:1821–1823. doi: 10.1002/mds.103
22. Petrucci S, Ginevrino M, Trezzi I, Monfrini E, Ricciardi L, Albanese A, et al. GBA-related Parkinson's disease: dissection of genotype-phenotype correlates in a large italian cohort. *Mov Disord.* (2020). 35:2106–11. doi: 10.1002/mds.28195
23. Brockmann K, Srulijes K, Pflederer S, Hauser AK, Schulte C, Maetzler W, et al. GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study. *Mov Disord.* (2015) 30:407–11. doi: 10.1002/mds.26071
24. Nichols WC, Pankratz N, Marek DK, Pauciulo MW, Elsaesser VE, Halter CA, et al. Parkinson study group. mutations in GBA are associated with familial Parkinson disease susceptibility and age at onset. *Neurology.* (2009) 72:310–6. doi: 10.1212/01.wnl.0000327823.81237.d1
25. Zhang Y, Shu L, Zhou X, Pan H, Xu Q, Guo J, et al. A meta-analysis of GBA-related clinical symptoms in Parkinson's disease. *Parkinsons Dis.* (2018) 2018:3136415. doi: 10.1155/2018/3136415
26. Lunde KA, Chung J, Dalen I, Pedersen KF, Linder J, Domellof ME, et al. Maple-Grodem J. Association of glucocerebrosidase polymorphisms and mutations with dementia in incident Parkinson's disease. *Alzheimers Dement.* (2018) 14:1293–301. doi: 10.1016/j.jalz.2018.04.006
27. Thaler A, Gurevich T, Bar Shira A, Gana Weisz M. Ash E, Shiner T, et al. A "dose" effect of mutations in the GBA gene on Parkinson's disease phenotype. *Parkinsonism Relat Disord.* (2017) 36:47–51. doi: 10.1016/j.parkreldis.2016.12.014
28. Lerche S, Wurster I, Roeben B, Zimmermann M, Riebenbauer B, Deuschle Cet al. Parkinson's disease: glucocerebrosidase 1 mutation severity is associated with CSF Alpha-synuclein profiles. *Mov Disord.* (2020) 35:495–499. doi: 10.1002/mds.27884
29. Lesage S, Anheim M, Condroyer C, Pollak P, Durif F, Dupuits C, et al. French Parkinson's disease genetics study, large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. *Hum Mol Genet.* (2011) 20:202–10. doi: 10.1093/hmg/ddq454
30. Liu G, Boot B, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, et al. International genetics of Parkinson disease progression. specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann Neurol.* (2016) 80:674–85. doi: 10.1002/ana.24781
31. Thaler A, Bregman N, Gurevich T, Shiner T, Dror Y, Zmira O, et al. Parkinson's disease phenotype is influenced by the severity of the mutations in the GBA gene. *Parkinsonism Relat Disord.* (2018) 55:45–49. doi: 10.1016/j.parkreldis.2018.05.009
32. Stoker TB, Camacho M, Winder-Rhodes S, Liu G, Scherzer CR, Foltynie T, et al. Impact of GBA1 variants on long-term clinical progression and mortality in incident Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (2020) 91:695–702. doi: 10.1136/jnnp-2020-322857
33. Thaler A, Kozlovski T, Gurevich T, Bar-Shira A, Gana-Weisz M, Orr-Urtreger A, et al. Survival rates among Parkinson's disease patients who carry mutations in the LRRK2 and GBA genes. *Mov Disord.* (2018) 33:1656–60. doi: 10.1002/mds.27490
34. Cilia R, Tunesi S, Marotta G, Cereda E, Siri C, Tesei S, et al. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann Neurol.* (2016) 80:662–73. doi: 10.1002/ana.24777
35. Olszewska DA, McCarthy A, Soto-Beasley AI, Walton RL, Magennis B, McLaughlin RL, et al. Association between glucocerebrosidase mutations and Parkinson's disease in Ireland. *Front Neurol.* (2020) 11:527. doi: 10.3389/fneur.2020.00527
36. McNeill A, Duran R, Proukakis C, Bras J, Hughes D, Mehta A, et al. Hyposmia and cognitive impairment in Gaucher disease patients and carriers. *Mov Disord.* (2012) 27:526–32. doi: 10.1002/mds.24945
37. Mullin S, Beavan M, Bestwick J, McNeill A, Proukakis C, Cox T, et al. Evolution and clustering of prodromal parkinsonian features in GBA1 carriers. *Mov Disord.* (2019) 34:1365–1373. doi: 10.1002/mds.27775
38. Beavan M, McNeill A, Proukakis C, Hughes DA, Mehta A, Schapira AH. Evolution of prodromal clinical markers of Parkinson disease in a GBA mutation-positive cohort. *JAMA Neurol.* (2015) 72:201–8. doi: 10.1001/jamaneurol.2014.2950
39. Avenali M, Toffoli M, Mullin S, McNeil A, Hughes DA, Mehta A, et al. Evolution of prodromal parkinsonian features in a cohort of GBA mutation-positive individuals: a 6-year longitudinal study. *J Neurol Neurosurg Psychiatry.* (2019) 90:1091–7. doi: 10.1136/jnnp-2019-320394
40. Malek N, Weil RS, Bresner C, Lawton MA, Grosset KA, Tan M, et al. Features of GBA-associated Parkinson's disease at presentation in the UK Tracking Parkinson's study. *J Neurol Neurosurg Psychiatry.* (2018) 89:702–9. doi: 10.1136/jnnp-2017-317348
41. Brockmann K, Srulijes K, Hauser AK, Schulte C, Csoti I, Gasser T, et al. GBA-associated PD presents with nonmotor characteristics. *Neurology.* (2011) 77:276–80. doi: 10.1212/WNL.0b013e318225ab77
42. Jesus S, Huertas I, Bernal-Bernal I, Bonilla-Toribio M, Caceres-Redondo MT, et al. GBA Variants Influence Motor and Non-Motor Features of Parkinson's Disease. *PLoS ONE.* (2016) 11:e0167749. doi: 10.1371/journal.pone.0167749
43. Chahine LM, Qiang J, Ashbridge E, Minger J, Yearout D, Horn S, et al. Chen-Plotkin Clinical A, and biochemical differences in patients having Parkinson disease with vs without GBA mutations. *JAMA Neurol.* (2013) 70:852–8. doi: 10.1001/jamaneurol.2013.1274
44. Wang C, Cai Y, Gu Z, Ma J, Zheng Z, Tang BS, et al. Chinese Parkinson Study. Clinical profiles of Parkinson's disease associated with common leucine-rich repeat kinase 2 and glucocerebrosidase genetic variants in Chinese individuals. *Neurobiol Aging.* (2014) 35:725 e1–6. doi: 10.1016/j.neurobiolaging.2013.08.012
45. Lythe V, Athauda D, Foley J, Mencacci NE, Jahanshahi M, Cipolotti L, et al. GBA-Associated Parkinson's Disease: Progression in a Deep Brain Stimulation Cohort. *J Parkinsons Dis.* (2017) 7:635–44. doi: 10.3233/JPD-171172
46. Mangone G, Bekadar S, Cormier-Dequaire F, Tahiri K, Welaratne A, Czernecki V, et al. Early cognitive decline after bilateral subthalamic deep brain stimulation in Parkinson's disease patients with GBA mutations. *Parkinsonism Relat Disord.* (2020) 76:56–62. doi: 10.1016/j.parkreldis.2020.04.002
47. Zokaei N, McNeill A, Proukakis C, Beavan M, Jarman P, Korlipara P, et al. Visual short-term memory deficits associated with GBA mutation and Parkinson's disease. *Brain.* (2014) 137(Pt 8):2303–11. doi: 10.1093/brain/awu143
48. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, Van Deerlin VM, et al. GBA Variants are associated with a distinct pattern of cognitive deficits in Parkinson's disease. *Mov Disord.* (2016) 31:95–102. doi: 10.1002/mds.26359
49. Davis MY, Johnson CO, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, et al. Association of GBA mutations and the E326K polymorphism with motor and cognitive progression in Parkinson Disease. *JAMA Neurol.* (2016) 73:1217–1224. doi: 10.1001/jamaneurol.2016.2245
50. Straniero L, Asselta R, Bonvegna S, Rimoldi V, Melistaccio G, Solda G, et al. The SPID-GBA study: sex distribution. penetrance, incidence, and dementia in GBA-PD. *Neurol Genet.* (2020). 6:e523. doi: 10.1212/NXG.0000000000000523

51. Barrett MJ, Shanker VL, Severt WL, Raymond D, Gross SJ, Schreiber-Agus N, et al. Cognitive and Antipsychotic Medication Use in Monoallelic GBA-Related Parkinson Disease. *JIMD Rep.* (2014) 16:31–8. doi: 10.1007/8904_2014_315
52. Giladi N, Mirelman A, Thaler A, Orr-Urtreger A. A personalized approach to Parkinson's disease patients based on founder mutation analysis. *Front Neurol.* (2016) 7:71. doi: 10.3389/fneur.2016.00071
53. Greuel A, Trezzi JP, Glaab E, Ruppert MC, Maier F, Jager C, et al. GBA variants in Parkinson's disease: clinical, metabolomic, and multimodal neuroimaging phenotypes. *Mov Disord.* (2020). 35:2201–10. doi: 10.1002/mds.28225
54. Simuni T, Brumm MC, Uribe L, Caspell-Garcia C, *Coffey* CS, Siderowf A, et al. Clinical and dopamine transporter imaging characteristics of leucine rich repeat kinase 2 (LRRK2) and glucosylceramidase beta (GBA) Parkinson's disease participants in the Parkinson's progression markers initiative: a cross-sectional study. *Mov Disord.* (2020) 35:833–844. doi: 10.1002/mds.27989
55. Simuni T, Uribe L, Cho HR, Caspell-Garcia C, *Coffey* CS, Siderowf A, et al. Clinical and dopamine transporter imaging characteristics of non-manifest LRRK2 and GBA mutation carriers in the Parkinson's Progression Markers Initiative (PPMI): a cross-sectional study. *Lancet Neurol.* (2020) 19:71–80. doi: 10.1016/S1474-4422(19)30319-9
56. Schindlbeck KA, Vo A, Nguyen N, Tang CC, Niethammer M, Dhawan V et al. LRRK2 and GBA Variants Exert Distinct Influences on Parkinson's Disease-Specific Metabolic Networks. *Cereb Cortex.* (2020) 30:2867–78. doi: 10.1093/cercor/bhz280
57. Arkadir D, Dinur T, Becker Cohen M, Revel-Vilk S, Tiomkin M, et al. Prodromal substantia nigra sonography undermines suggested association between substrate accumulation and the risk for GBA-related Parkinson's disease. *Eur J Neurol.* (2019) 26:1013–1018. doi: 10.1111/ene.13927
58. Thaler A, Kliper E, Maidan I, Herman T, Rosenberg-Katz K, Bregman N, et al. Cerebral imaging markers of GBA and LRRK2 related Parkinson's disease and their first-degree unaffected relatives. *Brain Topogr.* (2018) 31:1029–36. doi: 10.1007/s10548-018-0653-8
59. Lerche S, Schulte C, Srulijes K, Pilotto A, Rattay TW, Hauser AK, et al. Cognitive impairment in Glucocerebrosidase (GBA)-associated PD: not primarily associated with cerebrospinal fluid Abeta and Tau profiles. *Mov Disord.* (2017) 32:1780–1783. doi: 10.1002/mds.27199
60. Lerche S, Machetanz G, Wurster I, Roeben B, Zimmermann M, Pilotto A, et al. Dementia with lewy bodies: GBA1 mutations are associated with cerebrospinal fluid alpha-synuclein profile. *Mov Disord.* (2019) 34:1069–1073. doi: 10.1002/mds.27731
61. Kalia LV, Kalia SK, McLean PJ, Lozano AM, *Lang* AE. alpha-Synuclein oligomers and clinical implications for Parkinson disease. *Ann Neurol.* (2013) 73:155–69. doi: 10.1002/ana.23746
62. Pchelina S, Emelyanov A, Baydakova G, Andoskin P, Senkevich K, Nikolaev M, et al. Oligomeric alpha-synuclein and glucocerebrosidase activity levels in GBA-associated Parkinson's disease. *Neurosci Lett.* (2017) 636:70–76. doi: 10.1016/j.neulet.2016.10.039
63. Doppler K, Brockmann K, Sedghi A, Wurster I, Volkmann J, Oertel WH, et al. Dermal phospho-alpha-synuclein deposition in patients with Parkinson's disease and mutation of the glucocerebrosidase gene. *Front Neurol.* (2018) 9:1094. doi: 10.3389/fneur.2018.01094
64. Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain.* (2015) 138(Pt 9):2648–58. doi: 10.1093/brain/awv179
65. Huh YE, Chiang MSR, Locascio JJ, Liao Z, Liu G, Choudhury K, et al. beta-Glucocerebrosidase activity in GBA-linked Parkinson disease: The type of mutation matters. *Neurology.* (2020) 95:e685–e696. doi: 10.1212/WNL.0000000000009989
66. Parnetti L, Paciotti S, Eusebi P, Dardis A, Zampieri S, Chiasserini D, et al. Cerebrospinal fluid beta-glucocerebrosidase activity is reduced in parkinson's disease patients. *Mov Disord.* (2017) 32:1423–31. doi: 10.1002/mds.27136
67. Alecu I, Bennett SAL. Dysregulated lipid metabolism and its role in alpha-synucleinopathy in Parkinson's disease. *Front Neurosci.* (2019) 13:328. doi: 10.3389/fnins.2019.00328
68. Guedes LC, Chan RB, Gomes MA, Conceicao VA, Machado RB, Soares T, et al. Miltenberger-Miltenyi G. Serum lipid alterations in GBA-associated Parkinson's disease. *Parkinsonism Relat Disord.* (2017) 44:58–65. doi: 10.1016/j.parkreldis.2017.08.026
69. Pchelina S, Baydakova G, Nikolaev M, Senkevich K, Emelyanov A, Kopytova A, et al. Blood lysosphingolipids accumulation in patients with Parkinson's disease with glucocerebrosidase 1 mutations. *Mov Disord.* (2018) 33:1325–30. doi: 10.1002/mds.27393
70. Miliukhina IV, Usenko TS, Senkevich KA, Nikolaev MA, Timofeeva AA, Agapova EA, et al. Plasma cytokines profile in patients with Parkinson's disease associated with mutations in GBA gene. *Bull Exp Biol Med.* (2020) 168:423–26. doi: 10.1007/s10517-020-04723-x
71. Vieira SRL, Toffoli M, Campbell P, Schapira AHV. Biofluid biomarkers in Parkinson's disease: clarity amid controversy. *Mov Disord.* (2020) 35:1128–33. doi: 10.1002/mds.28030
72. Schierding W, Farrow S, Fadason T, Graham OEE, Pitcher TL, Qubisi S, et al. Common variants coregulate expression of GBA and modifier genes to delay Parkinson's disease onset. *Mov Disord.* (2020) 35:1346–1356. doi: 10.1002/mds.28144
73. Davis AA, Andruska KM, Benitez BA, Racette BA, Perlmutter JS, Cruchaga C. Variants in GBA, SNCA, and MAPT influence Parkinson disease risk, age at onset, and progression. *Neurobiol Aging.* (2016) 37:209 e1–209 e7. doi: 10.1016/j.neurobiolaging.2015.09.014
74. Gan-Or Z, Amshalom I, Bar-Shira A, Gana-Weisz M, *Mirelman* A, *Marder K* et al. The Alzheimer disease BIN1 locus as a modifier of GBA-associated Parkinson disease. *J Neurol.* (2015) 262:2443–7. doi: 10.1007/s00415-015-7868-3
75. Gan-Or Z, Bar-Shira A, Gurevich T, Giladi N, Orr-Urtreger A. Homozygosity for the MTX1 c.184T>A (p.S63T) alteration modifies the age of onset in GBA-associated Parkinson's disease. *Neurogenetics.* (2011) 12:325–32. doi: 10.1007/s10048-011-0293-6
76. Blauwendraat C, Reed X, Krohn L, Heilbron K, Bandres-Ciga S, Tan M, et al. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. *Brain.* (2020) 143:234–48. doi: 10.1093/brain/awz350
77. Stoker TB, Camacho M, Winder-Rhodes S, Liu G, Scherzer CR, Foltynie T, et al. A common polymorphism in SNCA is associated with accelerated motor decline in GBA-Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (2020) 91:673–4. doi: 10.1136/jnnp-2019-322210
78. Blandini F, Cilia R, Cerri S, Pezzoli G, Schapira AHV, Mullin S, et al. Glucocerebrosidase mutations and synucleinopathies: toward a model of precision medicine. *Mov Disord.* (2019) 34:9–21. doi: 10.1002/mds.27583
79. Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, et al. Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell.* (2011) 146:37–52. doi: 10.1016/j.cell.2011.06.001
80. Ysselstein D, Nguyen M, Young TJ, Severino A, Schwake M, Merchant K, et al. *LRRK2 kinase activity regulates lysosomal glucocerebrosidase in neurons derived from Parkinson's disease patients. Nat Commun.* (2019) 10:5570. doi: 10.1038/s41467-019-13413-w
81. Nam GE, Kim SM, Han K, Kim NH, Chung HS, Kim JW, et al. Metabolic syndrome and risk of Parkinson disease: A nationwide cohort study. *PLoS Med.* (2018) 15:e1002640. doi: 10.1371/journal.pmed.1002640
82. Thaler A, Shenhar-Tsarfaty S, Shaked Y, Gurevich T, Omer N, Bar-Shira A, et al. Metabolic syndrome does not influence the phenotype of LRRK2 and GBA related Parkinson's disease. *Sci Rep.* (2020) 10:9329. doi: 10.1038/s41598-020-66319-9
83. Simitsi A, Koros C, Moraitou M, Papagiannakis N, Antonellou R, Bozi M, et al. Phenotypic characteristics in GBA-associated Parkinson's disease: a study in a greek population. *J Parkinsons Dis.* (2018) 8:101–5. doi: 10.3233/JPD-171221

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BRIEF RESEARCH REPORT
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Clinical and Genetic Analysis of Costa Rican Patients With Parkinson's Disease

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Background: Most research in genomics of Parkinson's disease (PD) has been done in subjects of European ancestry, leading to sampling bias and leaving Latin American populations underrepresented. We sought to clinically characterize PD patients of Costa Rican origin and to sequence familial PD and atypical parkinsonism-associated genes in cases and controls.

Methods: We enrolled 118 PD patients with 97 unrelated controls. Collected information included demographics, exposure to risk and protective factors, and motor and cognitive assessments. We sequenced coding and untranslated regions in familial PD and atypical parkinsonism-associated genes including *GBA, SNCA, VPS35, LRRK2, GCH1, PRKN, PINK1, DJ-1, VPS13C*, and *ATP13A2*.

Results: Mean age of PD probands was 62.12 ± 13.51 years; 57.6% were male. The frequency of risk and protective factors averaged ∼45%. Physical activity significantly correlated with better motor performance despite years of disease. Increased years of education were significantly associated with better cognitive function, whereas hallucinations, falls, mood disorders, and coffee consumption correlated with worse cognitive performance. We did not identify an association between tested genes and PD or any damaging homozygous or compound heterozygous variants. Rare variants in *LRRK2* were nominally associated with PD; six were located between amino acids p.1620 and 1623 in the C-terminal-of-ROC (COR) domain of Lrrk2. Non-synonymous *GBA* variants (p.T369M, p.N370S, and p.L444P) were identified in three healthy individuals. One PD patient carried a pathogenic *GCH1* variant, p.K224R.

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Discussion: This is the first study that describes sociodemographics, risk factors, clinical presentation, and genetics of Costa Rican patients with PD, adding information to genomics research in a Latino population.

Keywords: Parkinson's disease, genotype, phenotype, Costa Rica, Latin America

INTRODUCTION

Parkinson's disease (PD) is a complex and heterogeneous movement disorder caused by a progressive degeneration of dopaminergic neurons. Main clinical motor symptoms associated with PD include tremor, rigidity, bradykinesia, and postural imbalance (1). Years before motor symptoms are manifested, there can be prodromal non-motor key features that include rapid eye movement (REM) sleep disorders, anosmia, and constipation (2). Cognitive impairment involving dysexecutive dysfunction with deficits in planning (3), shifting and sharing of attention (4), and problem solving (5), together with visuospatial dysfunction (6), can be also present from early stages of the disease. PD pathophysiology involves environmental factors as well as genetic variance, which provide insight into its molecular pathogenesis. Among environmental factors that contribute to PD risk are pesticide and herbicide exposure, welding, and well water consumption. There are also protective factors such as smoking, coffee consumption, and performing physical activity that may reduce the risk of developing PD (7).

Since the description of PD-associated mutations in the *SNCA* (8), other genes have been linked to autosomal dominant (AD) forms of familial PD, including *LRRK2* and *VPS35*. In addition, there are clinically and genetically diverse early-onset (EO) autosomal-recessive (AR) forms of PD with associated genes like *PRKN, PINK1*, and *DJ-1* that exhibit phenotypes similar to idiopathic PD, while other associated genes such as *VPS13C* and *ATP13A2* combine atypical features of parkinsonism like dystonia and early cognitive impairment, along with a poor response to levodopa (9). Large-scale genome-wide association studies (GWASs) have identified 90 variants for PD risk across 78 genomic regions, confirming *SNCA* and *GBA* as the most important ones (10). Different *GBA* locus present as strong risk factors for PD in both homozygous and heterozygous state, displaying a phenotype similar to idiopathic PD, yet with a faster rate of progression of cognitive and motor decline (11).

Clinical characterization of PD in Latin American and Hispanic populations has been scarce (12). Likewise, there is a lack of diversity in genomics with an overrepresentation of European-derived individuals, leading to sampling bias and leaving large populations underrepresented (13). Few genetic trials have been conducted in PD individuals from Latin American populations. Studies looking at *LRRK2* mutations have shown that their frequency varies across geographic areas and ethnicity groups. For the G2019S mutation in the *LRRK2* gene, frequencies range from 0.2 to 0.4% in Peruvian cohorts (14, 15), up to 4% in Uruguayans (14) and 5.45% in an Argentinian series (16–19). Likewise, the R1441G and R1441H mutations in this same gene seem to be uncommon in Latin American populations (0.3–0.8%) (14, 18). The LARGE-PD, a research consortium established among several Latin American countries, has been collecting data for what is the largest PD cohort in the region, allowing for large-scale genotyping as well as performing GWAS in these cohorts (20–22). This initiative aimed to estimate the frequency of *LRRK2* mutations in the region and reported varying frequencies of the G2019S and R1441G/C mutations, which strongly correlated with the European admixture of the samples analyzed (15, 20).

GBA mutations have also been studied in few Latin American cohorts but mainly focused on most frequently reported mutations in other populations. The observed frequency of these mutations varies across regions ranging from 0.2% (p.N370S) to 0.7% (p.E326K) in Ecuadorians (23) and up to 5.5% (p.L444P) in Mexican Mestizo and Brazilian cohorts (23–27). Few studies have studied the entire *GBA* gene in Latin America, showing a frequency similar to those reported in individuals of European descent (4–5%), but lower than frequencies reported in Ashkenazi patients (20%) (28). Moreover, the overall frequency of *GBA* mutations seems to be consistently higher than *LRRK2* mutations across different geographic areas, suggesting that *GBA* could play a more important role in PD genetics for Latin American populations. Velez-Pardo et al. found a mutation that was specific for a Colombian cohort (p.K198E) and in a much higher frequency (9.9%) highlighting the need to sequence the whole *GBA* gene rather than focusing only on assessing commonly reported mutations (27).

In this study, we sought to clinically characterize PD patients of Costa Rican origin and to sequence familial PD and atypical parkinsonism-associated genes in Costa Rican PD cases and controls.

MATERIALS AND METHODS

Study Subjects

We enrolled 118 consecutive unrelated PD patients (68 males, 50 females) with 97 unrelated controls (28 males, 69 females), matched according to age and gender whenever possible. Thirty-five patients (16.28%) reported having a relative ($\leq 2^{\circ}$) with any sort of movement disorder; of those, 21 (9.77%) had a formal PD diagnosis. All subjects resided and were originated from Costa Rica and were recruited at the Movement Disorders Unit of the Department of Neurology, Hospital San Juan de Dios, Caja Costarricense de Seguro Social. All patients fulfilled Gelb criteria for the clinical diagnosis of PD, while controls had no signs or personal history of any neurodegenerative disease and were mainly the spouses of the PD cases. We preferred using Gelb criteria over the United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) as it provided different clinical diagnostic levels of certainty (possible and probable) and it has

shown to have similar positive and negative predictive values, as well as sensitivity and global accuracy when compared to UKPDSBB (29). Albeit both diagnostic criteria sets have low specificity and are mainly focused on motor features, UKPDSBB criteria further err by challenging PD diagnosis in the presence of genetic risk factors (30). Our last patient was enrolled by 2011, which is 4 years earlier than when the Movement Disorder Society (MDS) task force proposed the new clinical diagnostic criteria for PD (MDS-PD criteria) (31); therefore, we were not able to use those for clinical diagnosis of patients enrolled in our study.

We gathered information concerning work and educational status as well as history of exposure to risk and protective factors of PD. We further obtained detailed information on PD history, comorbidities, and antiparkinsonian treatments. Additionally, motor disability of the patients was evaluated by means of the Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn & Yahr (H&Y), and Schwab & England (S&E) scales. Cognitive status was assessed using the Montreal Cognitive Assessment (MoCA) test.

Genetic Analysis

Molecular inversion probes were used to sequence coding and untranslated regions in familial PD and atypical parkinsonism-associated genes including *GBA, SNCA, VPS35, LRRK2, GCH1, PRKN, PINK1, DJ-1, VPS13C,* and *ATP13A2* at McGill University with Illumina HiSeq 4000 as previously described (32). The full protocol can be found at https://github.com/gan-orlab/MIP_protocol. All sequences were aligned using Burrows-Wheeler Aligner (BWA) using the reference genome hg19 (33). Genome Alignment Tool Kit (GATK v3.8) was used to call variants and perform quality control and ANNOVAR was used to annotate each variant (34, 35). Exons 10 and 11 of *GBA* were sequenced using Sanger sequencing as previously described (36), and *GBA* variants in other exons were also validated using Sanger sequencing. We decided to focus on genes that are involved in typical PD, as our selected cohort is of typical PD (10, 37, 38).

Quality Control

All samples and variants were filtered based on standard quality control process as previously reported (39). In brief, variants were separated into common and rare by minor allele frequency (MAF) in the cohort. Rare variants (MAF < 0.01) with a minimum depth of coverage of $>30\times$ were included in the analysis, along with common variants (MAF ≥ 0.01) with $>15\times$ coverage. We have established that for common variants, we get reliable reads at $15\times$; however, to get reliable reads for rare variants, we need $>30\times$; otherwise, there are many false positives (40). Variant calls with a genotype frequency of $<25\%$ of the reads or genotype quality of <30 were excluded. Samples and variants with more than 10% missingness were also excluded.

In silico Structural Analysis

The atomic coordinates of the human Lrrk2 C-terminal domain structure (a.a. 1327–2527) were downloaded from the Protein Data Bank (ID 6VP6). The figure was generated using PyMol v.2.4.0.

Statistics

We used Stata® (version 14) for the statistical analysis of sociodemographic and clinical variables. Normally distributed variables are reported as mean with its standard deviation (SD), whereas continuous but non-normally distributed variables are reported as median with the 25th and 75th percentile values (interquartile range, IQR). Normally distributed variables were compared with paired or unpaired *t*-tests, while non-normally distributed variables were compared with Mann–Whitney *U*-test or Wilcoxon match-paired signed-rank test. Frequencies were compared with χ^2 and Fisher's exact test. Tests were two-tailed, and significance was set at $p < 0.05$. We modeled through linear regression the association between demographic and clinical variables with the severity of the disease, as indexed by UPDRS and MoCA, as dependent variables in the models.

For genetic analysis, common and rare variants were analyzed separately. Association of common variants was tested using logistic regression adjusted for age and sex in PLINK v1.9. For rare variants' analysis, we examined the burden of rare variants in each gene using optimized sequence Kernel association test (SKAT-O) adjusted for age and sex (41). Rare variants were separated into different categories based on their potential pathogenicity to examine specific enrichment in different variant subgroups as described previously (40): (1) variants with Combined Annotation Dependent Depletion (CADD) score of $\geq$12.37 (representing the top 2% of potentially deleterious variants) (42); (2) regulatory variants predicted by ENCODE (43); (3) potentially functional variants including all non-synonymous variants, stop gain/loss variants, frameshift variants, and intronic splicing variants located within two base pairs of exon–intron junctions; (4) loss-of-function variants, which includes stop gain/loss, frameshift, and splicing variants; and (5) only non-synonymous variants. Bonferroni correction for multiple comparisons was applied as necessary.

This study was approved by the Ethics Committee of Hospital San Juan de Dios, Caja Costarricense de Seguro Social (CLOBI-HSJD #014-2015) and the University of Costa Rica (837-B5-304). Written informed consent was obtained from all participants.

RESULTS

Sociodemographic and Clinical Variables

At enrollment, PD probands had a mean age of 62.12 ± 13.51 years (range 25–86), and the mean age at onset was 54.62 ± 13.54 (range 16–83) years. Male PD patients comprised 57.63% of the sample. Despite the fact that a significantly larger proportion of the male PD patients reported current or previous jobs involving agricultural activities (19.40% male, 2.08% female; $p = 0.01$), the mean number of years of education of these men was significantly higher than women (10.74 ± 3.81 vs. 8.86 ± 4.01; $p = 0.03$). **Table 1** details subjects' baseline characteristics along with the frequency of exposure to main risk and protective factors for PD. Most of the risk and protective factors were more prevalent in men. **Tables 1**, **2** detail the frequency of clinical manifestations as well as the standardized scale scores reported for PD cases. The most frequent initial symptoms included resting tremor (71.30%), rigidity (24.07%), and pain (10.19%). Most of the

TABLE 1 | Baseline characteristics with frequency of risk and protective factors for PD in study subjects, with sex comparison.

	Men 96 (44.65%)	Women 119 (55.35%)	Total ($n = 215$)	*p*
Condition				**<0.001**
Cases	68/118 (57.63%)	50/118 (42.37%)	118/215 (54.88%)	
Controls	28/97 (28.87%)	69/97 (71.13%)	97/215 (45.12%)	
Age of onset (mean ± SD)	54.74 ± 12.03	54.46 ± 15.49	54.62 ± 13.54	0.91
Age of recruitment (mean ± SD)	62.75 ± 12.17	61.26 ± 15.22	62.12 ± 13.51	0.1
Years of education† (mean ± SD)	10.74 ± 3.81	8.86 ± 4.01	9.94 ± 3.98	**0.03**
Agricultural activities†, *n* (%)	13/67 (19.40%)	1/48 (2.08%)	14/115 (12.17%)	**0.01**
Risk factors†, *n* (%)				
Pesticides	24/67 (35.82%)	10/48 (20.83%)	34/115 (29.57%)	0.1
Herbicides	26/67 (38.81%)	9/48 (18.75%)	35/115 (30.43%)	**0.03**
Welding	22/65 (33.85%)	3/48 (6.25%)	25/113 (22.12%)	**<0.001**
Heavy metals	11/64 (17.19%)	2/48 (4.17%)	13/112 (11.61%)	**0.04**
Non-potable water	29/66 (43.94%)	19/48 (39.58%)	48/114 (42.11%)	0.7
Cardiovascular	41/58 (70.69%)	32/43 (74.42%)	73/101 (72.28%)	0.82
Years of exposure, median (IQR)	8 (1–20)	5 (1–19)	8 (1–20)	0.36
Protective factors†, *n* (%)				
Smoking	36/67 (53.73%)	9/48 (18.75%)	45/115 (39.13%)	**<0.001**
Coffee	61/65 (93.85%)	44/48 (91.67%)	105/113 (92.92%)	0.72
Alcohol	43/67 (64.18%)	9/48 (18.75%)	52/115 (45.22%)	**<0.001**
Physical activity	47/67 (70.15%)	17/48 (35.42%)	64/115 (55.65%)	**<0.001**
UPDRS "on" median (IQR)				
I	2 (0.5–4)	2 (0–5)	2 (0–5)	0.98
II	9 (3–17)	8 (3–14)	9 (3–16)	0.57
III	23 (10–35)	26 (14–37)	23 (12–36)	0.57
Total	35 (21–59)	36.5 (23–60)	36 (22–60)	0.97
Hoehn and Yahr				
1	5/59 (8.47%)	9/43 (20.93%)	14/102 (13.73%)	0.36
1.5	7/59 (11.86%)	6/43 (13.95%)	13/102 (12.75%)	
2	12/59 (20.34%)	5/43 (11.63%)	17/102 (16.67%)	
2.5	14/59 (23.73%)	9/43 (20.93%)	23/102 (22.55%)	
3	17/59 (28.81%)	8/43 (18.60%)	25/102 (24.51%)	
4	3/59 (5.08%)	4/43 (9.30%)	7/102 (6.86%)	
5	1/59 (1.69%)	2/43 (4.65%)	3/102 (2.94%)	
Schwab and England, median (IQR)	80 (80–90)	90 (80–90)	80 (80–90)	0.33
MoCA test, median (Q1–Q3)	22.5 (18–25.5)	22 (14–25)	22 (17–25)	0.38

†*Information available only for patients and does not include controls.*

IQR, interquartile range (Q1–Q3); MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; SD, standard deviation; UPDRS, Unified Parkinson's Disease Rating Scale. The significance was set at $p < 0.05$ were indicated in bold.

patients had asymmetric onset (94.12%) and a good response to levodopa (89.11%). Other frequently reported motor features comprised dystonia (46.08%), falls (39.22%), and dysphagia (36.27%). Common non-motor manifestations such as hyposmia, sleep disorders and depressive/anxious mood were seen in more than 50% of the cases. Overall median score of UPDRS "ON" was 36 (22–60), most of our patients were graded in the "2.5" and "3" categories of the H&Y scale with a median for S&E score of 80% (80–90%). The median value for the MoCA test was 22 (17–25). There were no statistically significant differences between sex, regarding these scores.

We were able to establish through multivariate linear regression modeling that an increased disease duration along with the presence of orthostasis, dysphagia, and mood disorders significantly correlated with increased scores in total ON UPDRS. Furthermore, we found an interaction between performing regular physical activity and duration of disease, where despite having increased years of evolution, patients that performed regular physical activity still scored less in the total ON UPDRS (see **Supplementary Figure 1**). Additionally, lower scores in MoCA testing significantly correlated with increased age, coffee consumption, and the presence of hallucinations, falls, and mood disorders (depression/anxiety), whereas increased years of education correlated with better MoCA scores (see **Supplementary Figure 2**).

TABLE 2 | Clinical manifestations of PD cases, with sex comparison.

	Men 68 (57.63%)	Women 50 (42.37%)	Total ($n = 118$)	p
Initial symptoms, *n* (%)				
Resting tremor	43/63 (68.25%)	34/45 (75.56%)	77/108 (71.30%)	0.52
Rigidity	17/63 (26.98%)	9/45 (20.00%)	26/108 (24.07%)	0.5
Postural instability	3/63 (4.76%)	1/45 (2.22%)	4/108 (3.70%)	0.64
Bradykinesia	3/63 (4.76%)	1/45 (2.22%)	4/108 (3.70%)	0.64
Pain	3/63 (4.76%)	8/45 (17.78%)	11/108 (10.19%)	**0.03**
Symptoms, *n* (%)				
Resting tremor	51/59 (86.44%)	40/43 (93.02%)	91/102 (89.22%)	0.35
Bradykinesia	55/59 (93.22%)	39/43 (90.70%)	94/102 (92.16%)	0.72
Rigidity	50/59 (84.75%)	36/43 (83.72%)	86/102 (84.31%)	0.89
Asymmetry	57/59 (96.61%)	39/43 (90.70%)	96/102 (94.12%)	0.24
Levodopa response	55/59 (93.22%)	35/42 (83.33%)	90/101 (89.11%)	0.19
Hallucinations	14/59 (23.73%)	8/43 (18.60%)	22/102 (21.57%)	0.63
Orthostatism	9/59 (15.25%)	12/43 (27.91%)	21/102 (20.59%)	0.14
Falls	23/59 (38.98%)	17/43 (39.53%)	40/102 (39.22%)	0.96
Syncope	1/59 (1.69%)	2/43 (4.65%)	3/102 (2.94%)	0.57
Dystonia	28/59 (47.46%)	19/43 (44.19%)	47/102 (46.08%)	0.84
Dysphagia	20/59 (33.90%)	17/43 (39.53%)	37/102 (36.27%)	0.68
Hyposmia	34/63 (53.97%)	23/47 (48.94%)	57/110 (51.82%)	0.7
Constipation	23/52 (44.23%)	13/41 (31.71%)	36/93 (38.71%)	0.29
Urinary symptoms	7/52 (13.46%)	5/41 (12.20%)	12/93 (12.90%)	0.86
Sleep disorders	52/65 (80.0%)	37/52 (71.2%)	89/116 (76.7%)	0.26
Insomnia	22/52 (42.31%)	16/37 (43.24%)	38/89 (42.70%)	0.93
Vivid dreams	26/52 (50.00%)	13/37 (35.14%)	39/89 (43.82%)	0.2
Mood disorders (depression or anxiety)	40/63 (63.49%)	29/47 (61.70%)	69/110 (62.73%)	0.85
Disease duration (years), median (IQR)	5 (3–10)	5 (3–7)	5 (3–9)	0.18

IQR, interquartile range (Q1–Q3). The significance was set at $p < 0.05$ were indicated in bold.

Quality of Coverage and Identified Variants

The average coverage of the 10 genes analyzed in this study was >588× for all genes. The coverage per gene and the percentage of nucleotides covered at >15× and >30× for each gene are detailed in **Supplementary Table 1**. There were no differences in the coverage across the samples (patients and controls). Overall, after quality control, we identified 163 rare variants (**Supplementary Table 2**) and 158 common variants (**Supplementary Table 3**) across all genes and all samples that were included in the analysis. Specific protein and DNA changes are listed in **Supplementary Tables 4, 5** for rare and common exonic variants, respectively.

Rare and Common Variants in PD and Parkinsonism-Related Genes

Burden and SKAT-O analyses did not identify an association of any of the tested genes and PD (**Table 3**) after correction for multiple comparisons, as expected given the small sample size. We also did not identify any PD patients with potentially damaging homozygous or compound heterozygous variants in any of these genes. Rare variants in *LRRK2* were nominally associated with PD, and 11 (9.2%) patients carried a rare non-synonymous variant, compared to four (4.1%) among the controls. Interestingly, six of these rare non-synonymous variants, all located between amino acids p.1620 and 1623 in the C-terminal-of-ROC (COR) domain of Lrrk2, were found in six patients and none in controls (**Table 4**).

Non-synonymous *GBA* variants were identified in three individuals: p.T369M was identified in a male patient with age at onset of 48 years, p.N370S was identified in a healthy female individual recruited at the age of 78 years, and p.L444P was identified in a healthy female individual recruited at the age of 64. While we cannot rule out that these healthy individuals will develop PD in the future, it is unlikely that *GBA* variants have a major role in PD among Costa Rican patients. One PD patient carried a pathogenic *GCH1* variant, p.K224R, further emphasizing the role of this gene in PD.

In the analysis of common variants, none of the variants was associated with PD after correction for multiple comparisons (**Supplementary Table 3**), which set the corrected p-value for statistical significance at $p < 0.00031$. One non-synonymous variant in *LRRK2*, p.I723V, was found with allele frequency of

TABLE 3 | Burden and SKAT-O analyses with no significant association found of any of the tested genes and PD, after Bonferroni correction for multiple comparisons.

	All		CADD		Encode		Funct		LOF		NS	
	Burden	**SKATO**	**Burden**	**SKATO**	**Burden**	**SKATO**	**Burden**	**SKATO**	**Burden**	**SKATO**	**Burden**	**SKATO**
LRRK2	0.017	0.030	0.127	0.265	0.400	0.682	0.061	0.123	NA	NA	0.087	0.160
VPS35	0.341	0.341	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SNCA	0.045	0.101	NA	NA	0.347	0.560	0.347	0.560	NA	NA	NA	NA
GCH1	0.764	0.880	0.209	0.209	0.722	0.722	0.753	0.624	NA	NA	0.209	0.209
PRKN	0.839	0.967	0.874	0.900	NA	NA	0.874	0.900	NA	NA	0.874	0.900
PINK1	0.722	0.860	0.200	0.355	NA	NA	0.352	0.582	NA	NA	0.352	0.582
PARK7	0.586	0.779	0.779	0.779	0.812	0.897	0.546	0.778	NA	NA	0.779	0.779
VPS13C	0.274	0.406	0.563	0.808	0.095	0.095	0.195	0.349	0.332	0.767	0.829	0.952
ATP13A2	0.054	0.137	0.791	0.397	NA	NA	0.791	0.397	NA	NA	0.791	0.397

The numbers represent the uncorrected p-values of the tests. Burden, burden test; SKATO, optimized sequence Kernel association test; CADD, Combined Annotation Dependent Depletion (CADD) score of ≥12.37 (representing the top 2% of potentially deleterious variants); Encode, regulatory variants predicted by ENCODE; Funct, Potentially functional variants including all non-synonymous variants, stop gain/loss variants, frameshift variants, and intronic splicing variants located within two base pairs of exon–intron junctions; LOF, loss-of-function variants, which include stop gain/loss, frameshift, and splicing variants; NS, only non-synonymous variants; NA, not applicable—not enough variants for analysis.

0.01 in patients and 0.09 in controls (OR = 0.11, 95% CI = 0.02–0.52, $p = 0.005$), yet this difference was not statistically significant after correction for multiple comparisons.

DISCUSSION

Clinical Features

PD prevalence has been increasing over time with a global age-standardized prevalence rate increase of 21.7% from the years 1990 to 2016 (44). Furthermore, PD prevalence seems to be lower in Eastern compared to Western countries (45). Few studies have explored the prevalence of PD in Latin America providing values that are similar either to other developing countries (46) or to European cohorts (47, 48). PD also becomes more common with advancing age (44, 45). Our sample average age of PD at onset and at diagnosis was lower when compared to other cohorts (49–51), although it could suggest that PD presents earlier in Costa Rica, and more epidemiological studies are needed as it could also be related to recruitment bias.

The majority of our patients fulfilled Group A Gelb criteria while up to 60% also reported at least one of Group B symptoms, the most frequent being dystonia, falls, and dysphagia. The median for years of evolution of the disease for both men and women was 5; thus, we would expect to find Group B criteria in these patients along with the evolution of the disease. Few studies have explored ethnic variations in motor symptoms of PD, suggesting increased atypical features in Black and South Asian PD patients (52, 53); however, there is not enough evidence available along with a lack of standardized methodology to determine motor subtypes across studies and to further establish ethnic patterns of motor features (12). Common non-motor manifestations such as hyposmia, sleep disorders, and depressive/anxious mood were seen in more than half of our PD cases. Regardless of ethnicity, non-motor features are commonly present in PD with subtle differences described. Gastrointestinal non-motor features along with depression seem to be high in East Asian cohorts (54, 55). Likewise, Latino populations, such as Mexican (56), Peruvian (57), and ours, also reported high frequency of mood disorders including depression and anxiety, when compared to studies from UK and USA (58, 59). We also observed in our sample a frequency of sleep disorders and hyposmia that is higher than those reported in other cohorts (12).

Overall, our patients had a low education, which has been previously associated with a higher hazard of incident parkinsonism (60). A reduced education has also been suggested as a risk factor for cognitive impairment in PD (61). A history of non-potable water consumption along with exposure to pesticides and herbicides was reported in up to 40% of our patients. This type of exposure agrees with a mostly rural origin and the fact that 12.2% of the subjects reported involvement in agricultural activities as a main income source. We did not assess the frequency of protective and risk factors in the control group; hence, we are not able to establish any comparison with PD cases. Previous exposure to pesticides and herbicides is associated with the development of PD (62); yet, the identification of a given specific agent and the exact timing and dosing of exposure are almost impossible to establish through observational studies (63, 64). Nonetheless, key work detailing specific mechanisms that render patients vulnerable to pesticide-induced injury has been elegantly shown in animal models, further establishing biologic and toxicological pathways for specific chemicals to potentially cause PD (65). A similar situation is present regarding the exposure to welding and heavy metals. Manganese (66), copper, iron (67), and mercury (68) have been proposed as possible agents associated with the development of PD. In this study, 22.1 and 11.6% of the patients reported frequent exposure to welding and other heavy metals, respectively; however, the exact timing and dosing of exposure was not possible to assess.

Other literature has underscored the presence of protective factors for PD development, among which the most notable and with the strongest evidence include tobacco (69) and coffee consumption (7, 70–73). For both protective factors, there is also a dosing effect described, where the protective effect increases along with an increasing exposure (74, 75). Paradoxically, over

TABLE 4 | Rare variants in *LRRK2* present in patients and controls.

SNP location/rs number and nucleotide change	Detailed annotation of the variant (DA)	Status	Family history
12:40709172:T:C	intronic:LRRK2:NM_198578	Control	
12:40709180:T:C	intronic:LRRK2:NM_198578	Affected	
12:40709181:T:C	intronic:LRRK2:NM_198578	Affected	
rs760912433:C:T	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.C4856T:p.P1619L	Control	
12:40713821:A:G†	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.A4859G:p.K1620R	Affected	
12:40713824:A:G†	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.A4862G:p.H1621R	Affected	Mother: • Epilepsy (type unknown) diagnosed in early adulthood. • Dementia associated to rigidity, diagnosed at 67 years old, death at 69 years old • Retinitis pigmentosa
rs765275134:C:A†	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.C4863A:p.H1621Q	Affected	
12:40713826:C:A†	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.C4864A:p.P1622T	Affected	Father: • Dementia (type unknown), not associated to hallucinations or motor symptoms. Diagnosed at 74 years old, death at 84 years old.
rs751492506:C:T†	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.C4865T:p.P1622L	Affected	Two sisters (from both parents): • Parkinson's disease diagnosed at ages 30 and 20 years old. Maternal aunt: • Bilateral hand tremor (described as intention tremor, does not have a definitive diagnosis). Maternal great-grandfather: • Bilateral hand tremor (type unknown).
12:40713828:T:C	exonic:synonymous_SNV:LRRK2:NM_198578:exon34:c.T4866C:p.P1622P	Control	
12:40713829:A:G†	exonic:non-synonymous_SNV:LRRK2:NM_198578:exon34:c.A4867G:p.K1623E	Affected	Mother: • Cirrhosis (not associated to neurologic symptoms). Maternal uncle: • Parkinson's disease (diagnosed at 50 years old). Maternal grandfather: • Tremor in both hands (type unknown).
rs73097447:A:C	intronic:LRRK2:NM_198578	Affected	
12:40716092:G:T	intronic:LRRK2:NM_198578	Affected and Control	

Six of these rare non-synonymous variants (†), all located between amino acids p.1620 and 1623 in the COR domain of LRRK2, were found only in patients and not in controls.

90% of our PD cases had been exposed to a protective factor in the past, most of them having a regular coffee intake (two to three cups per day for over 15 years), and yet they all developed PD.

Performing regular physical activity correlated with lower ON UPDRS scores in spite of increasing age. Physical activity has been established as a possible protective factor for incident Parkinsonism (76); our data would suggest that physical activity could determine reduced severity of disease, specifically concerning motor features. Although exercise has not been proven to slow the progression of akinesia, rigidity, and gait disturbances, it promotes a feeling of physical and mental well-being, and at the same time, it can alleviate rigidity-related pain and improve patients' motor (77) and non-motor symptoms (78).

Increasing age, coffee consumption, hallucinations, falls, and mood disorders along with reduced years of education significantly correlated with worse MoCA scores. Older age and duration of PD are determinant risk factors for incidence of dementia in PD (79). Furthermore, hallucinations have been established as risk factors for cognitive impairment (79, 80) along with gait disturbances (manifested by falls) (81) and depression (82). Reduced education years also have been proposed as a risk factor for cognitive impairment in patients with PD (61). Poor global cognition has been previously associated with a

higher risk of incident parkinsonism (60). Coffee consumption has been suggested to reduce risk of dementia (83) with a dosing effect (84, 85); however, there have been inconsistent findings regarding the effects of coffee consumption on specific cognitive domains. It has been suggested to be in association with improved executive performance but smaller hippocampal volume and worse memory function (86); nonetheless, this association is not sustained when cognition is analyzed longitudinally. Other literature suggested that coffee might be slightly beneficial on memory without a dose–response relationship (87). Recent large-scale genetic analysis using mendelian randomization did not find any evidence supporting any beneficial or adverse long-term effect of coffee consumption on global cognition or memory function (88) or AD incidence (89). To our knowledge, there is no literature evaluating the effect on cognition of coffee consumption, specifically for PD patients. Our findings suggest a possible deleterious effect that should be further explored in this population.

Genetic Assessment

After sequence coding familial PD and atypical parkinsonism-associated genes including *GBA, SNCA, VPS35, LRRK2, GCH1, PRKN, PINK1, DJ-1, VPS13C,* and *ATP13A2* and correcting for multiple comparisons, burden and SKAT-O analyses did not show an association of any of the tested genes and PD. We also did not identify any homozygous or compound heterozygous pathogenic variants in any of these genes.

Non-synonymous *GBA* variants were identified in three individuals including one patient and two unaffected controls. While we cannot rule out that these healthy individuals will develop PD in the future, it is unlikely that *GBA* variants have a major role in PD among Costa Rican patients especially when compared to other European and Ashkenazi Jewish populations where we find that 8–20% of the patients harbor *GBA* variants (28).

Finally, one PD patient carried a pathogenic variant, p.K224R, in the *GCH1* gene. *GCH1* encodes for GTP cyclohydrolase 1, which is a key enzyme for dopamine production in nigrostriatal neurons. Loss-of-function mutations such as p.K224R have been shown to cause Dopa-responsive dystonia (DRD); however, variants in this gene have also been implicated in PD, perhaps through regulation of *GCH1* expression (90, 91). It has been suggested that late-onset DRD might present clinically with parkinsonism, or alternatively, pathogenic *GCH1* mutations may predispose to both diseases and carriers will develop any or both depending on other genetic or environmental factors (92). Our patient did not present clinical features suggestive of DRD and did not have any family history of PD.

Rare variants in *LRRK2* were nominally associated with PD, observed only in affected individuals; six of these rare non-synonymous variants were located between amino acids p.1620 and 1623 in the COR domain of Lrrk2. *LRRK2* encodes a multiple domain protein that includes a Roc-COR tandem domain, a tyrosine kinase-like protein kinase domain, and at least four repeat domains located within the N-terminal and C-terminal regions. The Roc-COR domain classifies the Lrrk2 protein as part of the ROCO superfamily of Ras-like G proteins (93). Mutations in *LRRK2* are the most common cause of late-onset hereditary PD. Most frequently reported disease-causing mutations are located in the kinase domain (i.e., G2019S), increasing kinase activity, and in the Roc-COR tandem domain (i.e., R1441C/G and Y1699C), impairing its GTPase function. Alterations of both kinase and GTPase activity may mediate neurodegeneration in these forms of PD (94). Of the six patients found to have non-synonymous variants in the COR domain, two had first-degree relatives with dementia, one had a second-degree relative with PD, and one had two sisters with PD diagnosed at a very young age (20 and 30 years old) (see **Table 4**).

Methodological issues, such as size and composition of the sample (i.e., number of familial and sporadic cases), might explain the variations seen in the frequency of *LRRK2* mutations in case series from similar countries. However, there is a clear difference established among geographical regions, where North African Arabs (95), Ashkenazy-Jews (96) and certain Europeans cohorts (97–99) might report a higher prevalence than Latin American and Asian populations for these mutations (15, 100, 101).

Structural Analysis of *LRRK2* Pathogenic Mutations

The non-synonymous missense mutations described here are all found in the COR domain of Lrrk2. To gain insight into how these mutations may affect the function of Lrrk2, we investigated their locations in the structure of Lrrk2. The high-resolution cryoelectron microscopy (cryoEM) structure of the C-terminal domains of Lrrk2 in different states have recently been reported and shed light on how allosteric interactions between different domains regulate microtubule interactions (102). The structure notably shows interactions between the ROC GTPase domain and the COR-B domain, notably involving the pathogenic mutation sites p.Arg1441 and p.Tyr1699 (**Figure 1A**). These interdomain interactions enable the kinase activity to be regulated by GTP binding to the ROC domain. The mutations described here, found in the segment a.a. 1619–1623, are all located in a loop of the COR-A domain. This loop, which spans a.a. 1613–1624, is disordered in the cryoEM structure, and thus, no atomic resolution model is available for that segment (**Figure 1A**). It is therefore not possible to gain detailed insights into the effect of each individual missense mutation.

However, integrative modeling, based on cryoelectron tomography (cryoET) data collected from *in situ* and *in vitro*-reconstituted Lrrk2 filaments bound to microtubules, shows how the different domains of Lrrk2 dimerize and associate with microtubules (102, 103). Dimerization is mediated via two sites through reciprocal interactions: one involving WD40–WD40 interactions and another one involving COR–COR interactions. These interactions enable Lrrk2 C-terminal domains to form extended oligomeric filaments that form a helix around the microtubule. Of particular interest here, the COR–COR dimerization interface involves both the COR-A and COR-B domains, with the loop containing a.a. 1613–1624 at the center of this interface (**Figure 1B**). Mutations in this loop may thus affect dimerization. Given that the kinase activity and

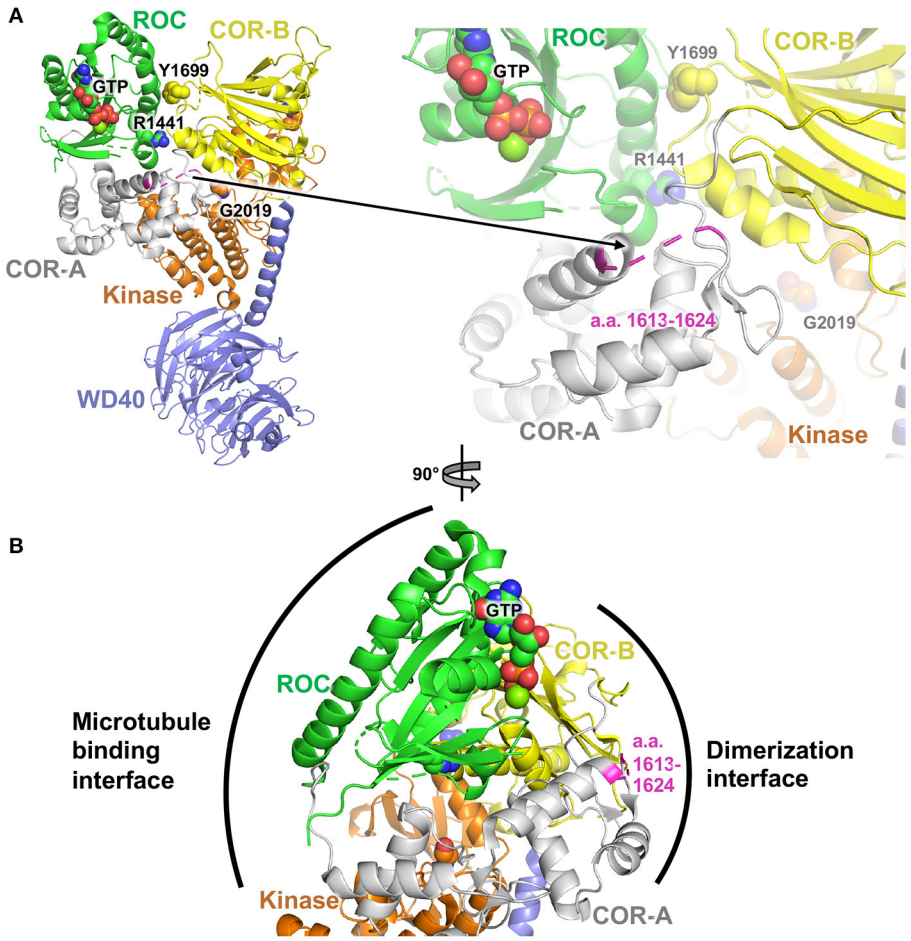

FIGURE 1 | Structural analysis of PD variants in LRRK2. **(A)** Cryoelectron microscopy structure of Lrrk2 C-terminal domains, PDB code 6vp6 (102). Parkinson-linked missense mutation sites R1441, Y1699, and G2019 are shown as spheres. The loop spanning a.a. 1612–1624 in the COR-A domain is shown in magenta. **(B)** Rotated view (90°) of the structure in **(A)**, showing the proposed dimerization and microtubule binding interfaces, based on integrative modeling of Lrrk2 filaments bound to microtubules (102).

conformation affect the ability of Lrrk2 to dimerize through the COR domain *via* allosteric interactions, it is possible that mutations in the COR-A loop in turn affect the kinase activity. Further experiments would be required to determine how the mutations described here affect the kinase, dimerization, and microtubule-binding activity of Lrrk2.

LIMITATIONS

Genome analysis from Mestizo populations in Latin America has previously shown in Costa Rica a European, Native American, and African admixture of 66.7, 28.7, and 4.6%, respectively (104). Therefore, we would have expected to observe a higher frequency of mutations, similar to other European series reported. However, our sample size is small and is more representative of the metropolitan area where most of the patients were recruited, thus warranting in the future a more comprehensive study involving a wider and more representative population of the whole country, particularly including more patients from the non-metropolitan and coastal zones. Moreover, the purpose of our study was to serve as an exploratory analysis in this population, which had not been studied before; likewise, we opted to cover as many genes as possible. We are aware that the sample size is limited, yet underrepresented populations with limited funding and resources that struggle to achieve large sample sizes should be studied and reported as well.

We did not gather information concerning protective and risk factors for subjects in the control group, therefore, we were not able to compare and discuss the frequency of these factors between cases and controls.

CONCLUSIONS

This is the first study that reports on sociodemographics, risk factors, clinical presentation, and genetics of Costa Rican patients with PD. We observed a high frequency of exposure to both risk factors (pesticides, herbicides, non-potable water, and low education) and protective factors (tobacco and coffee

intake). Regular physical activity significantly correlated with better UPDRS scores despite years of evolution of the disease. Increased years of education were significantly associated with better MoCA test scores, whereas the presence of hallucinations, falls, and mood disorders correlated with a worse performance in the MoCA test. Interestingly, coffee consumption also correlated significantly with worse MoCA test scoring.

We did not find an association between any of the tested familial PD and atypical parkinsonism-associated genes, including *GBA, SNCA, VPS35, LRRK2, GCH1, PRKN, PINK1, DJ-1, VPS13C,* and *ATP13A2*, and PD. We also did not identify any homozygous or compound heterozygous pathogenic variants in any of these genes. Rare variants in *LRRK2* were nominally associated with PD, with six of these rare non-synonymous variants all located in the COR domain of *LRRK2*. One PD patient carried a pathogenic *GCH1* variant, p.K224R, further emphasizing the role of this gene in PD.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NIH-dbGAP repository, accession number phs002495.v1.p1 (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs002495.v1.p1).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Hospital San Juan de Dios, Caja Costarricense de Seguro Social (CLOBI-HSJD #014-2015) and the University of Costa Rica (837-B5-304). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GT-A and EY conceptualized the report and made substantial contributions to the design, drafting, and revision of the work. J-FT performed *in silico* structural analysis and contributed to the discussion of these results. TL-P, JR-M, AG-P, ZG-O, KC-C, IF-M, and JF-T significantly contributed to drafting and critically reviewing the paper. All authors have contributed to the work and agree with the presented findings and that the work has not been published before nor is being considered for publication in another journal. All authors approved the final version of the manuscript and assume accountabilities for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.656342/full#supplementary-material

REFERENCES

1. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. *Nat Rev Dis Primers.* (2017) 3:17013. doi: 10.1038/nrdp.2017.13
2. Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB, et al. Update of the mDS research criteria for prodromal parkinson's disease. *Mov Disord.* (2019) 34:1464–70. doi: 10.1002/mds.27802
3. Taylor AE, Saint-Cyr JA, Lang AE. Frontal lobe dysfunction in parkinson's disease. The cortical focus of neostriatal outflow. *Brain.* (1986) 109:845–83. doi: 10.1093/brain/109.5.845
4. Cools R, Barker RA, Sahakian BJ, Robbins TW. Mechanisms of cognitive set flexibility in parkinson's disease. *Brain.* (2001) 124:2503–12. doi: 10.1093/brain/124.12.2503
5. Owen AM, James M, Leigh PN, Summers BA, Marsden CD, Quinn NP, et al. Fronto-striatal cognitive deficits at different stages of parkinson's disease. *Brain.* (1992) 115:1727–51. doi: 10.1093/brain/115.6.1727
6. Cronin-Golomb A, Braun AE. Visuospatial dysfunction and problem solving in parkinson's disease. *Neuropsychology.* (1997) 11:44–52. doi: 10.1037/0894-4105.11.1.44
7. Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for parkinson disease. *Ann Neurol.* (2012) 72:893–901. doi: 10.1002/ana.23687
8. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with parkinson's disease. *Science.* (1997) 276:2045–7. doi: 10.1126/science.276.5321.2045
9. Bonifati V. Genetics of parkinson's disease–state of the art, 2013. *Parkinsonism Relat Disord.* (2014) 1:S23–8. doi: 10.1016/S1353-8020(13)70009-9
10. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
11. Gan-Or Z, Liong C, Alcalay RN. GBA-Associated parkinson's disease and other synucleinopathies. *Curr Neurol Neurosci Rep.* (2018) 18:44. doi: 10.1007/s11910-018-0860-4
12. Ben-Joseph A, Marshall CR, Lees AJ, Noyce AJ. Ethnic variation in the manifestation of parkinson's disease: a narrative review. *J Parkinsons Dis.* (2020) 10:31–45. doi: 10.3233/JPD-191763

13. Popejoy AB, Fullerton SM. Genomics is failing on diversity. *Nature.* (2016) 538:161–4. doi: 10.1038/538161a
14. Mata IF, Cosentino C, Marca V, Torres L, Mazzetti P, Ortega O, et al. LRRK2 mutations in patients with parkinson's disease from peru and uruguay. *Parkinsonism Relat Disord.* (2009) 15:370–3. doi: 10.1016/j.parkreldis.2008.09.002
15. Cornejo-Olivas M, Torres L, Velit-Salazar MR, Inca-Martinez M, Mazzetti P, Cosentino C, et al. Variable frequency of lRRK2 variants in the latin american research consortium on the genetics of parkinson's disease (LARGE-PD), a case of ancestry. *NPJ Parkinsons Dis.* (2017) 3:19. doi: 10.1038/s41531-017-0020-6
16. Perez-Pastene C, Cobb SA, Diaz-Grez F, Hulihan MM, Miranda M, Venegas P, et al. Lrrk2 mutations in south america: a study of chilean parkinson's disease. *Neurosci Lett.* (2007) 422:193–7. doi: 10.1016/j.neulet.2007.06.021
17. Pimentel MM, Moura KC, Abdalla CB, Pereira JS, De Rosso AL, Nicaretta DH, et al. A study of lRRK2 mutations and parkinson's disease in brazil. *Neurosci Lett.* (2008) 433:17–21. doi: 10.1016/j.neulet.2007.12.033
18. Yescas P, Lopez M, Monroy N, Boll MC, Rodriguez-Violante M, Rodriguez U, et al. Low frequency of common lRRK2 mutations in mexican patients with parkinson's disease. *Neurosci Lett.* (2010) 485:79–82. doi: 10.1016/j.neulet.2010.08.029
19. Gatto EM, Parisi V, Converso DP, Poderoso JJ, Carreras MC, Marti-Masso JF, et al. The lRRK2 g2019S mutation in a series of argentinean patients with parkinson's disease: clinical and demographic characteristics. *Neurosci Lett.* (2013) 537:1–5. doi: 10.1016/j.neulet.2013.01.011
20. Zabetian CP, Mata IF, Latin American Research Consortium on the Genetics of PD (LARGE-PD). LARGE-PD: examining the genetics of parkinson's disease in latin america. *Mov Disord.* (2017) 32:1330–1. doi: 10.1002/mds.27081
21. Loesch D, Horimoto ARVR, Heilbron K, Sarihan EI, Inca-Martinez M, Mason E, et al. Characterizing the genetic architecture of parkinson's disease in latinos. *medRxiv [preprint].* (2020). doi: 10.1101/2020.11.09.20227124
22. Sarihan EI, Pérez-Palma E, Niestroj LM, Loesch D, Inca-Martinez M, Horimoto ARVR, et al. Genome-wide analysis of copy number variation in latin american parkinson's disease patients. *Mov. Disord.* (2021) 36:434–41. doi: 10.1002/mds.28353
23. Tipton PW, Soto-Beasley AI, Walton RL, Soler-Rangel S, Romero-Osorio Ó, Díaz C, et al. Prevalence of gBA p.K198E mutation in colombian and hispanic populations. *Parkinsonism Relat Disord.* (2020) 73:16–18. doi: 10.1016/j.parkreldis.2020.03.008
24. Dos Santos AV, Pestana CP, Diniz K. R. D. S., Campos M, Abdalla-Carvalho CB, de Rossob ALZ, et al. Mutational analysis of gIGYF2, aTP13A2 and gBA genes in brazilian patients with early-onset parkinson's disease. *Neurosci Lett.* (2010) 485:121–4. doi: 10.1016/j.neulet.2010.08.083
25. González-Del Rincón MDL, Monroy Jaramillo N, Suárez Martínez A, Yescas Gómez P, Boll Woehrlen M, López López M, et al. The l444P gBA mutation is associated with early-onset parkinson's disease in mexican mestizos. *Clin Genet.* (2013) 84:386–7. doi: 10.1111/cge.12084
26. Abreu GM, Valença DC, Campos MJ, Da Silva CP, Pereira JS, Araujo Leite MA, et al. Autosomal dominant parkinson's disease: incidence of mutations in lRRK2, sNCA, vPS35 and gBA genes in brazil. *Neurosci Lett.* (2016) 635:67–70. doi: 10.1016/j.neulet.2016.10.040
27. Velez-Pardo C, Lorenzo-Betancor O, Jimenez-Del-Rio M, Moreno S, Lopera F, Cornejo-Olivas M, et al. The distribution and risk effect of gBA variants in a large cohort of pD patients from colombia and peru. *Parkinsonism Relat Disord.* (2019) 63:204–8. doi: 10.1016/j.parkreldis.2019.01.030
28. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in parkinson's disease. *N Engl J Med.* (2009) 361:1651–61. doi: 10.1056/NEJMoa0901281
29. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of lewy body parkinson's disease. *Neurology.* (2001) 57:1497–9. doi: 10.1212/WNL.57.8.1497
30. Marsili L, Rizzo G, Colosimo C. Diagnostic criteria for parkinson's disease: from james parkinson to the concept of prodromal disease. *Front Neurol.* (2018) 9:156. doi: 10.3389/fneur.2018.00156
31. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for parkinson's disease. *Mov Disord.* (2015) 30:1591–601. doi: 10.1002/mds.26424
32. Ross JP, Dupre N, Dauvilliers Y, Strong S, Ambalavanan A, Spiegelman D, et al. Analysis of dNAJC13 mutations in french-Canadian/French cohort of parkinson's disease. *Neurobiol Aging.* (2016) 45:213–217. doi: 10.1016/j.neurobiolaging.2016.04.023
33. Li H, Durbin R. Fast and accurate short read alignment with burrows–Wheeler transform. *Bioinformatics.* (2009) 25:1754–60. doi: 10.1093/bioinformatics/btp324
34. Mckenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a mapReduce framework for analyzing next-generation dNA sequencing data. *Genome Res.* (2010) 20:1297–303. doi: 10.1101/gr.107524.110
35. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* (2010) 38:e164. doi: 10.1093/nar/gkq603
36. Ruskey JA, Greenbaum L, Roncière L, Alam A, Spiegelman D, Liong C, et al. Increased yield of full gBA sequencing in ashkenazi jews with parkinson's disease. *Eur J Med Genet.* (2019) 62:65–9. doi: 10.1016/j.ejmg.2018.05.005
37. Yang X, Xu Y. Mutations in the *ATP13A2* gene and parkinsonism: a Preliminary review. *BioMed Res Int.* (2014) 2014:371256. doi: 10.1155/2014/371256
38. Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, Van Der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new parkinson's disease risk loci. *Nat Genet.* (2017) 49:1511–6. doi: 10.1038/ng.3955
39. Mufti K, Rudakou U, Yu E, Krohn L, Ruskey JA, Asayesh F, et al. Comprehensive analysis of familial parkinsonism genes in rapid-eye-movement sleep behavior disorder. *Mov Disord.* (2021) 36:235–40. doi: 10.1002/mds.28318
40. Rudakou U, Yu E, Krohn L, Ruskey JA, Asayesh F, Dauvilliers Y, et al. Targeted sequencing of parkinson's disease loci genes highlights sYT11, fGF20 and other associations. *Brain.* (2021) 144:462–72. doi: 10.1093/brain/awaa401
41. Lee S, Emond MJ, Bamshad MJ, Barnes KC, Rieder MJ, Nickerson DA, et al. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *Am J Hum Genet.* (2012) 91:224–37. doi: 10.1016/j.ajhg.2012.06.007
42. Amendola LM, Dorschner MO, Robertson PD, Salama JS, Hart R, Shirts BH, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res.* (2015) 25:305–15. doi: 10.1101/gr.183483.114
43. Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, et al. Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature.* (2011) 473:43–9. doi: 10.1038/nature09906
44. GBD 2016 Parkinson's Disease Collaborators. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* (2018) 17:939–53. doi: 10.1016/S1474-4422(18)30295-3
45. Abbas MM, Xu Z, Tan LCS. Epidemiology of parkinson's disease-East versus west. *Mov Disord Clin Pract.* (2017) 5:14–28. doi: 10.1002/mdc3.12568
46. Nicoletti A, Sofia V, Bartoloni A, Bartalesi F, Gamboa Barahon H, Giuffrida S, et al. Prevalence of parkinson's disease: a door-to-door survey in rural bolivia. *Parkinsonism Relat Disord.* (2003) 10:19–21. doi: 10.1016/S1353-8020(03)00066-X
47. Melcon MO, Anderson DW, Vergara RH, Rocca WA. Prevalence of parkinson's disease in junin, buenos aires province, argentina. *Mov Disord.* (1997) 12:197–205. doi: 10.1002/mds.870120210
48. Barbosa MT, Caramelli P, Maia DP, Cunningham MC, Guerra HL, Lima-Costa MF, et al. Parkinsonism and parkinson's disease in the elderly: a community-based survey in brazil (the bambui study). *Mov Disord.* (2006) 21:800–8. doi: 10.1002/mds.20806
49. Viñes JJ, Larumbe R, Gaminde I, Artázcoz MT. [Incidence of idiopathic and secondary parkinson disease in navarre. *Population-based case registry*]. *Neurologia.* (1999) 14:16–22.
50. Mizuno Y. [Progress in the basic and clinical aspects of parkinson's disease]. *Rinsho Shinkeigaku.* (2004) 44:741–50.
51. Gan-Or Z, Rao T, Leveille E, Degroot C, Chouinard S, Cicchetti F, et al. The quebec parkinson network: a Researcher-Patient matching

platform and multimodal biorepository. *J Parkinsons Dis.* (2020) 10:301–13. doi: 10.3233/JPD-191775
52. Chaudhuri KR, Hu MT, Brooks DJ. Atypical parkinsonism in afro-Caribbean and indian origin immigrants to the uK. *Mov Disord.* (2000) 15:18–23. doi: 10.1002/1531-8257(200001)15:1<18::AID-MDS1005>3.0.CO;2-Z
53. Yu RL, Wu RM, Chan AY, Mok V, Wu YR, Tilley BC, et al. Cross-Cultural differences of the non-Motor symptoms studied by the traditional chinese version of the international parkinson and movement disorder society-unified parkinson's disease rating scale. *Mov Disord Clin Pract.* (2017) 4:68–77. doi: 10.1002/mdc3.12349
54. Cheon SM, Ha MS, Park MJ, Kim JW. Nonmotor symptoms of parkinson's disease: prevalence and awareness of patients and families. *Parkinsonism Relat Disord.* (2008) 14:286–90. doi: 10.1016/j.parkreldis.2007.09.002
55. Li HJ, Zhang MF, Chen MX, Hu AL, Li JB, Zhang B, et al. Validation of the nonmotor symptoms questionnaire for parkinson's disease: results from a chinese pilot study. *Int J Neurosci.* (2015) 125:929–35. doi: 10.3109/00207454.2014.986573
56. Rodríguez-Violante M, Cervantes-Arriaga A, Villar-Velarde A, Corona T. Relationship between the type and side of motor symptoms with the prevalence of non-motor symptoms in parkinson's disease. *Neurología (English Edition).* (2011) 26:319–24. doi: 10.1016/S2173-5808(11)70076-1
57. Cosentino C, Nuñez Y, Torres L. Frequency of non-motor symptoms in peruvian patients with parkinson's disease. *Arq Neuropsiquiatr.* (2013) 71:216–9. doi: 10.1590/0004-282X20130005
58. Romenets SR, Wolfson C, Galatas C, Pelletier A, Altman R, Wadup L, et al. Validation of the non-motor symptoms questionnaire (NMS-Quest). *Parkinsonism Relat Disord.* (2012) 18:54–8. doi: 10.1016/j.parkreldis.2011.08.013
59. Duncan GW, Khoo TK, Yarnall AJ, O'brien JT, Coleman SY, Brooks DJ, et al. Health-related quality of life in early parkinson's disease: the impact of nonmotor symptoms. *Mov Disord.* (2014) 29:195–202. doi: 10.1002/mds.25664
60. Darweesh SKL, Wolters FJ, Postuma RB, Stricker BH, Hofman A, Koudstaal PJ, et al. Association between poor cognitive functioning and risk of incident parkinsonism: the rotterdam study. *JAMA Neurol.* (2017) 74:1431–8. doi: 10.1001/jamaneurol.2017.2248
61. Kierzynka A, Kazmierski R, Kozubski W. Educational level and cognitive impairment in patients with parkinson disease. *Neurol Neurochir Pol.* (2011) 45:24–31. doi: 10.1016/S0028-3843(14)60056-6
62. Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS. Environmental risk factors and parkinson's disease: a metaanalysis. *Environ Res.* (2001) 86:122–7. doi: 10.1006/enrs.2001.4264
63. Firestone JA, Smith-Weller T, Franklin G, Swanson P, Longstreth WT, Jr., Checkoway H. et al. Pesticides and risk of parkinson disease: a population-based case-control study. *Arch Neurol.* (2005) 62:91–5. doi: 10.1001/archneur.62.1.91
64. Kasten M, Chade A, Tanner CM. Epidemiology of parkinson's disease. *Handb Clin Neurol.* (2007) 83:129–51. doi: 10.1016/S0072-9752(07)83006-5
65. Ritz BR, Paul KC, Bronstein JM. Of pesticides and men: a california story of genes and environment in parkinson's disease. *Curr Environ Health Rep.* (2016) 3:40–52. doi: 10.1007/s40572-016-0083-2
66. Powers KM, Smith-Weller T, Franklin GM, Longstreth WT, Jr., Swanson PD, Checkoway H. et al. Parkinson's disease risks associated with dietary iron, manganese, and other nutrient intakes. *Neurology.* (2003) 60:1761–6. doi: 10.1212/01.WNL.0000068021.13945.7F
67. Kaur D, Andersen J. Does cellular iron dysregulation play a causative role in parkinson's disease? *Ageing Res Rev.* (2004) 3:327–43. doi: 10.1016/j.arr.2004.01.003
68. Ngim CH, Devathasan G. Epidemiologic study on the association between body burden mercury level and idiopathic parkinson's disease. *Neuroepidemiology.* (1989) 8:128–41. doi: 10.1159/000110175
69. Grandinetti A, Morens DM, Reed D, Maceachern D. Prospective study of cigarette smoking and the risk of developing idiopathic parkinson's disease. *Am J Epidemiol.* (1994) 139:1129–38. doi: 10.1093/oxfordjournals.aje.a116960
70. Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of parkinson's disease. *Ann Neurol.* (2002) 52:276–84. doi: 10.1002/ana.10277
71. Ascherio A, Chen H. Caffeinated clues from epidemiology of parkinson's disease. *Neurology.* (2003) 61:S51–4. doi: 10.1212/01.WNL.0000095213.86899.21
72. Liu R, Guo X, Park Y, Huang X, Sinha R, Freedman ND, et al. Caffeine intake, smoking, and risk of parkinson disease in men and women. *Am J Epidemiol.* (2012) 175:1200–7. doi: 10.1093/aje/kwr451
73. Palacios N, Gao X, Mccullough ML, Schwarzschild MA, Shah R, Gapstur S, et al. Caffeine and risk of parkinson's disease in a large cohort of men and women. *Mov Disord.* (2012) 27:1276–82. doi: 10.1002/mds.25076
74. Quik M. Smoking, nicotine and parkinson's disease. *Trends Neurosci.* (2004) 27:561–8. doi: 10.1016/j.tins.2004.06.008
75. Costa J, Lunet N, Santos C, Santos J, Vaz-Carneiro A. Caffeine exposure and the risk of parkinson's disease: a systematic review and meta-analysis of observational studies. *J Alzheimers Dis.* (2010) 1:S221–38. doi: 10.3233/JAD-2010-091525
76. Fang X, Han D, Cheng Q, Zhang P, Zhao C, Min J, et al. Association of levels of physical activity with risk of parkinson disease: a Systematic review and meta-analysis. *JAMA Netw Open.* (2018) 1:e182421. doi: 10.1001/jamanetworkopen.2018.2421
77. Shulman LM, Katzel LI, Ivey FM, Sorkin JD, Favors K, Anderson KE, et al. Randomized clinical trial of 3 types of physical exercise for patients with parkinson disease. *JAMA Neurol.* (2013) 70:183–90. doi: 10.1001/jamaneurol.2013.646
78. Amara AW, Memon AA. Effects of exercise on non-motor symptoms in parkinson's disease. *Clin Ther.* (2018) 40:8–15. doi: 10.1016/j.clinthera.2017.11.004
79. Hobson P, Meara J. Risk and incidence of dementia in a cohort of older subjects with parkinson's disease in the united kingdom. *Mov Disord.* (2004) 19:1043–9. doi: 10.1002/mds.20216
80. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P. Prevalence and characteristics of dementia in parkinson disease: an 8-year prospective study. *Arch Neurol.* (2003) 60:387–92. doi: 10.1001/archneur.60.3.387
81. Reid WG, Hely MA, Morris JG, Broe GA, Adena M, Sullivan DJ, et al. A longitudinal of parkinson's disease: clinical and neuropsychological correlates of dementia. *J Clin Neurosci.* (1996) 3:327–33. doi: 10.1016/S0967-5868(96)90028-4
82. Stern Y, Marder K, Tang MX, Mayeux R. Antecedent clinical features associated with dementia in parkinson's disease. *Neurology.* (1993) 43:1690–2. doi: 10.1212/WNL.43.9.1690
83. Santos C, Costa J, Santos J, Vaz-Carneiro A, Lunet N. Caffeine intake and dementia: systematic review and meta-analysis. *J Alzheimers Dis.* (2010) 1:S187–204. doi: 10.3233/JAD-2010-091387
84. Liu QP, Wu YF, Cheng HY, Xia T, Ding H, Wang H, et al. Habitual coffee consumption and risk of cognitive decline/dementia: a systematic review and meta-analysis of prospective cohort studies. *Nutrition.* (2016) 32:628–36. doi: 10.1016/j.nut.2015.11.015
85. Wu L, Sun D, He Y. Coffee intake and the incident risk of cognitive disorders: a dose-response meta-analysis of nine prospective cohort studies. *Clin Nutr.* (2017) 36:730–6. doi: 10.1016/j.clnu.2016.05.015
86. Araujo LF, Mirza SS, Bos D, Niessen WJ, Barreto SM, Van Der Lugt A, et al. Association of coffee consumption with mRI markers and cognitive function: a Population-Based study. *J Alzheimers Dis.* (2016) 53:451–61. doi: 10.3233/JAD-160116
87. Araujo LF, Giatti L, Reis RC, Goulart AC, Schmidt MI, Duncan BB, et al. Inconsistency of association between coffee consumption and cognitive function in adults and elderly in a cross-Sectional study (ELSA-Brasil). *Nutrients.* (2015) 7:9590–601. doi: 10.3390/nu7115487
88. Zhou A, Taylor AE, Karhunen V, Zhan Y, Rovio SP, Lahti J, et al. Habitual coffee consumption and cognitive function: a mendelian randomization meta-analysis in up to 415,530 participants. *Sci Rep.* (2018) 8:7526. doi: 10.1038/s41598-018-25919-2

89. Kwok MK, Leung GM, Schooling CM. Habitual coffee consumption and risk of type 2 diabetes, ischemic heart disease, depression and alzheimer's disease: a mendelian randomization study. *Sci Rep.* (2016) 6:36500. doi: 10.1038/srep36500
90. Mencacci NE, Isaias IU, Reich MM, Ganos C, Plagnol V, Polke JM, et al. Parkinson's disease in gTP cyclohydrolase 1 mutation carriers. *Brain.* (2014) 137:2480–92. doi: 10.1093/brain/awu179
91. Guella I, Sherman HE, Appel-Cresswell S, Rajput A, Rajput AH, Farrer MJ. Parkinsonism in gTP cyclohydrolase 1 mutation carriers. *Brain.* (2015) 138:e349. doi: 10.1093/brain/awu341
92. Rudakou U, Ouled Amar Bencheikh B, Ruskey JA, Krohn L, Laurent SB, Spiegelman D, et al. Common and rare gCH1 variants are associated with parkinson's disease. *Neurobiol Aging.* (2019) 73 231–236. doi: 10.1016/j.neurobiolaging.2018.09.008
93. Bosgraaf L, Van Haastert PJ. Roc, a ras/GTPase domain in complex proteins. *Biochim Biophys Acta.* (2003) 1643:5–10. doi: 10.1016/j.bbamcr.2003.08.008
94. Tsika E, Moore DJ. Contribution of gTPase activity to lRRK2-associated parkinson disease. *Small GTPases.* (2013) 4:164–70. doi: 10.4161/sgtp.25130
95. Lesage S, Ibanez P, Lohmann E, Pollak P, Tison F, Tazir M, et al. G2019S lRRK2 mutation in french and north african families with parkinson's disease. *Ann Neurol.* (2005) 58:784–7. doi: 10.1002/ana.20636
96. Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, et al. LRRK2 g2019S as a cause of Parkinson's disease in Ashkenazi jews. *N Engl J Med.* (2006) 354:424–5. doi: 10.1056/NEJMc055509
97. Infante J, Rodriguez E, Combarros O, Mateo I, Fontalba A, Pascual J, et al. LRRK2 g2019S is a common mutation in spanish patients with late-onset parkinson's disease. *Neurosci Lett.* (2006) 395:224–6. doi: 10.1016/j.neulet.2005.10.083
98. Gonzalez-Fernandez MC, Lezcano E, Ross OA, Gomez-Esteban JC, Gomez-Busto F, Velasco F, et al. Lrrk2-associated parkinsonism is a major cause of disease in northern spain. *Parkinsonism Relat Disord.* (2007) 13:509–15. doi: 10.1016/j.parkreldis.2007.04.003
99. Gorostidi A, Ruiz-Martinez J, Lopez De Munain A, Alzualde A, Marti Masso JF. LRRK2 g2019S and r1441G mutations associated with parkinson's disease are common in the basque country, but relative prevalence is determined by ethnicity. *Neurogenetics.* (2009) 10:157–9. doi: 10.1007/s10048-008-0162-0
100. Tan EK, Shen H, Tan LC, Farrer M, Yew K, Chua E, et al. The g2019S lRRK2 mutation is uncommon in an asian cohort of parkinson's disease patients. *Neurosci Lett.* (2005) 384:327–9. doi: 10.1016/j.neulet.2005.04.103
101. Cho JW, Kim SY, Park SS, Kim HJ, Ahn TB, Kim JM, et al. The g2019S lRRK2 mutation is rare in korean patients with parkinson's disease. *Can J Neurol Sci.* (2007) 34:53–5. doi: 10.1017/S0317167100005783
102. Deniston CK, Salogiannis J, Mathea S, Snead DM, Lahiri I, Matyszewski M, et al. Structure of lRRK2 in parkinson's disease and model for microtubule interaction. *Nature.* (2020) 588:344–9. doi: 10.1038/s41586-020-2673-2
103. Watanabe R, Buschauer R, Bohning J, Audagnotto M, Lasker K, Lu TW, et al. The *in situ* structure of parkinson's disease-Linked lRRK2. *Cell.* (2020) 182:1508–18. doi: 10.1016/j.bpj.2019.11.2690
104. Wang S, Ray N, Rojas W, Parra MV, Bedoya G, Gallo C, et al. Geographic patterns of genome admixture in latin american mestizos. *PLoS Genet.* (2008) 4:e1000037. doi: 10.1371/journal.pgen.1000037
105. Torrealba-Acosta G, Yu E, Lobo-Prada T, Ruiz-Martínez J, Gorostidi-Pagola A, Gan-Or Z, et al. Clinical and genetic analysis of costa rican patients with Parkinson's disease. *medRxiv [preprint].* (2020). doi: 10.1101/2020.09.29.20202432

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Genotype-Phenotype Correlations in Monogenic Parkinson Disease: A Review on Clinical and Molecular Findings

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Parkinson disease (PD) is a complex neurodegenerative disorder, usually with multifactorial etiology. It is characterized by prominent movement disorders and non-motor symptoms. Movement disorders commonly include bradykinesia, rigidity, and resting tremor. Non-motor symptoms can include behavior disorders, sleep disturbances, hyposmia, cognitive impairment, and depression. A fraction of PD cases instead is due to Parkinsonian conditions with Mendelian inheritance. The study of the genetic causes of these phenotypes has shed light onto common pathogenetic mechanisms underlying Parkinsonian conditions. Monogenic Parkinsonisms can present autosomal dominant, autosomal recessive, or even X-linked inheritance patterns. Clinical presentations vary from forms indistinguishable from idiopathic PD to severe childhood-onset conditions with other neurological signs. We provided a comprehensive description of each condition, discussing current knowledge on genotype-phenotype correlations. Despite the broad clinical spectrum and the many genes involved, the phenotype appears to be related to the disrupted cell function and inheritance pattern, and several assumptions about genotype-phenotype correlations can be made. The interest in these assumptions is not merely speculative, in the light of novel promising targeted therapies currently under development.

Keywords: Parkinson's disease, phenotype, monogenic, early onset parkinsonism, Juvenile parkinsonism

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INTRODUCTION

Parkinson disease (PD) is a complex, progressive, neurodegenerative disorder with worldwide incidence of 5–35 in 100,000 cases per year and prevalence reaching 2–4% at the age of 85. PD prevalence is expected to double in the next two decades because of population aging. Mortality is higher in patients with a more than 10-year-long history of disease (1). Pathological hallmarks of the condition include Lewy bodies, Lewy neurites, and loss of dopaminergic neurons of the *substantia nigra* (SN) *pars compacta* (SNpc). However, PD neuropathology is pleomorphic, as Lewy bodies are absent in some monogenic forms of PD (2). Clinical manifestations include motor signs (resting tremor, rigidity, bradykinesia, and postural instability) and non-motor features such as hyposmia, constipation, mood disorders, and rapid eye movement sleep behavior disorder (RBD),

often preceding the motor signs. In later stages, cognitive decline and autonomic dysfunction may appear. The mean age of onset is in the sixth decade of life, ranging from <40 to more than 80 years. Early-onset PD (EOPD) is commonly defined as an age of onset under 45 years, while juvenile Parkinsonism (JOPD) refers to those cases with onset within 21 years (3).

A PD case is defined as familial or sporadic, according to the presence or absence of a clear family history. Approximately 5–10% can be classified as familial (4).

Most PD cases have a multifactorial etiology, resulting from the combined effects of environmental and genetic factors, while about 5–10% are caused by pathogenic variants in single genes. Monogenic forms of PD are more frequent in EOPD patients, being more than 10% of cases with onset before 45 years and more than 40% in those with onset before 30 years (5). Familial and monogenic PD must not be regarded as synonymous because many familial cases do not have a Mendelian transmission model. To date, more than 20 genes whose mutations cause autosomal dominant (AD), autosomal recessive (AR), and X-linked Parkinsonisms are known, with related phenotypes ranging from idiopathic PD-like (iPD-like) to young-onset Parkinsonisms, either pure or complicated by atypical motor and non-motor features (6). This review will focus on monogenic conditions with Parkinsonian signs as the prominent feature. Parkinsonisms presenting as an iPD-like condition will be classified as "typical PD." Cases presenting complex phenotypes, featuring prominent additional neurological signs, such as dementia, spasticity, dystonia, and/or abnormal ocular movements, will be classified as "atypical PD." We will discuss the clinical presentation and genetic cause of each condition, with insights into underlying molecular pathology, to provide genotype–phenotype correlations. Genetic variants will be identified with the most widely used traditional literature nomenclature. The phenotypes will be presented and discussed individually, based on transmission patterns and clinical features. A comprehensive overview of the discussed conditions is provided in **Table 1**.

Autosomal Dominant PD

Autosomal dominant PD (AD PD) includes different forms of Parkinsonisms that share many peculiarities such as incomplete penetrance, a mean age of onset during the fifth decade or later, and a good response to dopaminergic treatment. However, additional neurological signs, when present, distinguish iPD-like conditions from atypical Parkinsonism. Pathogenic variants in *LRRK2* and *VPS35* are usually related to Parkinsonisms resembling typical PD, while alterations in *SNCA* are more frequently found in atypical forms. However, phenotypes may overlap.

Idiopathic PD-Like

LRRK2

Pathogenic variants in the *LRRK2* gene (leucine-rich repeat kinase 2, MIM*609007), also known as *Dardarin*, are the most commonly known causes of AD PD, accounting for 5% of familial and 1% of sporadic cases (PARK8, MIM#607060) (7). They were identified more than 15 years ago by two independent groups in two unrelated families, with late-onset Parkinsonism resembling iPD (8, 9). Many rare *LRRK2* variants have been detected over the years but only six of them (p.R1441G/C/H, p.G2019S, p.Y1699C, and p.I2020T) are considered disease causing (8–13). Three additional variants (p.A211V, p.K544E, and p.T1410M), recently demonstrated to cause neurotoxicity, are waiting for confirmation (14). Two coding substitutions, p.G2385R and p.R1628P, mostly identified in Asian populations, act instead as genetic risk factors, each conferring a 2-fold risk of developing PD (15, 16). The p.G2019S variant is by far the most common, being detected in 4% of familial and 1% of sporadic PD cases worldwide (17). Its frequency is even higher among Mediterranean populations and some ethnic groups, including Ashkenazi Jews and North Africans, in which it is found in 23 and 37% of patients with familial PD, respectively (18). Conversely, p.G2019S is extremely rare in East Asia (17). The other known pathogenic *LRRK2* variants are very rare, with the exception of p.R1441G and p.R1441C, which are founder mutations in Basque and south Italian ethnicities, respectively (19, 20). The penetrance of the p.G2019S variant is incomplete and age-dependent, peaking at 42.5–74% at the age of ∼80 years (17, 21), but varies among different ethnicities (22). Additional genetic variants, acting as single or cumulative risk factors, have been demonstrated to contribute to such variability (23, 24). Age-related penetrance has also been reported for the p.R1441G mutation, ranging from 13% at age 65 to 83% at age 80 (25). Less is known about the penetrance of the other pathogenic variants.

The *LRRK2*-related phenotype, closely resembling iPD, is characterized by a late-onset progressive Parkinsonism, with resting tremor as a common presenting feature, good response to levodopa therapy, and, usually, positive outcomes with deep brain stimulation (DBS) (17, 26). However, the mean age of onset is slightly lower in *LRRK2*-related cases than iPD, as patients with PD onset before 40 years are more common among the p.G2019S carriers (7), and differs among different populations, for example, being 10 years earlier among Tunisian carriers compared to Norwegian ones (22). Further features differentiating *LRRK2* p.G2019S-related Parkinsonism from iPD are the absence of gender differences (27), the slower progression for motor signs and cognitive impairment (28), and the more frequent occurrence of postural-instability-gait-difficulty (29). Typical iPD non-motor features, such as hyposmia, sleep, and cognitive and dysautonomic alterations, occur in *LRRK2* PD cases, but with less frequency (30). Dementia is also rarer in *LRRK2*-related Parkinsonism (17). When present, cognitive deterioration is usually milder and more slowly progressive than in iPD (31) and is characterized by better attention, executive function, and language (32). Pathogenic variants in *LRRK2* were found to be extremely rare in multiple system atrophy (MSA), progressive supranuclear palsy, and corticobasal degeneration (33–36).

Prodromal and premotor symptoms in *LRRK2* carriers are still poorly known. In pre-diagnostic PD phases, asymptomatic cases may present subtle motor alterations or isolated Parkinsonian signs, including decreased arm swing, gait asymmetry, and voice changes (37). Non-motor symptoms, such as constipation and urinary urgency, anxiety, daytime sleepiness or poorer

TABLE 1 | Genes causing Parkinsonian conditions and related phenotypes.

Gene	MIM	Function	Disease onset	Phenotype	Additional features	Neuropathology[#]
Autosomal dominant, confirmed						
LRRK2	609007	Lysosomal	Late/variable	Typical	–	±Lewy body; ±tau
VPS35	601501	Vesicular trafficking	Late	Typical	–	–
SNCA	163890	Unknown	Late/early*	Atypical/typical*	D; PS; PYR; MYO	Lewy body
GCH1	600225	Monoamine synthesis	Variable	Typical	–	Lewy body
ATXN2	601517	mRNA transport/regulation	Early	Typical	–	Lewy body
Autosomal dominant, to be confirmed						
HTRA2	610297	Mitochondrial	Late	Typical	–	Unknown
GIGYF2	607688	Possibly IGF-1 signaling	Late	Typical	–	Unknown
UCHL1	613643	Ubiquitin-proteasome	Late	Typical	–	Unknown
EIF4G1	614251	Protein synthesis	Late	Typical	–	Unknown
CHCHD2	616244	Mitochondrial	Variable	Typical	–	Lewy body, tau
DNAJC13	614334	Vesicular formation	Late	Typical	–	Lewy body, tau
TMEM230	617919	Vesicular trafficking	Late	Typical	–	Lewy body, tau
RIC3	610509	Acetylcholine receptor assembly/expression	Variable	Typical	–	Unknown
Risk factor for PD						
GBA	606463	Lysosomal	Late/variable	Atypical/typical	D	Lewy body
Autosomal recessive						
PRKN	602544	Mitochondrial	Early/juvenile	Typical	–	–
PINK1	608309	Mitochondrial	Early	Typical	–	± Lewy body
DJ-1	602533	Mitochondrial	Early	Typical/typical	–	± Lewy Body
VPS13C	608879	Mitochondrial/vesicular trafficking	Early	Atypical	D; PYR	Lewy body
PTRHD1	617342	Ubiquitin-proteasome	Early	Atypical	ID; PYR; PSY	Unknown
PODXL	602632	Neurite outgrowth	Juvenile	Typical	–	Unknown
DNAJC6	608375	Synaptic endocytosis	Juvenile	Atypical	D; PSY; SZ; PYR	Unknown
SYNJ1	604297	Synaptic endocytosis	Juvenile	Atypical	D; SZ; DYS; OM	Unknown
ATP13A2	610513	Lysosomal	Juvenile	Atypical	D; PYR; SVGP; mini-MYO	Lipofuscin deposits
PLA2G6	603604	Membrane homeostasis/mitochondrial	Juvenile	Atypical	D; PYR; ATX; PSY; OM	Lewy body
FBX7	605648	Ubiquitin-proteasome/mitochondrial	Juvenile	Atypical	PYR; PSY	Unknown
X-linked						
RAB39B	300774	Vesicular trafficking	Early in males	Atypical in males	ID; MC	Lewy body

*D, dementia; PSY, psychiatric disorders; PYR, pyramidal signs; MYO, myoclonus; ID, intellectual disability; SZ, seizures; OM, ocular motion disorders; DYS, dystonia; SVGP, supranuclear vertical gaze palsy; ATX, ataxia. * = in peculiar gene alterations; # = beyond loss of dopamine neurons.*

performances in executive functioning, and subtle gait changes, are more frequent in asymptomatic *LRRK2* variant carriers than in heathy controls (7).

Brain imaging alterations have been found in *LRRK2* mutation carriers. Increased gray matter volume of different anatomical structures associated with motor loops has been documented in symptomatic and asymptomatic *LRRK2* carriers compared to controls. In contrast, a decreased basal ganglia gray matter volume has been found in *LRRK2* PD cases (38). Inversion recovery MRI sequences, assessing brain iron content, showed excessive iron deposition in the SN of brains from *LRRK2* carriers (39). Abnormal DAT-SPECT scans have been found in all *LRRK2* patients manifesting PD, as well as in some carriers showing prodromal signs and in a subgroup of non-manifesting carriers (40). Moreover, PET studies showed increased dopaminergic and cholinergic activity in *LRRK2* non-manifesting carriers compared to sporadic PD cases, possibly reflecting compensatory changes preceding the motor onset of PD (41, 42).

The neuropathology of *LRRK2*-related disease, mainly investigated in p.G201S carriers, is characterized by neuronal loss in the SNpc and *locus coeruleus* in all cases, with or without protein aggregation. Typical Lewy-type pathology and alpha-synuclein (α-syn) aggregates are present in 65–80% of *LRRK2* manifesting carriers, at a lower frequency than iPD cases (43). Tau inclusions, with variable distribution and severity, are also common, being found in about half the brains from patients with *LRRK2*- PD (43). a-syn aggregates prevail in p.G2019S carriers, while pure nigral degeneration has been described in about half

of p.I2020T patients. Nevertheless, neuropathology can differ among relatives carrying the same pathogenic variant (44). Neuropathological and clinical features are strictly correlated, as Lewy-type pathology has been associated with the occurrence of non-motor symptoms, while pure neurodegeneration has been found in brains from patients with PD with only motor signs (43, 44).

More recently, genome-wide association studies (GWAS) confirmed the linkage of *LRRK2* locus variants to sporadic PD (45–47). Contrary to rare pathogenic mutations, these common variants confer modest risk for PD, suggesting a possible role of *LRRK2* in influencing iPD susceptibility (37).

LRRK2 is a large and multifunctional protein with multiple domains for various protein–protein interactions and enzymatic serine–threonine kinase and GTPase activities. All the clearly pathogenic mutations cause a toxic gain-of-function. The increase in the *LRRK2* kinase activity mainly compromises neuronal vesicular trafficking, through an aberrant excessive phosphorylation of Rab GTPases (48). These findings have suggested that *LRRK2* kinase inhibitors might be a therapeutic target in *LRRK2*-related PD not only for monogenic *LRRK2*-related Parkinsonisms but also for the more common iPD (49).

VPS35

Late-onset PD with autosomal dominant inheritance (PARK17, MIM#614203) is also the phenotypic picture related to pathogenic variants in the *VPS35* gene (vacuolar protein sorting 35, MIM*601501). The phenotype is quite similar to iPD, mostly homogeneous and with a benign course. It may differ for an earlier age of onset (mean age at onset 50 years) (50). No atypical signs have been described in the *VPS35* p.D620N variant carriers since its discovery in two unrelated families with Swiss and Austrian origin, respectively (51, 52). Scarce cognitive and neuropsychiatric features, hyposmia in about 50% of patients, and excellent response to levodopa are reported (52, 53). Among these kindreds, the penetrance was high but incomplete (53). Beyond the p.D620N, identified in a few other familial or sporadic PD cases worldwide, no other proved pathogenic variants have been reported (54). The only patient who underwent neuropathology after death did not show signs of α-syn (55).

The *VPS35* protein is part of the retromer complex, involved in the neuronal vesicular recycling from endosomes to the trans-Golgi network. Altered *VPS35* is supposed to compromise the intracellular localization and stability of these organelles (56).

Atypical AD PD

SNCA

Pathogenic variants in *SNCA* (alpha-synuclein, MIM*163890), encoding α-syn, were identified 20 years ago as the first genetic cause of AD PD (PARK1, MIM#168601; PARK4, MIM#605543). In the large "Contursi kindred" the *SNCA* p.A53T mutation segregated with a PD and dementia with Lewy bodies phenotype (DLB, MIM#127750) (57, 58). Many other point mutations and whole gene multiplications have been detected in hundreds of patients with hereditary forms of autosomal dominant forms of PD, DLB, and other neurodegenerative conditions thus far (14). The identification of α-syn as a major component of Lewy bodies (LBs) and Lewy neurites (LNs), the neuropathological pathognomonic hallmarks of PD and DLB, confirmed the role of *SNCA* in the pathogenesis of PD. The detection of Lewy-type pathology in sporadic PD supported the involvement of α-syn in iPD, too (44, 59). Damaging mutations and whole gene multiplications favor α-syn aggregation with potential deleterious consequences at both the synaptic level and lysosomal/endosomal compartments, by a gain-of-function mechanism (60).

Duplication and triplications of an otherwise normal *SNCA* gene have been described in more than 100 patients (61). *SNCA* missense mutations (p.A53T, p.A30P, p.E46K, p.H50Q, and p.G51D) are instead very rare worldwide.

Many different PD phenotypes have been related to *SNCA* mutations, ranging from the more common late-onset Parkinsonism, either with or without non-motor symptoms, to the rarer early-onset aggressive diseases with atypical signs.

In whole-gene multiplications, the number of *SNCA* copies clearly correlates with the disease severity, supporting the notion of a "dosage effect" (62). Indeed, patients with four copies of *SNCA* (heterozygous triplication or homozygous duplication carriers) have a 10-year earlier age of onset, a more rapid progression and a more severe phenotype, often complicated by myoclonus, severe cognitive impairment, and psychiatric features, compared to heterozygous duplication carriers (61). Marked weight loss, dysautonomia, and fatigue can precede motor symptoms onset with death occurring within 7 years. Brain imaging reveals frontoparietal atrophy and a severe striatal dopaminergic deficit (63, 64). *SNCA* duplications cause a more variable phenotype, even within the same family, ranging from asymptomatic carriers to iPD-like or, more rarely, to severe forms resembling *SNCA* triplication carrier phenotypes (61, 65). The mean age of onset for *SNCA* duplication-related PD is 50 years. Non-motor symptoms are inconstantly present, and death occurs after 15 years from onset (66, 67). Atypical phenotypes have been described, including fronto-temporal dementia (FTD) with marked anxiety and obsessive–compulsive disorder, and a singular head shaking movement disorder (68, 69).

Compared to copy-gain variants, missense mutations cause more complex phenotypes with mutation-specific trends in clinical presentations (70). In most cases, despite similar ages of onset (average age 47.6 + 12.9 years), motor and non-motor features differ among patients with different specific mutations.

The p.A53T PD is characterized by marked intra-familial and inter-familial variability (67, 71). Penetrance is incomplete but high (80–90%). Parkinsonism resembles iPD, with a more aggressive and rapid course. Tremor is not common. Motor signs are initially L-dopa responsive but worsen early because of the occurrence of motor complications. Non-motor features, including hyposmia, orthostatic hypotension, RBD, and depression are inconstantly present. Myoclonic jerks and central hypoventilation have been reported. Cognitive impairment may vary, but dementia usually occurs within 5–7 years from disease onset (67, 72, 73). Parkinson dementia disease (PDD) and DLB have been described (74). More rarely, prominent language dysfunction resembling primary progressive aphasia

and frontotemporal dementia with behavioral dysregulation and speech-related problems have been reported (75). Olfactory dysfunction, RBD, and dopaminergic deficit at DATSCAN have been proposed as possible premotor signs after their identification in otherwise asymptomatic p.A53T carriers (71, 72).

Conversely, a late-onset Parkinsonism with tremor as a rather constant motor sign and cognitive impairment ranging from mild cognitive decline to frank dementia have been reported in p.H50Q carriers, all with English ancestry (76, 77).

Patients with the p.A30P mutation, found in a single German family, present a condition similar to iPD, with onset around 60 years, incomplete penetrance, and a benign course of disease. Cognitive impairment and hallucination occur, although inconsistently (78, 79).

More severe phenotypes are related to the p. E46K and p.G51D variants. The first one, identified in a Basque family, causes a high penetrant and severe Parkinsonism, presenting at 50–65 years (80). Dementia with LB phenotype and autonomic dysregulation occur a few years after the onset of motor signs. However, disease severity may vary among families. Marked cardiac denervation has been found in patients and in p.E46K asymptomatic carriers (81).

Conversely, the Parkinsonian condition related to p.G51D, found in a few European and Asian cases, strongly differs from the late-onset PD caused by the other *SNCA* damaging missense mutations. The age of onset is very early, before 20 years in one case, and pyramidal signs, myoclonus and seizures coexist, inconstantly complicated by psychiatric symptoms, dementia, and autonomic dysfunction (82, 83).

Another early onset *SNCA*-related Parkinsonism, with no atypical signs, has been described in a Finnish family with the p.A53E *SNCA* mutation (84).

A proper definition of phenotypes related to *SNCA* pathogenic missense variants is limited by the extreme rarity of such mutations and by the lack of thorough clinical evaluations for each individual case. A comprehensive international database considering complete and standardized information from individual patients would help to overcome these limits.

Pathogenic *SNCA* variants are localized in exons 2 and 3, which encode for an α-helical domain with lipid binding activity and for a hydrophobic domain (85). These mutations probably prolong α-syn half-life by interfering with lysosomal degradation (86). The tendency of some of these variants to accelerate α-syn aggregation and to recruit tau proteins into inclusions has been demonstrated with *in vitro* and *in vivo* studies. Further investigation will clarify if phenotype differences for distinct missense mutations depend on toxic gain-of-function mechanisms or another prolonged mutated protein half-life (87).

Soon after the discovery of the role of *SNCA* in AD PD, common variants at this locus were investigated for association with sporadic PD. A positive association emerged between iPD and expanded alleles at the *NACP*-Rep1 repeat, located 10 kb upstream of the transcriptional start site of *SNCA* (88, 89). Many additional GWAS analyses since then have supported a statistically significant association between the risk for PD and several single-nucleotide polymorphisms (SNP) located both at the 5′ end and the 3′ end of the *SNCA* gene (46). Although the effect of each SNP is individually low (odd ratio 1.3), the cumulative risk can be substantial. The *NACP*-Rep1 alleles and the rs356168 SNP increase α-syn expression both *in vitro* and *in vivo*, supporting the hypothesis that common *SNCA* variants increasing α-syn expression also increase the risk for apparently sporadic PD (87).

AD Genes Awaiting Confirmation

In addition to these AD PD genes, many others have been proposed as monogenic causes of hereditary iPD-like conditions. However, their pathogenicity is still debated or waiting for confirmation. *GIGYF2* (GRB10-interacting GYF protein 2, MIM*612003; PARK11, MIM#607688), *HTRA2* (HTRA serine peptidase 2, MIM*610297; PARK13, MIM#606441), *UCHL1* (ubiquitin carboxyl-terminal esterase L1, MIM*191342; PARK15, MIM#613643), and *EIF4G1* (eukaryotic translation initiation factor 4-G, MIM*614251; PARK18, MIM#614251) variants were detected in families with late-onset Parkinsonism resembling iPD and segregating with autosomal dominant fashion. However, many studies denied their pathogenic role in PD and none of them is still considered as a PD gene (90, 91). With the advent of NGS and the increasing availability of whole exome/genome sequencing (WES/WGS) many genes such as *DNAJC13*, *CHCHD2*, *TMEM230*, *LRP10,* and *RIC3* have been identified in AD PD families. Variants in *CHCHD2* (coiled-coil-helix domain containing protein 2, MIM*616244), a gene involved in the mitochondrial respiratory function, were identified in a few families with AD PD (PARK22, MIM#616710). Patients presented variable disease onset (mean age 52 years), good response to levodopa, depression, and the absence of cognitive impairment. A brain autopsy of a *CHCHD2* PD patient revealed widespread LB pathology with amyloid plaques and neurofibrillary tangles in the brainstem, limbic regions, and cortex (92–94). Despite these results, large-scale studies did not support the causative role of *CHCHD2* in PD [reviewed in (95)]. A definitive confirmation of its pathogenicity is still lacking.

Evidence is less robust for the remaining four genes. The c.2564A>G mutation in *DNAJC13* (DNAJ/HSP40 homolog subfamily C, member 13, MIM*614334) gene was identified in patients with late onset (mean age 63 years) and slow progressive PD from a large Mennonite kindred. Brain pathology of three mutation carriers showed LB with cell loss in Meynert nucleus and SN, as well as tau pathology. However, no other PD cases with pathogenic variants in *DNAJC13* have been detected to date and its role in PD etiology has been recently reconsidered (91). An independent study in the same Mennonite kindred identified the c.422G>T variant in *TMEM230* (Transmembrane protein 230, MIM*617019) as the cause of the Parkinsonism segregating in that family. The same variant was also detected in a few sporadic PD cases (96). As no other rare pathogenic mutation in *TMEM230* has been detected in familial and sporadic PD patients, pathogenicity for this gene still needs confirmation (97).

Recently, the c.169C>A mutation in *RIC3* (resistance to inhibitors of cholinesterase 3, MIM*610509) was found to segregate with a variable onset (30–68 years) Parkinsonism in a large family from South India. Nine mutated patients

across three consecutive generations presented typical PD with RBD, depression, and restless leg syndrome (98). *RIC3*, not detected in other PD cases, is also waiting for confirmation (99). Heterozygous mutations in *LRP10* (low-density lipoprotein receptor-related protein 10, MIM*609921) were detected in Italian kindred with late-onset PD and in other unrelated patients with Parkinsonism and dementia PDD and DLB (100). However, inconsistent findings emerged by replications studies worldwide, with no differences in *LRP10* variant frequency between patients and controls, and for the incongruity of segregation analysis (101). Other population and functional studies are required to elucidate the role of this gene in PD.

Other Disease-Causing Genes Associated With PD

Other Movement Disorder Genes Possibly Manifesting as AD PD

An iPD phenotype can be related to genes known to cause other movement disorders. In particular, pathogenic variants in *GCH1* (GTP cyclohydrolase I, MIM*600225), the most common cause of levodopa-responsive dystonia (MIM#128230), have been found in patients and families with AD PD characterized by variable disease onset (mean age 43 years), long-term motor complications, and non-motor signs such as cognitive impairment, sleep disorders, hyposmia, and autonomic dysfunction, without dystonia. Nigrostriatal degeneration in affected patients was also proved by a reduced tracer uptake in SPECT studies (102). The association between PD phenotype and *GCH1* pathogenic variants was confirmed by further studies (103, 104). Also, triplet expansion variants in *ATXN2* (MIM*601517), responsible for autosomal dominant cerebellar ataxia type 2 (SCA2, MIM#183090), may cause a form of typical PD with onset after 40 years, good response to levodopa therapy without cognitive impairment, and cerebellar signs. LB pathology and neuronal loss have been documented, in the absence of cerebellar atrophy. A CAG repeat expansion exceeding 33 is considered pathogenic. However, while in SCA2 phenotypes the mean of repeats is 43; in PD cases, repeats are lower in number (mean 36 ± 1) and with CAA interruptions (105, 106).

GBA

Heterozygous variants in the *GBA* (acid beta glucosidase, MIM*606463) gene are the most common genetic risk factor for PD (MIM#168600) worldwide. *GBA* encodes the β-glucocerebrosidase (GCase), a lysosomal enzyme that cleaves the glucosylceramide sphingolipid into glucose and ceramide. Biallelic mutations in *GBA* cause Gaucher's disease (GD), the most common AR lysosomal storage disease with a variable involvement of the central nervous system. About 300 different pathogenic *GBA* variants have been found, many of them resulting in a significant loss of GCase activity (107). Different mutations can lead to different phenotypes of GD. Variants are overall classified according to the GD subtype they are related to. Mutations causing the non-neuronopathic GD type 1 are defined as mild (e.g., the c.1226A>G, also known as N370S in the traditional nomenclature); those causing neuronopathic GD type 2 and 3 are classified as severe (e.g., c.1448T > C, L444P) (108).

The association between *GBA* variants and increased risk of Parkinsonism arose after clinical observations of higher incidence of PD among GD type 1 patients and their heterozygous parents. This suggestion was confirmed by a large, worldwide, multicenter association study, and by many other papers which demonstrated higher frequencies of the GBA variants in PD patients compared to healthy controls (109). The N370S and the L444P mutations are the two most common alleles worldwide, accounting for 70–80% of *GBA* variants associated with PD in some populations. In particular, the N370S is the most frequent among Ashkenazi Jews from eastern Europe whereas L444P is more common among non-Jew European descendants (110). Nevertheless, *GBA* mutations represent only a risk factor for PD. Population studies showed that a little more than 9% of *GBA* mutation carriers develop PD (111). Heterozygotes for a *GBA* mutation have a 5-fold increased risk of developing PD compared to the age-matched general population. For homozygotes, the risk increase is 10- to 20-fold (108). Pathogenic variants in *GBA* are detected in 8.5% of PD patients (112). However, carrier frequency varies across different ethnic groups, ranging from 10 to 31% in Ashkenazi Jews, 2.9–12% in non-Jewish Europeans, and 2.3% in Norwegians (113). PD cases with *GBA* mutations are usually similar to iPD. However, some peculiarities are emerging by large population studies comparing carrier and non-carrier PD phenotypes and clinical features related to mild and severe mutations. In heterozygous *GBA* PD, the first symptoms manifest 3–6 years earlier than in iPD, a rigid akinetic motor phenotype is more common, and the response to levodopa is good. However, at least in a subset of patients, the progression of the disease can be faster and therapeutic outcomes are often limited by earlier development of motor fluctuations and dyskinesias, as well as cognitive decline after DBS (114, 115). *GBA* mutation carriers are also more likely to manifest non-motor symptoms. Cognitive involvement is more frequent, and the risk of developing dementia is at least 3-fold higher than in iPD (116, 117). Non-motor symptoms, such as hallucinations, depression and anxiety, impulse control disorders, RBD, and autonomic dysfunctions, are also more common among *GBA* patients, and more frequent in severe mutation carriers. Similarly, motor complications, including dysphagia, dysarthria, and freezing of gait, are more frequent in *GBA* carriers (118).

Compared to milder variants, severe *GBA* mutations are associated with a higher risk of developing PD, younger onset, worse motor progression, more frequent cognitive involvement, and more complex non-motor phenotype (27, 119).

GBA PD patients have a distributed pattern of white matter abnormalities involving the interhemispheric, frontal cortico-cortical, and parahippocampal tracts, and no gray matter atrophy at structural MRI (120). Spectroscopic MRI shows a neurodegeneration pattern more pronounced in the putamen than in the midbrain (121). *GBA* mutation carriers also differ from iPD cases in PET studies, showing reduced cerebral blood flow in the parieto-occipital cortex and reduced nigrostriatal function. This resembles the

pattern typically seen in DLB, especially in severe mutation carriers (119).

Mutations in *GBA* are also a significant risk factor for DLB, conferring a more than 8-fold increased risk of developing this condition compared to controls (122). The association between *GBA* pathogenic variants and other Parkinsonian conditions is instead less consistent. Correlations with PSP are still weak (123, 124), but recent studies demonstrated an association between *GBA* variants and MSA (125, 126).

The exact mechanism by which *GBA* mutations lead to PD is still unclear. It is well-known that GCase is part of the endo-lysosomal pathway, particularly crucial in many pathogenetic pathways leading to PD. Mutated GCase is not able to fold properly and accumulates in different cellular compartments of the dopaminergic neurons, causing a cell stress response, damage, and neuronal death. In addition, the entrapment of the beta GCase in the endoplasmic reticulum reduces enzyme levels in the cell, triggering α-syn accumulation (127). Intriguingly, LB pathology has been found in cortical areas of brains from 10 PD patients with Parkinsonism and from almost all *GBA*-related PD cases who underwent autopsy. Less is known about the distribution of neuronal loss or additional neuropathology (128).

At present, clinical trials assessing the safety and the efficacy of molecules aiding proper GCase folding, improving enzymatic activity, or reducing the GCase substrates accumulation are ongoing (108).

Autosomal Recessive Early-Onset Parkinsonisms

Among AR parkinsonisms, forms caused by biallelic pathogenic variants in the *PRKN*, *PINK1*, and *DJ-1* genes are thus far considered pure forms of EOPD. Together, they account for ∼13% of EOPD cases (73). *VPS13C* and *PTRHD1* are mutated in families with EOPD complicated by pyramidal signs and cognitive involvement. Other genes such as *APT13A2*, *PLA2G6*, *FBXO7*, and the more recently identified *DNAJC6*, *SYNJ1*, and *VPSC13* are usually related to younger onset Parkinsonism, complicated by atypical motor and non-motor phenotypes. Biallelic mutations in another gene, *PODXL,* recently have been detected in siblings with a juvenile form of pure PD. However, this gene is still waiting for confirmation.

Pure EOPD Forms

The *PRKN*, *PINK1*, and *DJ1* genes share similar PD phenotypes and the same cellular pathway. They are involved in mitochondrial homeostasis and mitophagy. Variants in these genes can impair mitochondrial function, leading to cellular stress and neurotoxicity. *PRKN* encodes the E3-ubiquitin ligase, involved in the proteasome pathway for damaged proteins degradation and in mitochondrial homeostasis. *PINK1* encodes a mitochondrial kinase involved in mitophagy, acting upstream of Parkin. *DJ-1* encodes for a protein involved in the antioxidant response that shares biochemical pathways with *PINK1* and Parkin (129–131).

PRKN

Biallelic pathogenic variants in *PRKN* (Parkin, MIM*602544), are the most common genetic cause of early-onset pure Parkinsonism (PARK2, MIM#602544) (132). First identified in a Japanese family with AR PD, *PRKN* variants currently account for 10–20% of PD with onset within 40 years (133). The occurrence of biallelic mutation is inversely related to the age of the disease onset: the younger the onset, the higher the probability to detect homozygous or compound heterozygous *PRKN* carriers. The onset is usually before 40 years, with a median age of 31 years for the first motor signs. Juvenile onset, with the first symptoms within 20 years, is described in 16% of PD patients with biallelic *PRKN* mutations (73). Cardinal signs of *PRKN*-related PD are a more common bradykinetic motor phenotype, benign and slow progression, and good response to levodopa therapy, although frequently complicated by iatrogenic dyskinesia, to anticholinergic medication, and to DBS (26, 73, 134). Hyperreflexia and/or dystonia may occur, and lower-limb dystonia can be a presenting sign. Cognitive decline is uncommon, being as frequent as in the general population, and dementia is extremely rare. Sense of smell is usually well-preserved and additional features, such as psychiatric manifestations and dysautonomia, are also rare (134, 135). Disease duration can reach 50 years. Later disease stages may be complicated by freezing of gait, postural deformities, and motor fluctuations (136).

Unlike iPD, women and men are equally affected and the loss of dopaminergic striatal innervation, revealed by 18F-DOPA PET/SPECT, is rather symmetric and slowly progressive (134).

Neuronal loss in pigmented nuclei of the brainstem is the prominent feature found in brains of *PRKN* PD patients. Neurodegeneration is more prominent in the SNpc than in the *locus coeruleus*. Typical LBs containing α-syn have been identified in very few affected individuals (136, 137). Pathogenic variants are highly diverse, including exonic deletions or multiplications and missense, nonsense, and frameshift variants, described in homozygous or compound heterozygous states. Exonic rearrangements are the most common anomalies. Functional studies demonstrated protein loss-of-function or absence of protein due to nonsense mRNA decay for most of them (138, 139).

PINK1

The phenotype related to biallelic pathogenic variants in *PINK1* (PTEN-induced putative kinase 1, MIM*608309; PARK6 MIM#605909) is similar to *PRKN* PD, with early onset, good response to levodopa, and rare cognitive compromission. However, hyperreflexia is less common and hyposmia, dysautonomic features, and psychiatric symptoms including anxiety, psychosis, and affective disorders may occur (73). Neuronal loss is prevalent in SN and, contrary to *PRKN*-related disease, LBs have been found on neuropathological examinations (140).

PINK1 biallelic mutations are the second most common cause of EOPD worldwide, accounting for 1–5% of cases, but with variations among ethnic groups, being more frequent in north

Africa (90). More than 60 variants, including deletions and missense and nonsense variations, have been reported (73).

DJ-1

Biallelic variants in *DJ-1* (oncogene Dj1, *MIM**602533) cause a rare Parkinsonism (PARK7, MIM#606324), the third most common AR PD after the *PRKN*- and *PINK1*-related forms, accounting for the 0.4 and 1% of EOPD cases, respectively (1). Patients usually share the same phenotype of both *PRKN* and *PINK1* cases, presenting early onset slowly progressive Parkinsonism (mean age 27 years), good response to dopaminergic therapy, frequent focal dystonia, motor complications upon treatment, and psychiatric symptoms, in particular anxiety that often presents as the first symptom (73, 132). Unlike *PRKN* and *PINK1*, additional features, including depression, cognitive decline, motor neuron disease, and bulbar signs are seldom described (141, 142). The only reported neuropathological brain examination showed loss of neurons in the substantia nigra and locus coeruleus with widespread α-syn, akin to sporadic PD brains (142).

Heterozygous Variants in PRKN, PINK1, and DJ-1

While biallelic pathogenic variants in *PRKN*, *PINK1*, and *DJ-1* are clearly causative for EOPD, the role of single heterozygous mutations in these genes is debated. *PRKN* and *PINK1* heterozygous mutations have been detected in a substantial number of PD patients but also in healthy controls, raising the question of whether they may contribute to the disease or are incidental findings. The results of many case-controlled studies suggest that they may represent minor susceptibility factors that mildly contribute to the risk of sporadic PD (Parkin odds ratio 2:53; *PINK1* odds ratio 1:65) (143, 144). Interestingly, multimodal neuroimaging and electrophysiologic studies disclosed a latent nigrostriatal impairment in otherwise healthy subjects carrying heterozygous Parkin or *PINK1* mutations (145–147). However, the role of heterozygous variants in these AR genes cannot be conclusively established as prospective studies of healthy heterozygous carriers are still lacking (144).

Atypical EOPD Forms

VPS13C

VPS13C (vacuolar protein sorting 13 homolog C, MIM* 608879) is the most recently identified gene causing a rare, atypical form of early-onset Parkinsonism (PARK 23, MIM#616840). To date, only three families have been described. All affected patients presented asymmetric akinetic-rigid Parkinsonism starting from age 25 to 45, with initially good response to dopaminergic treatment. The disease progression is severe and rapid with dramatic early cognitive involvement, dysautonomia, limb dystonia, hyperreflexia, and pyramidal signs leading to tetraplegia. The patients are bedridden after about 10 years of disease. MRI documented asymmetric brain atrophy. Neuropathology of a single case showed widespread and abundant α-syn positive LBs and neuritis, together with tau pathology with neurofibrillary tangles (148). *VPS13C* encodes the vacuolar protein sorting 13 protein involved in mitochondrial activity and vesicular trafficking. It has been shown that *VPS13C* mutations alter mitochondrial function and *PINK1*-Parkin-dependent mitophagy (149).

PTRHD1

Homozygous missense mutations and 28-nucleotid frameshift deletion in *PTRHD1* (peptidyl-tRNA hydrolase domain-containing 1, MIM*617342) have been recently identified in two unrelated consanguineous Iranian families and in a sub-Saharan African kindred with early onset Parkinsonism and intellectual disability (150, 151). Motor signs of Parkinsonism appeared, at 20–30 years of age. Phenotypes were variably complicated by muscle stiffness, postural tremor, pyramidal signs, sensory-motor polyneuropathy, behavioral disorders, and hypersomnia. No other *PTRHD1* cases have been identified to date (152). Chromosomal microdeletions encompassing the *PTRHD1* gene have been previously related to many syndromes with intellectual disability. *PDRHT1* encodes a protein involved in the ubiquitin proteasome pathway. Intriguingly, the pathogenic variants causing Parkinsonism are in the ubiquitin-like (UBL) domain-binding site of the protein. The suppression of the ubiquitin protein degradation is a well-known mechanism of PD. Despite these findings, further population and functional studies are needed to confirm the role of this rare gene in determining PD.

Juvenile Parkinsonism

Juvenile Parkinsonism includes those forms of PD with onset within 21 years, often combined with other hyperkinetic movement disorders and neurological and imaging abnormalities (153). With the exclusion of *PRKN* mutations, responsible for 77% of juvenile PD (see section Other Movement Disorder Genes Possibly Manifesting as AD PD) and the extremely rare *PODXL*-related cases, more than 90% of patients have a complex or atypical presentation, with dystonia, pyramidal signs, neuropsychiatric disorders, abnormal eye movements, and brain imaging. Many genes have been associated with AR young JOPD. This section will focus on those forms in which Parkinsonism is the predominant sign.

Pure JOPD

PODXL

A homozygous frameshift variant in the *PODXL* gene (podocalyxin-like, MIM*602632) has been described in three siblings from an Indian consanguineous family, who developed a pure levodopa-responsive Parkinsonism manifested at 13–17 years of age, later complicated by dyskinesia and off-dystonia, with no additional signs. *PODXL* encodes a glycoprotein involved in the regulation of neurite outgrowth. The frameshift mutation (c.89_90insGTCGCCCC) resulted in a complete loss of protein function (154). Replications of these findings and confirmation of *PODXL* as a causative PD gene are still awaited.

Atypical

JOPD

DNAJC6 Biallelic damaging variants in *DNAJC6* (DNAJ/HSP40 homolog, subfamily c, member 6, MIM* 608375) are associated with a form of juvenile Parkinsonism (PARK19, MIM#615528),

with a mean age of onset at 11 years (range 7–42 years). The wide phenotypic spectrum ranges from typical pallido-pyramidal syndrome to pure EOPD. Motor signs at onset vary from tremor to bradykinesia but the disease later manifests with Parkinsonism, postural instability, and usually good response to levodopa, often limited by treatment-induced dyskinesia, psychosis, and hallucinations (155, 156). In complex forms, Parkinsonism is often complicated by atypical features such as cognitive decline, spasticity/pyramidal signs, dysarthria/anarthria, seizures, and hallucinations. Disease progression is severe, especially in cases with younger onset, who need a wheelchair after 2–10 years of disease (155, 157, 158). Brain atrophy has been described in all but one case (158). Conversely, those cases with pure Parkinsonism show variable age of onset, slow disease progression, and good response to dopaminergic therapies (156). *DNAJC6* encodes for Auxilin, a protein involved in calthrin-mediated endocytosis. Mutations in *DNAJC6* cause an impairment in synaptic vesicle recycling, compromising endocytosis (155). A clear genotype-phenotype correlation has been defined: nonsense mutations have been related to juvenile complex Parkinsonism, while patients with missense mutations or variants resulting in reduced protein production have been found in pure Parkinsonism cases (156, 157).

SYNJ1

SYNJ1 (synaptojanin, MIM*604297) mutations cause another AR form of JOPD (PARK20, MIM#615530) characterized by motor PD features presenting at a median age of 21 years (range 12–31 years), poor response to levodopa treatment, early induced dyskinesias, gait disorders, and dysarthria/anarthria (159, 160). In most cases, dystonia, cognitive decline, seizures, and oculomotor abnormalities were described as well (33). Cerebral cortical atrophy, quadrigeminal plate thinning, and hippocampal T2-hyperintensity at MRI have been inconsistently reported (95). Recently, a good response to clonazepam therapy, especially for trunk dystonia, has been reported in two *SYNJ1* compound heterozygous siblings with diplopia, dystonia, and Parkinsonism (161). At present, 12 families with atypical Parkinsonism and biallelic *SYNJ1* variants have been described (33, 161). *SYNJ1* encodes for a poly-phosphoinositide phosphatase, which contains two consecutive phosphatase domains. Mutations affecting the SAC1-like domain are responsible for Parkinsonism while variants impairing the dual phosphatase domain cause a recessive infantile epilepsy syndrome with severe and progressive neurodegeneration (MIM# 617389) (162). No pathology from *SYNJ1* patients with Parkinsonism is available today. However, brain neuropathology on a child with biallelic *SYNJ1* pathogenic variants affected by refractory epilepsy and severe neurodegeneration showed white matter atrophy and prominent cell loss, tau pathology, and neurofibrillary tangles in SN and, although less represented, in basal ganglia (163).

ATP13A2

ATP13A2 (ATPase 13A2, MIM*610513) causes Kufor Rakeb syndrome (MIM#606693), an AR atypical JOPD with onset before 20 years (164, 165). The disease is characterized by a combination of partially levodopa-responsive Parkinsonism and pyramidal signs, complicated in most cases by dementia, hallucinations, dystonia, impaired saccadic movements, vertical gaze palsy, and mini-myoclonus. Symptoms at onset are variable, encompassing akineto-rigid syndrome, learning disability, cognitive deterioration during school years, or behavioral dysfunctions. Progression of the disease is slow, sometimes associated with cerebellar dysfunction (33). Brain MRI shows diffuse atrophy and, in many cases, iron accumulation in the putamen and caudate (165, 166). *ATP13A2* encodes for a lysosomal P5-type ATPase protein that transports inorganic cations and regulates endo-lysosomal cargo sorting and neuronal integrity. Homozygous or compound heterozygous variants in *ATP13A2* also have been associated with complex hereditary spastic paraplegia (SPG78, MIM# 617225), with no Parkinsonism in most reported cases and to neuronal ceroid lipofuscinosis. In brains from Kufor Rakeb patients, neuronal and glial lipofuscin deposits at the cortex, cerebellum, and basal ganglia have been described (167, 168).

PLA2G6

Biallelic mutations in *PLA2G6* (phospholipase A2, group 6, MIM*603604) cause an early-onset dystonia-Parkinsonism (PARK14, MIM#612953) with variable age at onset (10–30 years) (169, 170). The disease is also characterized by rapid cognitive decline, pyramidal signs, eye movement abnormalities, psychiatric and behavioral problems, cerebellar ataxia, and autonomic dysfunction. The response to levodopa therapy is good but compromised by early-onset treatment-induced dyskinesias. The progression of the disease is rapid and severe, leading to loss of autonomy (171). MRI may show brain iron accumulations and frontal lobe and general white matter atrophy (172). Beyond atypical Parkinsonism, biallelic pathogenic variants in *PLA2G6* are also the cause of other neurodegenerative diseases, including infantile neuroaxonal dystrophy (INAD, MIM#256600) and idiopathic neurodegeneration with brain iron accumulation, type 2 (NBIA2, MIM# 610217), all sharing many pathological and clinical features. They are characterized by spheroid axonal inclusions in the brain and motor regression, progressive cognitive decline, axial hypotonia, spasticity, bulbar and ophthalmic dysfunctions, dystonia, and cerebellar atrophy, starting in the first year of life (171). *PLA2G6* encodes for calcium-independent phospholipase A2 beta enzyme ($iPLA_2\beta$), which participates in cell membrane homeostasis, mitochondrial function, fatty acid oxidation, and calcium signaling. Defects of this protein lead to membrane fluidity alteration and neuronal function impairment. a-syn with LBs, Lewy neurites, and neuroaxonal dystrophy are documented in patients with PARK14 and in INAD cases. Differences in phenotypes are related to the effects of the mutations: variants causing loss of *PLA2G6* catalytic activity leads to INAD/NBIA2, whereas mutations altering substrate specificity or regulatory domains are responsible for PARK14 (173).

FBXO7

A form of juvenile pallido-pyramidal syndrome (PARK15, MIM#260300) is caused by biallelic mutations in *FBXO7* (F-box

only protein, MIM*605648). Parkinsonism is usually the first manifestation with rigidity, bradykinesia, postural instability, and, less frequently, tremor, around the age of 17 (range 10–52) years. Pyramidal signs are common and cognitive decline, vertical gaze palsy, and autonomic dysfunctions also occur, although less frequently. Response to levodopa is good but often limited by psychiatric and dyskinetic complications. No abnormalities have been detected in brain MRI (174, 175).

FBXO7 encodes an adaptor protein involved in substrate degradation and in mitochondrial maintenance interacting with *PINK1* and Parkin.

X-Linked PD

Pathogenic whole gene deletion, missense, and splicing mutations in *RAB39B* (RAS-associated protein RAB39B, MIM* 300774) were identified in a few families with non-progressive intellectual disability, macrocephaly, and early-onset Parkinsonism in males (Waisman syndrome, MIM# 311510). Tremor was the presenting motor sign, followed by a levodopa-responsive akinetic-rigid Parkinsonism. Seizures were inconsistently present. Brain atrophy of one affected patient documented a clear α-syn with LBs in the SN and in the cortex, together with tau positive neurofibrillary tangles in the SN and axonal spheroid in the white matter of basal ganglia (176–178). Other mutations of *RAB39B* were detected in patients with intellectual disability of variable degree, autism, seizure, and macrocephaly without PD (95), as well as in male and female patients with early- or later-onset Parkinsonism, respectively, without intellectual disability (179). *RAB39B* encodes a neuronal protein involved in vesicular recycling trafficking and in the maintenance of α-syn homeostasis. Parkinsonian phenotypes have been mainly related to loss of function mutations (179). To date, *RAB39B* is a proven but rare cause of Parkinsonism and intellectual disability in males. Many studies failed to identify pathogenic alterations of this gene in large cohorts of PD patients (180).

DISCUSSION

The advent of next generation sequencing (NGS) increased the number of genes known to cause Mendelian forms of Parkinsonism. They are involved in many specific biological pathways, suggesting that several cellular functions are critical in the pathogenesis of PD. *SNCA* is directly responsible for altering the expression of α-syn, the main component of LBs (181, 182). *PRKN*, *PINK1,* and *DJ-1* are related to mitochondrial function and mitophagy (183–185), as well as other genes linked to classical and atypical PD such as *FBXO7*, *PLA2G6*, *VPS13C*, and *CHCHD2*, which are involved in the mitochondrial quality control system (186). The impairment of lysosomal function has also been linked to the pathogenesis of PD. Altered *LRRK2* compromises cell autophagy, reducing α-syn degradation (187, 188), *ATP13A2* mutations cause lysosomal dysfunctions (189), and reduced enzymatic activity of GCAse determinates α-syn accumulation, endoplasmic reticulum stress, and mitochondrial dysfunction (190). Recently, a novel disease mechanism involving vesicular trafficking and synaptic endocytosis has been proposed after the identification of many other PD-related genes, such as *VPS35*, *DNAJC6*, *SYNJ1*, and *LRP10* (49).

A precise genetic diagnosis enables proper genetic counseling according to the mode of inheritance and penetrance of the mutation and may help define the disease prognosis and influence therapeutic choices.

Some genes and some mutations are associated with specific phenotypes. The relationship between clinical phenotypes and their molecular bases is depicted in **Figure 1**. The *LRRK2* p.G2019S mutation usually causes a slow progressive iPD-like condition with variable age of onset. *SNCA* pathogenic variants are responsible for a more aggressive Parkinsonism with cognitive decline and other non-motor features. Instead, *GBA* variant carriers can present more heterogeneous phenotypes, ranging from absence of disease to severe Parkinsonian conditions. In affected carriers, the disease usually manifests as classical late-onset levodopa-responsive PD. However, in a subgroup of patients, the condition can be more severe, sometimes presenting with cognitive decline as PDD or DLB (122, 191). Among EOPD cases, *PRKN*, *PINK1*, and *DJ-1*, sharing mitochondria and mitophagy related functions, are usually responsible for pure PD forms with only motor signs, slow progression, and good response to dopaminergic therapy. Complex EOPD forms with rapid progression, early cognitive deterioration, and additional movement disorders instead are usually related to new AR genes. Except for *PODXL*, mutations in genes causing juvenile Parkinsonisms are always related to complex phenotypes in which pallido-pyramidal signs, oculomotor abnormalities, cognitive impairment, and seizures variably occur. Intellectual disability in males is a red flag for *RAB39B* mutations. However, phenotypes rarely overlap, hindering the achievement of the correct diagnostic assumptions. Early onset *LRRK2*-related PD as well as milder forms of *DNAJC6*-related Parkinsonism may present with a phenotype resembling pure *PRKN/PINK1* recessive cases (132, 156). Clinical features in *SNCA* triplication carriers are similar to the *VPS13C*-related phenotype (63, 148), while the complex phenotype of *SNCA* p.G51D patients, characterized by precocious disease onset, pyramidal signs, myoclonus, seizures, and, inconstantly, cognitive decline, significantly overlaps with *DNAJC6*- and *SYNJ1*-related phenotypes (82, 155, 157–160). Differential diagnosis among complex juvenile Parkinsonisms is more tangled and only partially helped by ancillary tests (33). The easier availability of NGS in clinical practice has notably unraveled the emerging genotype and phenotype heterogeneity in Parkinsonian conditions. However, the clinical reasoning is too often overshadowed by excessive reliance on the diagnostic power of this technique. Exonic, multiexonic, and/or whole gene rearrangements, frequently implied in many PD genes, including *PRKN*, *PINK1*, *DJ-1,* and *SNCA*, and even pathogenic repeat expansions of *ATXN2*, are not generally identified by NGS techniques. The request of the proper molecular analysis, and thus the possibility to thereby reach the correct diagnosis still requires a scrupulous clinical observation and, whenever possible, a focused clinical diagnostic suspicion, within the frame of accurate genetic counseling.

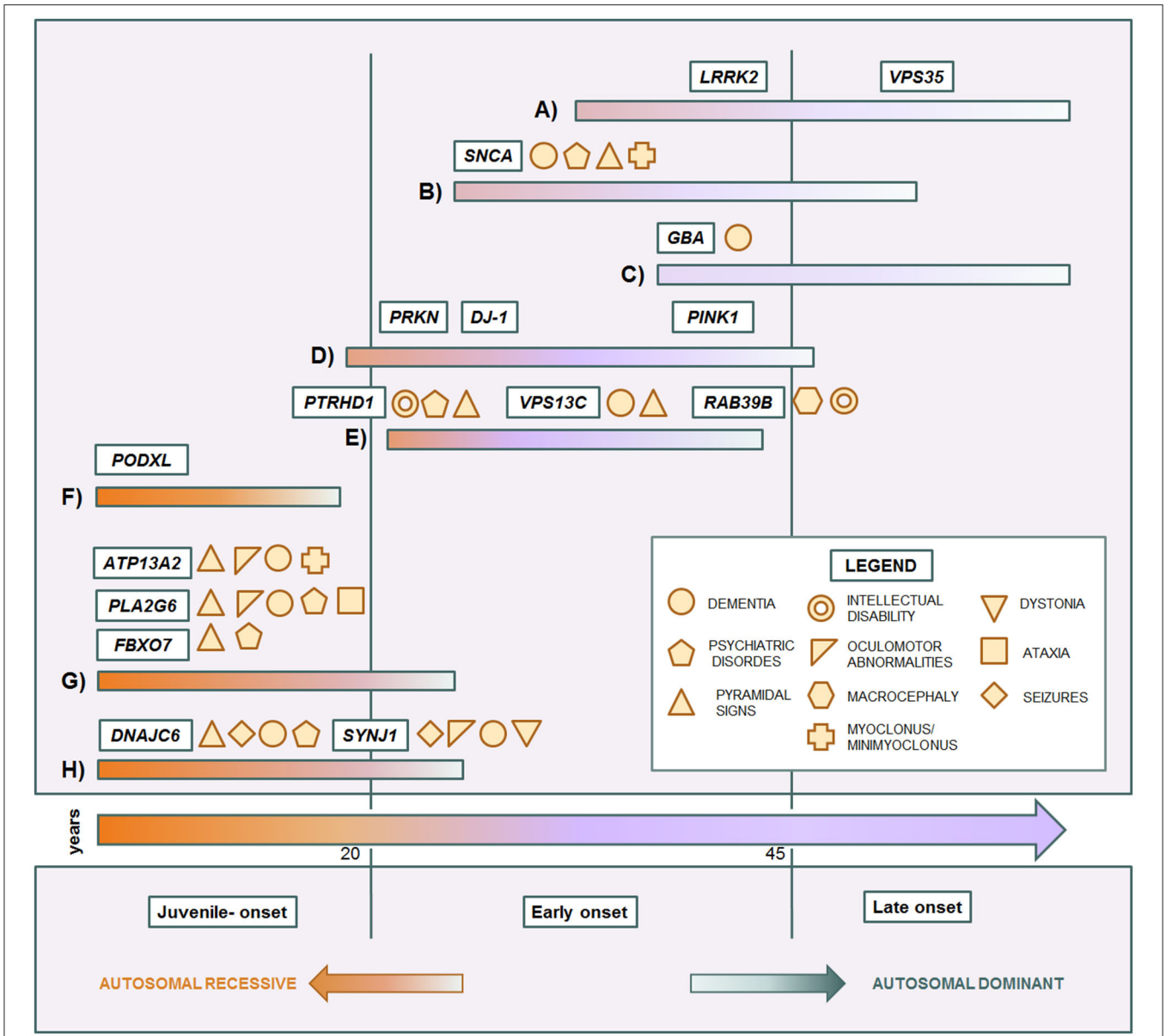

FIGURE 1 | Genotype-phenotype correlation in monogenic Parkinsonian conditions. Central arrow: time of onset. Each colored bar represents a subset of conditions. **(A)** iPD-like, late-onset autosomal dominant Parkinsonisms. **(B)** Complicated autosomal dominant Parkinsonisms. **(C)** Genetic risk factor causing late-onset Parkinsonism. **(D)** Typical autosomal recessive early-onset Parkinsonisms. **(E)** Complicated autosomal recessive early onset Parkinsonisms. **(F)** Juvenile uncomplicated Parkinsonism. **(G)** Juvenile pallido-pyramidal syndromes. **(H)** Juvenile atypical Parkinsonisms.

Precision medicine is becoming a reality as well for PD. The presence of defined pathogenic mechanisms, identifiable with genetic testing, is appealing for targeted therapies. Ongoing clinical trials are mainly recruiting participants carrying mutations in specific genes. However, some of these therapies might also be used for broader iPD cohorts. GCse, α-syn, *LRRK2*, and mitochondrial functions are currently seen as targets for personalized treatments.

The reduction of α-syn accumulation has been the first target of precision medicine. Many different therapeutic approaches have been proposed and even investigated in clinical trials.

One of them aims to reduce the synthesis of α-syn before its aggregation by neutralizing mRNA molecules with RNA interference (RNAi) technologies (192, 193), others by reducing *SNCA* transcription with molecules that interfere with histones acetylation (2-adrenergic receptor agonists) (194), or by using antisense oligonucleotide (ASO) therapy (49). A second therapeutic approach, aimed at preventing *SNCA* misfolding or aggregation, utilizes small antibody fragments (intrabodies) that prevent oligomerization by binding intracellular α-syn (195). Two such molecules are currently in early clinical trials (196, 197). An alternative strategy is the enhancement of α-syn

clearance by immunotherapies or the activation of autophagy. The first method is based on passive immunization with α-syn specific antibodies or active immunization with injections of modified α-syn stimulating the production of endogenous antibodies. The second one is based on the administration of autophagy enhancers such as rapamycin, lithium, or the antineoplastic drug nilotinib (198). Clinical trials are ongoing for these compounds as well.

Another target of great interest is GCase. Clinical trials for *GBA*-targeted therapies are studying drugs able to increase GCase production or activity. The glucosylceramide synthase inhibitor GZ/SAR402671, which reduces glucocerebrosidase substrates, and ambroxol hydrochloride, a small chaperone molecule able to increase GCase activity, are present research targets. Other small molecular chaperones have been shown to reduce α-syn accumulation in GBA PD patients and for one of them a clinical trial is currently running (Netherlands Trial Register: NTR6960). Another way to restore GCase activity is to introduce wild-type *GBA* genes into the genome of *GBA* mutation carriers. Gene therapy, at present under investigation in other neuromuscular diseases, also will be tested in *GBA*-mutated patients (49, 199).

The evidence that *LRRK2* kinase activity inhibition reverses the pathological features and reduces α-syn accumulation in cellular and animal models makes this protein another candidate for a target therapy. Preclinical research investigated many *LRRK2* inhibitors that failed in brain penetration or in pharmacokinetic properties. Recently, two molecules with exceptional potency and selectivity in inhibiting *LRRK2* kinase activity and a good safety profile have been identified (200–202). Based on the good results on animal models, the administration of this molecule started in healthy and affected human subjects, with and without *LRRK2* mutations (ClinicalTrials.gov Identifier: NCT03710707; ClinicalTrials.gov Identifier: NCT04056689).

Finally, various therapeutic approaches focusing on mitochondrial dysfunctions have been proposed. Molecules improving mitochondrial functions, such as kinetin triphosphate, able to ameliorate the kinase activity of mutated *PINK1*, and selective MAO-B inhibitors, including selegilin and regasilin, are being studied. Good results of these treatments have been reported only after a proper stratification of patients, with the administration of these compounds in the "mitochondrial subtypes PDs" (203).

Currently, genetically determined PD offers the unique framework of a constant dialogue between cell biology, clinical acumen, medical genetics, and targeted therapy. Only appropriate genetic testing allows identification of the specific condition in patients, pointing which pathway, or pathways, is involved in the underlying pathogenesis for that case. Most of the ongoing trials about target therapy involve PD cases with molecular diagnosis as positive controls for the different forms of PD. In the era of precision medicine, the importance of genetics in PD is overstepping the boundaries of the research to play an increasingly pivotal role in clinical practice. Nevertheless, the identification of the compromised pathways and genes, driving the choice of the proper therapy, is still strictly dependent on clinical reasoning that, despite the availability of innovative diagnostic techniques, continues to be the irreplaceable key in reaching a genetic diagnosis.

AUTHOR CONTRIBUTIONS

DG: conceptualization, investigation, data curation, original draft, and visualization. MP: data curation, methodology, supervision, review, and editing. MT: resources, methodology, supervision, review, and editing. AP: methodology, data curation, supervision, review, and editing. SP: conceptualization, investigation, data curation, original draft, methodology, and supervision. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Simon DK, Tanner CM, Brundin P. Parkinson disease epidemiology, pathology, genetics, and pathophysiology. *Clin Geriatr Med.* (2020) 36:1–12. doi: 10.1016/j.cger.2019.08.002
2. Johansen KK, Torp SH, Farrer MJ, Gustavsson EK, Aasly JO. A case of Parkinson's disease with no lewy body pathology due to a homozygous exon deletion in Parkin. *Case Rep Neurol Med.* (2018) 2018:6838965. doi: 10.1155/2018/6838965
3. Schrag A, Schott JM. Epidemiological, clinical, and genetic characteristics of early-onset parkinsonism. *Lancet Neurol.* (2006) 5:355–63. doi: 10.1016/S1474-4422(06)70411-2
4. Kumar KR, Djarmati-Westenberger A, Grunewald A. Genetics of Parkinson's disease. *Semin Neurol.* (2011) 31:433–40. doi: 10.1055/s-0031-1299782
5. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. *Nat Rev Dis Primers.* (2017) 3:17013. doi: 10.1038/nrdp.2017.13
6. Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. *Lancet Neurol.* (2020) 19:170–8. doi: 10.1016/S1474-4422(19)30287-X
7. Kestenbaum M, Alcalay RN. Clinical features of lrrk2 carriers with Parkinson's disease. *Adv Neurobiol.* (2017) 14:31–48. doi: 10.1007/978-3-319-49969-7_2
8. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* (2004) 44:595–600. doi: 10.1016/j.neuron.2004.10.023
9. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron.* (2004) 44:601–7. doi: 10.1016/j.neuron.2004.11.005
10. Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, et al. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. *Neurology.* (2005) 65:741–4. doi: 10.1212/01.WNL.0000172630.22804.73
11. Di Fonzo A, Rohe CF, Ferreira J, Chien HF, Vacca L, Stocchi F, et al. A frequent LRRK2 gene mutation associated with

autosomal dominant Parkinson's disease. *Lancet.* (2005) 365:412–5. doi: 10.1016/S0140-6736(05)17829-5
12. Hernandez DG, Paisan-Ruiz C, McInerney-Leo A, Jain S, Meyer-Lindenberg A, Evans EW, et al. Clinical and positron emission tomography of Parkinson's disease caused by LRRK2. *Ann Neurol.* (2005) 57:453–6. doi: 10.1002/ana.20401
13. Funayama M, Hasegawa K, Ohta E, Kawashima N, Komiyama M, Kowa H, et al. An LRRK2 mutation as a cause for the parkinsonism in the original PARK8 family. *Ann Neurol.* (2005) 57:918–21. doi: 10.1002/ana.20484
14. Koros C, Simitsi A, Stefanis L. Genetics of Parkinson's disease: genotype-phenotype correlations. *Int Rev Neurobiol.* (2017) 132:197–231. doi: 10.1016/bs.irn.2017.01.009
15. Li C, Ting Z, Qin X, Ying W, Li B, Guo Qiang L, et al. The prevalence of LRRK2 Gly2385Arg variant in Chinese Han population with Parkinson's disease. *Mov Disord.* (2007) 22:2439–43. doi: 10.1002/mds.21763
16. Ross OA, Wu YR, Lee MC, Funayama M, Chen ML, Soto AI, et al. Analysis of Lrrk2 R1628P as a risk factor for Parkinson's disease. *Ann Neurol.* (2008) 64:88–92. doi: 10.1002/ana.21405
17. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* (2008) 7:583–90. doi: 10.1016/S1474-4422(08)70117-0
18. Monfrini E, Di Fonzo A. Leucine-rich repeat kinase (LRRK2) genetics and Parkinson's Disease. *Adv Neurobiol.* (2017) 14:3–30. doi: 10.1007/978-3-319-49969-7_1
19. Simon-Sanchez J, Marti-Masso JF, Sanchez-Mut JV, Paisan-Ruiz C, Martinez-Gil A, Ruiz-Martinez J, et al. Parkinson's disease due to the R1441G mutation in Dardarin: a founder effect in the Basques. *Mov Disord.* (2006) 21:1954–9. doi: 10.1002/mds.21114
20. De Rosa A, De Michele G, Guacci A, Carbone R, Lieto M, Peluso S, et al. Genetic screening for the LRRK2 R1441C and G2019S mutations in Parkinsonian patients from Campania. *J Parkinsons Dis.* (2014) 4:123–8. doi: 10.3233/JPD-130312
21. Lee AJ, Wang Y, Alcalay RN, Mejia-Santana H, Saunders-Pullman R, Bressman S, et al. Penetrance estimate of LRRK2 p.G2019S mutation in individuals of non-Ashkenazi Jewish ancestry. *Mov Disord.* (2017) 32:1432–8. doi: 10.1002/mds.27059
22. Hentati F, Trinh J, Thompson C, Nosova E, Farrer MJ, Aasly JO. LRRK2 parkinsonism in Tunisia and Norway: a comparative analysis of disease penetrance. *Neurology.* (2014) 83:568–9. doi: 10.1212/WNL.0000000000000675
23. Fernandez-Santiago R, Garrido A, Infante J, Gonzalez-Aramburu I, Sierra M, Fernandez M, et al. alpha-synuclein (SNCA) but not dynamin 3 (DNM3) influences age at onset of leucine-rich repeat kinase 2 (LRRK2) Parkinson's disease in Spain. *Mov Disord.* (2018) 33:637–41. doi: 10.1002/mds.27295
24. Iwaki H, Blauwendraat C, Makarious MB, Bandres-Ciga S, Leonard HL, Gibbs JR, et al. Penetrance of Parkinson's disease in LRRK2 p.G2019S carriers is modified by a polygenic risk score. *Mov Disord.* (2020) 35:774–80. doi: 10.1101/738260
25. Ruiz-Martinez J, Gorostidi A, Ibanez B, Alzualde A, Otaegui D, Moreno F, et al. Penetrance in Parkinson's disease related to the LRRK2 R1441G mutation in the Basque country (Spain). *Mov Disord.* (2010) 25:2340–5. doi: 10.1002/mds.23278
26. Rizzone MG, Martone T, Balestrino R, Lopiano L. Genetic background and outcome of deep brain stimulation in Parkinson's disease. *Parkinsonism Relat Disord.* (2019) 64:8–19. doi: 10.1016/j.parkreldis.2018.08.006
27. Gan-Or Z, Leblond CS, Mallett V, Orr-Urtreger A, Dion PA, Rouleau GA. LRRK2 mutations in Parkinson disease; a sex effect or lack thereof? A meta-analysis. *Parkinsonism Relat Disord.* (2015) 21:778–82. doi: 10.1016/j.parkreldis.2015.05.002
28. Saunders-Pullman R, Mirelman A, Alcalay RN, Wang C, Ortega RA, Raymond D, et al. Progression in the LRRK2-Asssociated Parkinson disease population. *JAMA Neurol.* (2018) 75:312–9. doi: 10.1001/jamaneurol.2017.4019
29. Mirelman A, Heman T, Yasinovsky K, Thaler A, Gurevich T, Marder K, et al. Fall risk and gait in Parkinson's disease: the role of the LRRK2 G2019S mutation. *Mov Disord.* (2013) 28:1683–90. doi: 10.1002/mds.25587
30. Marras C, Schule B, Munhoz RP, Rogaeva E, Langston JW, Kasten M, et al. Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers. *Neurology.* (2011) 77:325–33. doi: 10.1212/WNL.0b013e318227042d
31. Aasly JO, Toft M, Fernandez-Mata I, Kachergus J, Hulihan M, White LR, et al. Clinical features of LRRK2-associated Parkinson's disease in central Norway. *Ann Neurol.* (2005) 57:762–5. doi: 10.1002/ana.20456
32. Alcalay RN, Mejia-Santana H, Mirelman A, Saunders-Pullman R, Raymond D, Palmese C, et al. Neuropsychological performance in LRRK2 G2019S carriers with Parkinson's disease. *Parkinsonism Relat Disord.* (2015) 21:106–10. doi: 10.1016/j.parkreldis.2014.09.033
33. Weissbach A, Wittke C, Kasten M, Klein C. 'Atypical' Parkinson's disease - genetic. *Int Rev Neurobiol.* (2019) 149:207–35. doi: 10.1016/bs.irn.2019.10.011
34. Sanchez-Contreras M, Heckman MG, Tacik P, Diehl N, Brown PH, Soto-Ortolaza AI, et al. Study of LRRK2 variation in tauopathy: progressive supranuclear palsy and corticobasal degeneration. *Mov Disord.* (2017) 32:115–23. doi: 10.1002/mds.26815
35. Tan EK, Skipper L, Chua E, Wong MC, Pavanni R, Bonnard C, et al. Analysis of 14 LRRK2 mutations in Parkinson's plus syndromes and late-onset Parkinson's disease. *Mov Disord.* (2006) 21:997–1001. doi: 10.1002/mds.20875
36. Cho JW, Kim SY, Park SS, Jeon BS. The G2019S LRRK2 mutation is rare in Korean patients with Parkinson's disease and multiple system atrophy. *J Clin Neurol.* (2009) 5:29–32. doi: 10.3988/jcn.2009.5.1.29
37. Tolosa E, Vila M, Klein C, Rascol O. LRRK2 in Parkinson disease: challenges of clinical trials. *Nat Rev Neurol.* (2020) 16:97–107. doi: 10.1038/s41582-019-0301-2
38. Brockmann K, Groger A, Di Santo A, Liepelt I, Schulte C, Klose U, et al. Clinical and brain imaging characteristics in leucine-rich repeat kinase 2-associated PD and asymptomatic mutation carriers. *Mov Disord.* (2011) 26:2335–42. doi: 10.1002/mds.23991
39. Pyatigorskaya,N, Sharman M, Corvol JC, Valabregue R, Yahia-Cherif L, Poupon F, et al. High nigral iron deposition in LRRK2 and Parkin mutation carriers using R2* relaxometry. *Mov Disord.* (2015) 30:1077–84. doi: 10.1002/mds.26218
40. Hustad E, Aasly JO. Clinical and imaging markers of prodromal Parkinson's disease. *Front Neurol.* (2020) 11:395. doi: 10.3389/fneur.2020.00395
41. Wile DJ, Agarwal PA, Schulzer M, Mak E, Dinelle K, Shahinfard E, et al. Serotonin and dopamine transporter PET changes in the premotor phase of LRRK2 parkinsonism: cross-sectional studies. *Lancet Neurol.* (2017) 16:351–9. doi: 10.1016/S1474-4422(17)30056-X
42. Liu SY, Wile DJ, Fu J, Valerio FJ, Shahinfard E, McCormick S, et al. The effect of LRRK2 mutations on the cholinergic system in manifest and premanifest stages of Parkinson's disease: a cross-sectional PET study. *Lancet Neurol.* (2018) 17:309–16. doi: 10.1016/S1474-4422(18)30032-2
43. Kalia LV, Lang AE, Hazrati LN, Fujioka S, Wszolek ZK, Dickson DW, et al. Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. *JAMA Neurol.* (2015) 72:100–5. doi: 10.1001/jamaneurol.2014.2704
44. Poulopoulos M, Levy OA, Alcalay RN. The neuropathology of genetic Parkinson's disease. *Mov Disord.* (2012) 27:831–42. doi: 10.1002/mds.24962
45. Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: mendelian versus non-Mendelian inheritance. *J Neurochem.* (2016) 139(Suppl 1):59–74. doi: 10.1111/jnc.13593
46. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* (2014) 46:989–93. doi: 10.1038/ng.3043
47. Chang D, Nalls MA, Hallgrimsdottir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet.* (2017) 49:1511–6. doi: 10.1038/ng.3955
48. Roosen DA, Cookson MR. LRRK2 at the interface of autophagosomes, endosomes and lysosomes. *Mol Neurodegener.* (2016) 11:73. doi: 10.1186/s13024-016-0140-1
49. Toffoli M, Vieira SRL, Schapira AHV. Genetic causes of PD: a pathway to disease modification. *Neuropharmacology.* (2020) 170:108022. doi: 10.1016/j.neuropharm.2020.108022

50. Struhal W, Presslauer S, Spielberger S, Zimprich A, Auff E, Bruecke T, et al. VPS35 Parkinson's disease phenotype resembles the sporadic disease. *J Neural Transm.* (2014) 121:755–9. doi: 10.1007/s00702-014-1179-1
51. Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, Offman MN, et al. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am J Hum Genet.* (2011) 89:168–75. doi: 10.1016/j.ajhg.2011.06.008
52. Vilarino-Guell C, Wider C, Ross OA, Dachsel JC, Kachergus JM, Lincoln SJ, et al. VPS35 mutations in Parkinson disease. *Am J Hum Genet.* (2011) 89:162–7. doi: 10.1016/j.ajhg.2011.06.001
53. Sharma M, Ioannidis JP, Aasly JO, Annesi G, Brice A, Bertram L, et al. A multi-centre clinico-genetic analysis of the VPS35 gene in Parkinson disease indicates reduced penetrance for disease-associated variants. *J Med Genet.* (2012) 49:721–6. doi: 10.1136/jmedgenet-2012-101155
54. Trinh J, Zeldenrust FMJ, Huang J, Kasten M, Schaake S, Petkovic S, et al. Genotype-phenotype relations for the Parkinson's disease genes SNCA, LRRK2, VPS35: MDSGene systematic review. *Mov Disord.* (2018) 33:1857–70. doi: 10.1002/mds.27527
55. Wider C, Wszolek ZK. Etiology and pathophysiology of frontotemporal dementia, Parkinson disease and Alzheimer disease: lessons from genetic studies. *Neurodegener Dis.* (2008) 5:122–5. doi: 10.1159/000113680
56. Mohan M, Mellick GD. Role of the VPS35 D620N mutation in Parkinson's disease. *Parkinsonism Relat Disord.* (2017) 36:10–18. doi: 10.1016/j.parkreldis.2016.12.001
57. Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE. Di Iorio G, et al. Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. *Science.* (1996) 274:1197–9. doi: 10.1126/science.274.5290.1197
58. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science.* (1997) 276:2045–7. doi: 10.1126/science.276.5321.2045
59. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature.* (1997) 388:839–40. doi: 10.1038/42166
60. Vekrellis K, Xilouri M, Emmanouilidou E, Rideout HJ, Stefanis L. Pathological roles of alpha-synuclein in neurological disorders. *Lancet Neurol.* (2011) 10:1015–25. doi: 10.1016/S1474-4422(11)70213-7
61. Konno T, Ross OA, Puschmann A, Dickson DW, Wszolek ZK. Autosomal dominant Parkinson's disease caused by SNCA duplications. *Parkinsonism Relat Disord.* (2016) 22 (Suppl 1):S1–6. doi: 10.1016/j.parkreldis.2015.09.007
62. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. *Science.* (2003) 302:841. doi: 10.1126/science.1090278
63. Gwinn K, Devine MJ, Jin LW, Johnson J, Bird T, Muenter M, et al. Clinical features, with video documentation, of the original familial lewy body parkinsonism caused by alpha-synuclein triplication (Iowa kindred). *Mov Disord.* (2011) 26:2134–6. doi: 10.1002/mds.23776
64. Olgiati S, Thomas A, Quadri M, Breedveld GJ, Graafland J, Eussen H, et al. Early-onset parkinsonism caused by alpha-synuclein gene triplication: clinical and genetic findings in a novel family. *Parkinsonism Relat Disord.* (2015) 21:981–6. doi: 10.1016/j.parkreldis.2015.06.005
65. Elia AE, Petrucci S, Fasano A, Guidi M, Valbonesi S, Bernardini L, et al. Alpha-synuclein gene duplication: marked intrafamilial variability in two novel pedigrees. *Mov Disord.* (2013) 28:813–7. doi: 10.1002/mds.25518
66. Nishioka K, Ross OA, Ishii K, Kachergus JM, Ishiwata K, Kitagawa M, et al. Expanding the clinical phenotype of SNCA duplication carriers. *Mov Disord.* (2009) 24:1811–9. doi: 10.1002/mds.22682
67. Kasten M, Klein C. The many faces of alpha-synuclein mutations. *Mov Disord.* (2013) 28:697–701. doi: 10.1002/mds.25499
68. Kara E, Kiely AP, Proukakis C, Giffin N, Love S, Hehir J, et al. A 6.4 Mb duplication of the alpha-synuclein locus causing frontotemporal dementia and Parkinsonism: phenotype-genotype correlations. *JAMA Neurol.* (2014) 71:1162–71. doi: 10.1001/jamaneurol.2014.994
69. Itokawa K, Sekine T, Funayama M, Tomiyama H, Fukui M, Yamamoto T, et al. A case of alpha-synuclein gene duplication presenting with head-shaking movements. *Mov Disord.* (2013) 28:384–7. doi: 10.1002/mds.25243
70. Tambasco N, Nigro P, Romoli M, Prontera P, Simoni S, Calabresi P. A53T in a parkinsonian family: a clinical update of the SNCA phenotypes. *J Neural Transm.* (2016) 123:1301–7. doi: 10.1007/s00702-016-1578-6
71. Ricciardi L, Petrucci S, Di Giuda D, Serra L, Spano B, Sensi M, et al. The contursi family 20 years later: intrafamilial phenotypic variability of the SNCA p.A53T mutation. *Mov Disord.* (2016) 31:257–8. doi: 10.1002/mds.26549
72. Papadimitriou D, Antonelou R, Miligkos M, Maniati M, Papagiannakis N, Bostantjopoulou S, et al. Motor and nonmotor features of carriers of the p.A53T alpha-synuclein mutation: a longitudinal study. *Mov Disord.* (2016) 31:1226–30. doi: 10.1002/mds.26615
73. Kasten M, Hartmann C, Hampf J, Schaake S, Westenberger A, Vollstedt EJ, et al. Genotype-phenotype relations for the Parkinson's disease genes Parkin, PINK1, DJ1: MDSGene systematic review. *Mov Disord.* (2018) 33:730–41. doi: 10.1002/mds.27352
74. Morfis L, Cordato DJ. Dementia with Lewy bodies in an elderly Greek male due to alpha-synuclein gene mutation. *J Clin Neurosci.* (2006) 13:942–4. doi: 10.1016/j.jocn.2005.11.040
75. Bougea A, Koros C, Stamelou M, Simitsi A, Papagiannakis N, Antonelou R, et al. Frontotemporal dementia as the presenting phenotype of p.A53T mutation carriers in the alpha-synuclein gene. *Parkinsonism Relat Disord.* (2017) 35:82–7. doi: 10.1016/j.parkreldis.2016.12.002
76. Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, et al. Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Mov Disord.* (2013) 28:811–3. doi: 10.1002/mds.25421
77. Proukakis C, Dudzik CG, Brier T, MacKay DS, Cooper JM, Millhauser GL, et al. A novel alpha-synuclein missense mutation in Parkinson disease. *Neurology.* (2013) 80:1062–4. doi: 10.1212/WNL.0b013e31828727ba
78. Kruger R, Kuhn W, Leenders KL, Sprengelmeyer R, Muller T, Woitalla D, et al. Familial parkinsonism with synuclein pathology: clinical and PET studies of A30P mutation carriers. *Neurology.* (2001) 56:1355–62. doi: 10.1212/WNL.56.10.1355
79. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet.* (1998) 18:106–8. doi: 10.1038/ng0298-106
80. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ro,s R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol.* (2004) 55:164–73. doi: 10.1002/ana.10795
81. Tijero B, Gomez-Esteban JC, Lezcano E, Fernandez-Gonzalez C, Somme J, Llorens V, et al. Cardiac sympathetic denervation in symptomatic and asymptomatic carriers of the E46K mutation in the alpha synuclein gene. *Parkinsonism Relat Disord.* (2013) 19:95–100. doi: 10.1016/j.parkreldis.2012.08.001
82. Lesage S, Anheim M, Letournel F, Bousset L, Honore A, Rozas N, et al. G51D alpha-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. *Ann Neurol.* (2013) 73:459–71. doi: 10.1002/ana.23894
83. Tokutake T, Ishikawa A, Yoshimura N, Miyashita A, Kuwano R, Nishizawa M, et al. Clinical and neuroimaging features of patient with early-onset Parkinson's disease with dementia carrying SNCA p.G51D mutation. *Parkinsonism Relat Disord.* (2014) 20:262–4. doi: 10.1016/j.parkreldis.2013.11.008
84. Martikainen MH, Paivarinta M, Hietala M, Kaasinen V. Clinical and imaging findings in Parkinson disease associated with the A53E SNCA mutation. *Neurol Genet.* (2015) 1:e27. doi: 10.1212/NXG.0000000000000027
85. George JM. The synucleins. *Genome Biol.* (2002) 3:REVIEWS3002. doi: 10.1186/gb-2001-3-1-reviews3002
86. Fraldi A, Klein AD, Medina DL, Settembre C. Brain disorders due to lysosomal dysfunction. *Annu Rev Neurosci.* (2016) 39:277–95. doi: 10.1146/annurev-neuro-070815-014031
87. Nussbaum RL. Genetics of synucleinopathies. *Cold Spring Harb Perspect Med.* (2018) 8:a024109. doi: 10.1101/cshperspect.a024109
88. Tan EK, Matsuura T, Nagamitsu S, Khajavi M, Jankovic J., Ashizawa T. Polymorphism of NACP-Rep1 in Parkinson's disease: an etiologic link with essential tremor? *Neurology.* (2000) 54:1195–8. doi: 10.1212/WNL.54.5.1195
89. Farrer M, Maraganore DM, Lockhart P, Singleton A, Lesnick TG, de Andrade M, et al. alpha-Synuclein gene haplotypes are associated with Parkinson's disease. *Hum Mol Genet.* (2001) 10:1847–51. doi: 10.1093/hmg/10.17.1847
90. Lunati A, Lesage S, Brice A. The genetic landscape of Parkinson's disease. *Rev Neurol.* (2018) 174:628–43. doi: 10.1016/j.neurol.2018.08.004
91. Saini P, Rudakou U, Yu E, Ruskey JA, Asayesh F, Laurent SB, et al. Association study of DNAJC13, UCHL1, HTRA2, GIGYF2, and

EIF4G1 with Parkinson's disease. *Neurobiol Aging.* (2021) 100:119.e7-119.e13. doi: 10.1016/j.neurobiolaging.2020.10.019
92. Funayama M, Ohe K, Amo T, Furuya N, Yamaguchi J, Saiki S, et al. CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: a genome-wide linkage and sequencing study. *Lancet Neurol.* (2015) 14:274–82. doi: 10.1016/S1474-4422(14)70266-2
93. Ikeda A, Matsushima T, Daida K, Nakajima S, Conedera S, Li Y, et al. A novel mutation of CHCHD2 p.R8H in a sporadic case of Parkinson's disease. *Parkinsonism Relat Disord.* (2017) 34:66–8. doi: 10.1016/j.parkreldis.2016.10.018
94. Ikeda A, Nishioka K, Meng H, Takanashi M, Hasegawa I, Inoshita T, et al. Mutations in CHCHD2 cause alpha-synuclein aggregation. *Hum Mol Genet.* (2019) 28:3895–911. doi: 10.1093/hmg/ddz241
95. Puschmann A. New genes causing hereditary Parkinson's disease or parkinsonism. *Curr Neurol Neurosci Rep.* (2017) 17:66. doi: 10.1007/s11910-017-0780-8
96. Deng HX, Shi Y, Yang Y, Ahmeti KB, Miller N, Huang C, et al. Identification of TMEM230 mutations in familial Parkinson's disease. *Nat Genet.* (2016) 48:733–9. doi: 10.1038/ng.3589
97. Wang X, Whelan E, Liu Z, Liu CF, Smith WW. Controversy of TMEM230 Associated with Parkinson's Disease. *Neuroscience.* (2021) 453:280–6. doi: 10.1016/j.neuroscience.2020.11.004
98. Sudhaman S, Muthane UB, Behari M, Govindappa ST, Juyal RC, Thelma BK. Evidence of mutations in RIC3 acetylcholine receptor chaperone as a novel cause of autosomal-dominant Parkinson's disease with non-motor phenotypes. *J Med Genet.* (2016) 53:559–66. doi: 10.1136/jmedgenet-2015-103616
99. Ross JP, Dupre N, Dauvilliers Y, Strong S, Dionne-Laporte A, Dion PA, et al. RIC3 variants are not associated with Parkinson's disease in French-Canadians and French. *Neurobiol Aging.* (2017) 53:194 e9–11. doi: 10.1016/j.neurobiolaging.2017.01.005
100. Quadri M, Mandemakers W, Grochowska MM, Masius R, Geut H, Fabrizio E, et al. LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study. *Lancet Neurol.* (2018) 17:597–608. doi: 10.1016/S1474-4422(18)30408-3
101. Chen Y, Cen Z, Zheng X, Pan Q, Chen X, Zhu, et al. LRP10 in autosomal-dominant Parkinson's disease. *Mov Disord.* (2019) 34:912–6. doi: 10.1002/mds.27693
102. Mencacci NE, Isaias IU, Reich MM, Ganos C, Plagnol V, Polke JM, et al. Parkinson's disease in GTP cyclohydrolase 1 mutation carriers. *Brain.* (2014) 137(Pt 9):2480–92. doi: 10.1093/brain/awu179
103. Guella I, Sherman HE, Appel-Cresswell S, Rajput A, Rajput, Farrer AH, et al. Parkinsonism in GTP cyclohydrolase 1 mutation carriers. *Brain.* (2015) 138(Pt 5):e349. doi: 10.1093/brain/awu341
104. Pan HX, Zhao YW, Mei JP, Fang ZH, Wang Y, Zhou X, et al. GCH1 variants contribute to the risk and earlier age-at-onset of Parkinson's disease: a two-cohort case-control study. *Transl Neurodegener.* (2020) 9:31. doi: 10.1186/s40035-020-00212-3
105. Kim YE, Jeon B, Farrer MJ, Scott E, Guella I, Park SS, et al. SCA2 family presenting as typical Parkinson's disease: 34 year follow up. *Parkinsonism Relat Disord.* (2017) 40:69–72. doi: 10.1016/j.parkreldis.2017.04.003
106. Takao M, Aoyama M, Ishikawa K, Sakiyama Y, Yomono H, Saito Y, et al. Spinocerebellar ataxia type 2 is associated with Parkinsonism and Lewy body pathology. *BMJ Case Rep.* (2011) 2011:bcr0120113685. doi: 10.1136/bcr.01.2011.3685
107. Smith L, Mullin S, Schapira AHV. Insights into the structural biology of Gaucher disease. *Exp Neurol.* (2017) 298(Pt B):180–90. doi: 10.1016/j.expneurol.2017.09.010
108. Riboldi GM, Di Fonzo AB. GBA, Gaucher disease, and Parkinson's disease: from genetic to clinic to new therapeutic approaches. *Cells.* (2019) 8:364. doi: 10.3390/cells8040364
109. Sidransky E, Samaddar T, Tayebi N. Mutations in GBA are associated with familial Parkinson disease susceptibility and age at onset. *Neurology.* (2009) 73:1424–5, author reply 1425–6. doi: 10.1212/WNL.0b013e3181b28601
110. Lesage S, Anheim M, Condroyer C, Pollak P, Durif F, Dupuits C, et al. Large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. *Hum Mol Genet.* (2011) 20:202–10. doi: 10.1093/hmg/ddq454
111. Anheim M, Elbaz A, Lesage S, Durr A, Condroyer C, Viallet F, et al. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. *Neurology.* (2012) 78:417–20. doi: 10.1212/WNL.0b013e318245f476
112. Skrahina V, Gaber H, Vollstedt EJ, Forster TM, Usnich T, Curado F, et al. The Rostock International Parkinson's Disease (ROPAD) study: protocol and initial findings. *Mov Disord.* (2021) 36:1005–10. doi: 10.1002/mds.28416
113. Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, et al. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain.* (2009) 132(Pt 7):1783–94. doi: 10.1093/brain/awp044
114. Mangone G, Bekadar S, Cormier-Dequaire F, Tahiri K, Welaratne A, Czernecki V, et al. Early cognitive decline after bilateral subthalamic deep brain stimulation in Parkinson's disease patients with GBA mutations. *Parkinsonism Relat Disord.* (2020) 76:56–62. doi: 10.1016/j.parkreldis.2020.04.002
115. Pal G, Robertson E, O'Keefe J, Hall D. The neuropsychiatric and motor profile of GBA-Associated Parkinson's disease: a review. *Mov Disord Clin Pract.* (2016) 3:4–8. doi: 10.1002/mdc3.12229
116. Thaler A, Gurevich T, Bar Shira A, Gana Weisz M, Ash E, Shiner T, et al. A "dose" effect of mutations in the GBA gene on Parkinson's disease phenotype. *Parkinsonism Relat Disord.* (2017) 36:47–51. doi: 10.1016/j.parkreldis.2016.12.014
117. Davis MY, Johnson CO, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, et al. Association of GBA mutations and the E326K polymorphism with motor and cognitive progression in Parkinson disease. *JAMA Neurol.* (2016) 73:1217–24. doi: 10.1001/jamaneurol.2016.2245
118. Petrucci S, Ginevrino M, Trezzi I, Monfrini E, Ricciardi L, Albanese A, et al. GBA-Related Parkinson's disease: dissection of genotype-phenotype correlates in a large Italian Cohort. *Mov Disord.* (2020) 35:2106–11. doi: 10.1002/mds.28200
119. Cilia R, Tunesi S, Marotta G, Cereda E, Siri C, Tesei S, et al. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann Neurol.* (2016) 80:662–73. doi: 10.1002/ana.24777
120. Agosta F, Kostic VS, Davidovic K, Kresojevic N, Sarro L, Svetel M, et al. White matter abnormalities in Parkinson's disease patients with glucocerebrosidase gene mutations. *Mov Disord.* (2013) 28:772–8. doi: 10.1002/mds.25397
121. Brockmann K, Hilker R, Pilatus U, Baudrexel S, Srulijes K, Magerkurth J, et al. GBA-associated PD. Neurodegeneration, altered membrane metabolism, and lack of energy failure. *Neurology.* (2012) 79:213–20. doi: 10.1212/WNL.0b013e31825dd369
122. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA Neurol.* (2013) 70:727–35. doi: 10.1001/jamaneurol.2013.1925
123. Pilotto A, Schulte C, Hauser AK, Biskup S, Munz M, Brockmann K, et al. GBA-associated parkinsonism and dementia: beyond alpha-synucleinopathies? *Eur J Neurol.* (2016) 23:520–6. doi: 10.1111/ene.12894
124. Picillo M, Petrucci S, Valente EM, Pappata S, Squame F, Ginevrino M, et al. Progressive supranuclear palsy-like phenotype in a GBA E326K mutation carrier. *Mov Disord Clin Pract.* (2017) 4:444–6. doi: 10.1002/mdc3.12406
125. Mitsui J, Matsukawa T, Sasaki H, Yabe I, Matsushima M, Durr A, et al. Variants associated with Gaucher disease in multiple system atrophy. *Ann Clin Transl Neurol.* (2015) 2:417–26. doi: 10.1002/acn3.185
126. Sklerov M, Kang UJ, Liong C, Clark L, Marder K, Pauciulo M, et al. Frequency of GBA variants in autopsy-proven multiple system atrophy. *Mov Disord Clin Pract.* (2017) 4:574–81. doi: 10.1002/mdc3.12481
127. McNeill A, Magalhaes J, Shen C, Chau KY, Hughes D, Mehta A, et al. Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. *Brain.* (2014) 137(Pt 5):1481–95. doi: 10.1093/brain/awu020
128. Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with parkinsonism: review of the literature. *Mov Disord.* (2017) 32:1504–23. doi: 10.1002/mds.27193
129. Ryan BJ, Hoek S, Fon EA, Wade-Martins R. Mitochondrial dysfunction and mitophagy in Parkinson's: from familial to sporadic disease. *Trends Biochem Sci.* (2015) 40:200–10. doi: 10.1016/j.tibs.2015.02.003
130. van der Merwe C, Jalali Sefid Dashti Z, Christoffels A, Loos B, Bardien S. Evidence for a common biological pathway linking three Parkinson's disease-causing genes: Parkin, PINK1 and DJ-1. *Eur J Neurosci.* (2015) 41:1113–25. doi: 10.1111/ejn.12872

131. Voigt A, Berlemann LA, Winklhofer KF. The mitochondrial kinase PINK1: functions beyond mitophagy. *J Neurochem.* (2016) 139(Suppl 1):232–9. doi: 10.1111/jnc.13655
132. Kilarski LL, Pearson JP, Newsway V, Majounie E, Knipe MD, Misbahuddin A, et al. Systematic review and UK-based study of PARK2 (parkin), PINK1, PARK7 (DJ-1) and LRRK2 in early-onset Parkinson's disease. *Mov Disord.* (2012) 27:1522–9. doi: 10.1002/mds.25132
133. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature.* (1998) 392:605–8. doi: 10.1038/33416
134. Khan NL, Graham E, Critchley P, Schrag AE, Wood NW, Lees AJ, et al. Parkin disease: a phenotypic study of a large case series. *Brain.* (2003) 126(Pt 6):1279–92. doi: 10.1093/brain/awg142
135. Alcalay RN, Siderowf A, Ottman R, Caccappolo E, Mejia-Santana H, Tang MX, et al. Olfaction in Parkin heterozygotes and compound heterozygotes: the CORE-PD study. *Neurology.* (2011) 76:319–26. doi: 10.1212/WNL.0b013e31820882aa
136. Doherty KM, Silveira-Moriyama L, Parkkinen L, Healy DG, Farrell M, Mencacci NE, et al. Parkin disease: a clinicopathologic entity? *JAMA Neurol.* (2013) 70:571–9. doi: 10.1001/jamaneurol.2013.172
137. Farrer M, Chan P, Chen R, Tan L, Lincoln S, Hernandez D, et al. Lewy bodies and parkinsonism in families with parkin mutations. *Ann Neurol.* (2001) 50:293–300. doi: 10.1002/ana.1132
138. Fiesel FC, Caulfield TR, Moussaud-Lamodiere EL, Ogaki K, Dourado DF, Flores SC, et al. Structural and functional impact of Parkinson disease-associated mutations in the E3 Ubiquitin Ligase Parkin. *Hum Mutat.* (2015) 36:774–86. doi: 10.1002/humu.22808
139. Sriram SR, Li X, Ko HS, Chung KK, Wong E, Lim KL, et al. Familial-associated mutations differentially disrupt the solubility, localization, binding and ubiquitination properties of parkin. *Hum Mol Genet.* (2005) 14:2571–86. doi: 10.1093/hmg/ddi292
140. Samaranch L, Lorenzo-Betancor O, Arbelo JM, Ferrer I, Lorenzo E, Irigoyen J, et al. PINK1-linked parkinsonism is associated with Lewy body pathology. *Brain.* (2010) 133(Pt 4):1128–42. doi: 10.1093/brain/awq051
141. Annesi G, Savettieri G, Pugliese P, D'Amelio M, Tarantino P, Ragonese P, et al. DJ-1 mutations and parkinsonism-dementia-amyotrophic lateral sclerosis complex. *Ann Neurol.* (2005) 58:803–7. doi: 10.1002/ana.20666
142. Taipa R, Pereira C, Reis I, Alonso I, Bastos-Lima A, Melo-Pires M, et al. DJ-1 linked parkinsonism (PARK7) is associated with Lewy body pathology. *Brain.* (2016) 139(Pt 6):1680–7. doi: 10.1093/brain/aww080
143. Klein C, Lohmann-Hedrich K, Rogaeva E, Schlossmacher MG, Lang AE. Deciphering the role of heterozygous mutations in genes associated with parkinsonism. *Lancet Neurol.* (2007) 6:652–62. doi: 10.1016/S1474-4422(07)70174-6
144. Marongiu R, Ferraris A, Ialongo T, Michiorri S, Soleti F, Ferrari F, et al. PINK1 heterozygous rare variants: prevalence, significance and phenotypic spectrum. *Hum Mutat.* (2008) 29:565. doi: 10.1002/humu.20719
145. Bruggemann N, Mitterer M, Lanthaler AJ, Djarmati A, Hagenah J, Wiegers K, et al. Frequency of heterozygous Parkin mutations in healthy subjects: need for careful prospective follow-up examination of mutation carriers. *Parkinsonism Relat Disord.* (2009) 15:425–9. doi: 10.1016/j.parkreldis.2008.11.014
146. Pavese N, Khan NL, Scherfler C, Cohen L, Brooks DJ, Wood NW, et al. Nigrostriatal dysfunction in homozygous and heterozygous parkin gene carriers: an 18F-dopa PET progression study. *Mov Disord.* (2009) 24:2260–6. doi: 10.1002/mds.22817
147. van Nuenen BF, van Eimeren T, van der Vegt JP, Buhmann C, Klein C, Bloem BR, et al. Mapping preclinical compensation in Parkinson's disease: an imaging genomics approach. *Mov Disord.* (2009) 24(Suppl. 2):S703–10. doi: 10.1002/mds.22635
148. Lesage S, Drouet V, Majounie E, Deramecourt V, Jacoupy M, Nicolas A, et al. Loss of VPS13C function in autosomal-recessive Parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. *Am J Hum Genet.* (2016) 98:500–13. doi: 10.1016/j.ajhg.2016.01.014
149. Schreglmann SR, Houlden H. VPS13C-another hint at mitochondrial dysfunction in familial Parkinson's disease. *Mov Disord.* (2016) 31:1340. doi: 10.1002/mds.26682
150. Khodadadi H, Azcona LJ, Aghamollaii V, Omrani MD, Garshasbi M, Taghavi S, et al. PTRHD1 (C2orf79) mutations lead to autosomal-recessive intellectual disability and parkinsonism. *Mov Disord.* (2017) 32:287–91. doi: 10.1002/mds.26824
151. Kuipers DJS, Carr J, Bardien S, Thomas P, Sebate B, Breedveld GJ, et al. PTRHD1 Loss-of-function mutation in an african family with juvenile-onset Parkinsonism and intellectual disability. *Mov Disord.* (2018) 33:1814–9. doi: 10.1002/mds.27501
152. Chen SJ, Ho CH, Lin HY, Lin CH, Wu RM. Lack of PTRHD1 mutation in patients with young-onset and familial Parkinson's disease in a Taiwanese population. *Neurobiol Aging.* (2021) 100:188e15–118e16. doi: 10.1016/j.neurobiolaging.2020.09.002
153. Niemann N, Jankovic J. Juvenile Parkinsonism: differential diagnosis, genetics, and treatment. *Parkinsonism Relat Disord.* (2019) 67:74–89. doi: 10.1016/j.parkreldis.2019.06.025
154. Sudhaman S, Prasad K, Behari M, Muthane UB, Juyal RC, Thelma BK. Discovery of a frameshift mutation in podocalyxin-like (PODXL) gene, coding for a neural adhesion molecule, as causal for autosomal-recessive juvenile Parkinsonism. *J Med Genet.* (2016) 53:450–6. doi: 10.1136/jmedgenet-2015-103459
155. Edvardson S, Cinnamon Y, Ta-Shma A, Shaag A, Yim YI, Zenvirt S, et al. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. *PLoS ONE.* (2012) 7:e36458. doi: 10.1371/journal.pone.0036458
156. Olgiati S, Quadri M, Fang M, Rood JP, Saute JA, Chien HF, et al. DNAJC6 mutations associated with early-onset Parkinson's disease. *Ann Neurol.* (2016) 79:244–56. doi: 10.1002/ana.24553
157. Elsayed LE, Drouet V, Usenko T, Mohammed IN, Hamed AA, Elseed MA, et al. A Novel nonsense mutation in DNAJC6 expands the phenotype of autosomal-recessive juvenile-onset Parkinson's disease. *Ann Neurol.* (2016) 79:335–7. doi: 10.1002/ana.24591
158. Koroglu C, Baysal L, Cetinkaya M, Karasoy H, Tolun A. DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. *Parkinsonism Relat Disord.* (2013) 19:320–4. doi: 10.1016/j.parkreldis.2012.11.006
159. Quadri M, Fang M, Picillo M, Olgiati S, Breedveld GJ, Graafland J, et al. Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset Parkinsonism. *Hum Mutat.* (2013) 34:1208–15. doi: 10.1002/humu.22373
160. Krebs CE, Karkheiran S, Powell JC, Cao M, Makarov V, Darvish H, et al. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. *Hum Mutat.* (2013) 34:1200–7. doi: 10.1002/humu.22372
161. Hong D, Cong L, Zhong S, He Y, Xin L, Gao X, et al. Clonazepam improves the symptoms of two siblings with novel variants in the SYNJ1 gene. *Parkinsonism Relat Disord.* (2019) 62:221–25. doi: 10.1016/j.parkreldis.2018.11.020
162. Hardies K, Cai Y, Jardel C, Jansen AC, Cao M, May P, et al. Loss of SYNJ1 dual phosphatase activity leads to early onset refractory seizures and progressive neurological decline. *Brain.* (2016) 139(Pt 9):2420–30. doi: 10.1093/brain/aww180
163. Dyment DA, Smith AC, Humphreys P, Schwartzentruber J, Beaulieu CL, Consortium FC, et al. Homozygous nonsense mutation in SYNJ1 associated with intractable epilepsy and tau pathology. *Neurobiol Aging.* (2015) 36:1222 e1–5. doi: 10.1016/j.neurobiolaging.2014.09.005
164. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet.* (2006) 38:1184–91. doi: 10.1038/ng1884
165. Di Fonzo A, Chien HF, Socal M, Giraudo S, Tassorelli C, Iliceto G, et al. ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. *Neurology.* (2007) 68:1557–62. doi: 10.1212/01.wnl.0000260963.08711.08
166. Schneider SA, Paisan-Ruiz C, Quinn NP, Lees AJ, Houlden H, Hardy J, et al. ATP13A2 mutations (PARK9) cause neurodegeneration with brain iron accumulation. *Mov Disord.* (2010) 25:979–84. doi: 10.1002/mds.22947
167. Estiar MA, Leveille E, Spiegelman D, Dupre N, Trempe JF, Rouleau GA, et al. Clinical and genetic analysis of ATP13A2 in hereditary spastic paraplegia expands the phenotype. *Mol Genet Genomic Med.* (2020) 8:e1052. doi: 10.1002/mgg3.1052
168. Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ. Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. *Hum Mol Genet.* (2012) 21:2646–50. doi: 10.1093/hmg/dds089

169. Paisan-Ruiz C, Guevara R, Federoff M, Hanagasi H, Sina F, Elahi E, et al. Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and spatacsin mutations. *Mov Disord.* (2010) 25:1791–800. doi: 10.1002/mds.23221
170. Bohlega SA, Al-Mubarak BR, Alyemni EA, Abouelhoda M, Monies D, et al. Clinical heterogeneity of PLA2G6-related Parkinsonism: analysis of two Saudi families. *BMC Res Notes.* (2016) 9:295. doi: 10.1186/s13104-016-2102-7
171. Guo YP, Tang BS, Guo JF. PLA2G6-associated neurodegeneration (PLAN): review of clinical phenotypes and genotypes. *Front Neurol.* (2018) 9:1100. doi: 10.3389/fneur.2018.01100
172. Kurian MA, Morgan NV, MacPherson L, Foster K, Peake D, Gupts. R, et al. Phenotypic spectrum of neurodegeneration associated with mutations in the PLA2G6 gene (PLAN). *Neurology.* (2008) 70:1623–9. doi: 10.1212/01.wnl.0000310986.48286.8e
173. Engel LA, Jing Z, O'Brien DE, Sun M, Kotzbauer PT. Catalytic function of PLA2G6 is impaired by mutations associated with infantile neuroaxonal dystrophy but not dystonia-parkinsonism. *PLoS ONE.* (2010) 5:e12897. doi: 10.1371/journal.pone.0012897
174. Di Fonzo A, Dekker MC, Montagna P, Baruzzi A, Yonova EH, Correia Guedes L, et al. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology.* (2009) 72:240–5. doi: 10.1212/01.wnl.0000338144.10967.2b
175. Wei L, Ding L, Li H, Lin Y, Dai Y, Xu X, et al. Juvenile-onset parkinsonism with pyramidal signs due to compound heterozygous mutations in the F-Box only protein 7 gene. *Parkinsonism Relat Disord.* (2018) 47:76–9. doi: 10.1016/j.parkreldis.2017.11.332
176. Wilson GR, Sim JC, McLean C, Giannandrea M, Galea CA, Riseley JR, et al. Mutations in RAB39B cause X-linked intellectual disability and early-onset Parkinson disease with alpha-synuclein pathology. *Am J Hum Genet.* (2014) 95:729–35. doi: 10.1016/j.ajhg.2014.10.015
177. Shi CH, Zhang SY, Yang ZH, Yang J, Shang DD, Mao CY, et al. A novel RAB39B gene mutation in X-linked juvenile parkinsonism with basal ganglia calcification. *Mov Disord.* (2016) 31:1905–9. doi: 10.1002/mds.26828
178. Lesage S, Bras J, Cormier-Dequaire F, Condroyer C, Nicolas A, Darwent L, et al. Loss-of-function mutations in RAB39B are associated with typical early-onset Parkinson disease. *Neurol Genet.* (2015) 1:e9. doi: 10.1212/NXG.0000000000000009
179. Mata IF, Jang Y, Kim CH, Hanna DS, Dorschner MO, Samii A, et al. The RAB39B p.G192R mutation causes X-linked dominant Parkinson's disease. *Mol Neurodegener.* (2015) 10:50. doi: 10.1186/s13024-015-0045-4
180. Gao Y, Wilson GR, Salce N, Romano A, Mellick GD, Stephenson SEM, et al. Genetic analysis of RAB39B in an early-onset Parkinson's disease Cohort. *Front Neurol.* (2020) 11:523. doi: 10.3389/fneur.2020.00523
181. Robak LA, Jansen IE, van Rooij J, Uitterlinden AG, Kraaij R, Jankovic J, et al. Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. *Brain.* (2017) 140:3191–203. doi: 10.1093/brain/awx285
182. Bandres-Ciga S, Saez-Atienzar S, Bonet-Ponce L, Billingsley K, Vitale D, Blauwendraat C, et al. The endocytic membrane trafficking pathway plays a major role in the risk of Parkinson's disease. *Mov Disord.* (2019) 34:460–8. doi: 10.1002/mds.27614
183. Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol.* (2014) 205:143–53. doi: 10.1083/jcb.201402104
184. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature.* (2006) 441:1162–6. doi: 10.1038/nature04779
185. Cookson MR. DJ-1, PINK1, and their effects on mitochondrial pathways. *Mov Disord.* (2010) 25(Suppl. 1):S44–8. doi: 10.1002/mds.22713
186. Bandres-Ciga S, Diez-Fairen M, Kim JJ, Singleton AB. Genetics of Parkinson's disease: an introspection of its journey towards precision medicine. *Neurobiol Dis.* (2020) 137:104782. doi: 10.1016/j.nbd.2020.104782
187. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. *Ann Neurol.* (2002) 51:296–301. doi: 10.1002/ana.10113
188. Yue Z, Yang XW. Dangerous duet: LRRK2 and alpha-synuclein jam at CMA. *Nat Neurosci.* (2013) 16:375–7. doi: 10.1038/nn.3361
189. Dehay B, Ramirez A, Martinez-Vicente M, Perier C, Canron MH, Doudnikoff E, et al. Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. *Proc Natl Acad Sci USA.* (2012) 109:9611–6. doi: 10.1073/pnas.1112368109
190. Balestrino R, Schapira AHV. Glucocerebrosidase and Parkinson disease: molecular, clinical, and therapeutic implications. *Neuroscientist.* (2018) 24:540–59. doi: 10.1177/1073858417748875
191. Winder-Rhodes SE, Evans JR, Ban M, Mason SL, Williams-Gray CH, Foltynie T, et al. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. *Brain.* (2013) 136(Pt 2):392–9. doi: 10.1093/brain/aws318
192. Lewis J, Melrose H, Bumcrot D, Hope A, Zehr C, Lincoln, et al. *In vivo* silencing of alpha-synuclein using naked siRNA. *Mol Neurodegener.* (2008) 3:19. doi: 10.1186/1750-1326-3-19
193. McCormack AL, Mak SK, Henderson JM, Bumcrot D, Farrer MJ, et al. Alpha-synuclein suppression by targeted small interfering RNA in the primate substantia nigra. *PLoS ONE.* (2010) 5:e12122. doi: 10.1371/journal.pone.0012122
194. Gronich N, Abernethy DR, Auriel E, Lavi I, Rennert G, Saliba W. beta2-adrenoceptor agonists and antagonists and risk of Parkinson's disease. *Mov Disord.* (2018) 33:1465–71. doi: 10.1002/mds.108
195. Chatterjee D, Bhatt M, Butler D, De Genst E, Dobson CM, Messer A, et al. Proteasome-targeted nanobodies alleviate pathology and functional decline in an alpha-synuclein-based Parkinson's disease model. *NPJ Parkinsons Dis.* (2018) 4:25. doi: 10.1038/s41531-018-0062-4
196. McFarthing K, Simuni T. Clinical trial highlights: targetting alpha-synuclein. *J Parkinsons Dis.* (2019) 9:5–16. doi: 10.3233/JPD-189004
197. Krishnan R, Tsubery H, Proschitsky MY, Asp E, Lulu M, Gilead S, et al. A bacteriophage capsid protein provides a general amyloid interaction motif (GAIM) that binds and remodels misfolded protein assemblies. *J Mol Biol.* (2014) 426:2500–19. doi: 10.1016/j.jmb.2014.04.015
198. Wang Z, Gao G, Duan C, Yang H. Progress of immunotherapy of anti-alpha-synuclein in Parkinson's disease. *Biomed Pharmacother.* (2019) 115:108843. doi: 10.1016/j.biopha.2019.108843
199. Dangouloff T, Servais L. Clinical evidence supporting early treatment of patients with spinal muscular atrophy: current perspectives. *Ther Clin Risk Manag.* (2019) 15:1153–61. doi: 10.2147/TCRM.S172291
200. Andersen MA, Wegener KM, Larsen S, Badolo L, Smith GP, Jeggo R, et al. PFE-360-induced LRRK2 inhibition induces reversible, non-adverse renal changes in rats. *Toxicology.* (2018) 395:15–22. doi: 10.1016/j.tox.2018.01.003
201. Fell MJ, Mirescu C, Basu K, Cheewatrakoolpong B, DeMong DE, Ellis JM, et al. MLi-2, a potent, selective, and centrally active compound for exploring the therapeutic potential and safety of LRRK2 kinase inhibition. *J Pharmacol Exp Ther.* (2015) 355:397–409. doi: 10.1124/jpet.115.227587
202. Scott JD, DeMong DE, Greshock TJ, Basu K, Dai X, Harris J, et al. Discovery of a 3-(4-Pyrimidinyl) indazole (MLi-2), an orally available and selective leucine-rich repeat kinase 2 (LRRK2) inhibitor that reduces brain kinase activity. *J Med Chem.* (2017) 60:2983–92. doi: 10.1021/acs.jmedchem.7b00045
203. Borsche M, Pereira SL, Klein C, Grunewald A. Mitochondria and Parkinson's disease: clinical, molecular, and translational aspects. *J Parkinsons Dis.* (2021) 11:45–60. doi: 10.3233/JDP-201981

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