frontiers | Frontiers in Cell and Developmental Biology

OPINION
published: 28 April 2022
doi: 10.3389/fcell.2022.886448

The Future of Genetic Disease Studies: Assembling an Updated Multidisciplinary Toolbox

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Keywords: genetic diseases, vesicle trafficking, protein sorting, cell biology, gene mutation

OPEN ACCESS

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Specialty section:
This article was submitted to Membrane Traffic, a section of the journal Frontiers in Cell and Developmental Biology

Received: *28 February 2022*
Accepted: *04 April 2022*
Published: *28 April 2022*

Citation:
Ramadesikan S, Lee J and Aguilar RC (2022) The Future of Genetic Disease Studies: Assembling an Updated Multidisciplinary Toolbox. Front. Cell Dev. Biol. 10:886448. doi: 10.3389/fcell.2022.886448

INTRODUCTION AND SUMMARY

Over the past decades, we witnessed impressive progress in our understanding of the mechanisms behind multiple genetic diseases including many considered to be "rare." Importantly, these advances coupled with new technological developments have increased the potential to translate research into tangible improvements in diagnosis and treatment. Here, we argue that to further accelerate discovery and translational applications in the field, the classical genetic, biochemical, and cell biological toolbox must be updated by systematic integration of rising methodologies from different disciplines. This short opinion article aims to encourage clinicians, geneticists, biochemists, and cell biologists to discover the impact and consider the methodical integration of each other's state-of-the-art disciplines in single comprehensive studies. Indeed, studies systematically integrating such emergent powerful approaches (see below) are not frequent in the field yet, perhaps because such strategies require investment in expanding one's expertise/repertoire of technologies (some of them seemingly very specialized) and in building communication bridges between disciplines perceived as distant (Love et al., 2021). Nevertheless, we consider that these are worthy efforts as the full use of an updated multidisciplinary methodological toolbox will assure future breakthrough studies. These diverse approaches, commonly used at the different steps of the scientific inquiry, are needed to formulate mechanistic hypotheses and consequently, to develop therapeutic strategies. Further, these methodologies are also expected to be used *a posteriori* to evaluate the efficacy of such therapeutic strategies. In fact, several studies using at least a subset of powerful and complementary approaches have already yielded important observations and conclusions, some of which are exemplified below.

CANDIDATE COMPONENTS FOR AN UPDATED MULTIDISCIPLINARY TOOLBOX: EXAMPLES AND LESSONS LEARNED

Although the role of proteins in specific diseases has often been studied by production of gene knock-out (KO) cells or animals, many patients present mutated protein variants rather than complete absence of gene product. The application of NGS technologies such as whole genome/exome sequencing (WGS/WES) has been (Ng et al., 2009; Ng et al., 2010), and continues to be (Macken et al., 2021; Marom et al., 2021; Usmani et al., 2021) instrumental in the identification of novel mutations including those leading to splicing defects. Further, this mutational information can be efficiently analyzed using advanced algorithms to predict non-tolerated/pathogenic changes (Ng and Henikoff, 2003; Schwarz et al., 2010; Adzhubei et al., 2013; Ioannidis et al., 2016; Rentzsch et al., 2019; Ge et al., 2021) and by molecular dynamics to infer putative structural alterations (Kellogg et al., 2011; Adolf-Bryfogle and Dunbrack, 2013).

Nevertheless, to understand the mechanisms underlying such pathologies, we must establish the potential impact of specific gene mutations on the *function* (or regulation of function) and/or on the *availability* (from absence to altered dosage, but also by incorrect localization) of the resulting protein. Moreover, examples from literature indicate that mutations assumed to be similar and affecting the same protein domains can display phenotype heterogeneity (Arystarkhova et al., 2019; Arystarkhova et al., 2021; Ramadesikan et al., 2021). The necessity of firmly establishing a genotype-phenotype relationship is one reason why the articulation of genetic/biochemical methods with cell biological approaches in particular, are a must.

Indeed, the use of classical functional biochemical assays (Davies et al., 2013; Fausther et al., 2014; Guttmann et al., 2018; Saveri et al., 2020) has been successfully complemented with powerful functional cell biological assays. Further, the latter provides context and physiological relevance for the functional impact of mutations in different genes, can be used as a diagnostic tool and for the evaluation of therapeutics (Bergamin et al., 2013; Visentin et al., 2013; Kwiatkowska et al., 2014; Vanier and Latour, 2015; Aoki et al., 2017; Carrion et al., 2017; Saveri et al., 2020; Wanikawa et al., 2020; Hung et al., 2021; Ramadesikan et al., 2021; Romano et al., 2021).

Moreover, the cumulative work of several groups has provided clues about the suitability of different disease models. On one hand, due to adaptation and "dominant" negative effects, the presence of a mutated variant can cause different and/or more severe phenotypes than the absence of the protein (as modeled by KO) (Tucker et al., 2012; Barnes et al., 2018; Lizarraga et al., 2021; Ramadesikan et al., 2021). On the other hand, cell biologists have long known that phenotypes observed under siRNA-mediated knock-down conditions (*i.e.,* acute depletion over a short period of time) often lead to more severe phenotype scenarios due to lack of cell/organism adaptation. Therefore, this confirms that when appropriate, *complete lack of function scenarios* can be better modeled by gene KO or (in some cases) by stable expression of shRNAs rather than by use of siRNAs.

Further, the CRISPR revolution has provided the means to reproduce and study pathological protein variants within meaningful and physiologically relevant contexts (Freedman et al., 2015; Ponomareva et al., 2016) as well as perform targeted rescue of disease-causing mutations in model systems (Min et al., 2020). These developments become synergistic with the possibility of reprogramming patient-derived induced Pluripotent Stem Cells (iPSCs) to study cell biological behavior of mutated variants expressed at endogenous levels in disease-relevant cell types and organoids (Parfitt et al., 2016; Boutry et al., 2018; Dvela-Levitt et al., 2019; Flemming et al., 2020; De Rus Jacquet et al., 2021; Romano et al., 2021).

An important contribution of cell biology analyses to our understanding of the impact of missense and truncation mutations to pathological conditions relates to protein *availability*. For example, mislocalization and delayed traffic leads to lack of protein availability when and where the target is needed causing phenotypic manifestations (Jacoby et al., 2009; Braun and Schweizer, 2017; Dunmore et al., 2020; Ramadesikan et al., 2021). Therefore, steady state and time-lapse microscopy localization studies are required (Wiegerinck et al., 2014; Bono et al., 2020; Murakami et al., 2020; Rodger et al., 2020; Mamais et al., 2021). These methodologies also open the possibility to be used as readouts for high throughput screens to identify affected biochemical circuits and/or candidate therapeutic agents.

Although currently used in only a few studies, techniques such as superresolution (Sumya et al., 2021; Zimmer et al., 2022) and light-sheet (Adhya et al., 2021) microscopies are already showing high value for genetic disease research. In addition, other important technical approaches used in various disciplines such as intravital microscopy (Frattolin et al., 2021), cell tracking (Betjes et al., 2021), atomic force microscopy (Dufrêne and Pelling, 2013; Sharma et al., 2018), single molecule imaging (Liu et al., 2022) and cryoelectron microscopy (Douguet et al., 2019) or tomography (Shoemark, 2017) are expected to increase in utilization for mechanistic studies related to genetic diseases.

Moreover, the specific case of mutations affecting protein sorting signals (Mukherjee et al., 2012; Aguilar, 2015) is likely not to be detected by routine sequence analysis and standard biochemical approaches, requiring appropriate studies and interpretation of intracellular localization data to reveal cellular abnormalities. Along the same lines, evaluating the specific role of a mutated protein potentially involved in protein traffic/sorting in a disease (e.g., coatopathies (Dell'angelica and Bonifacino, 2019)) also requires cell biological analysis and expertise. Indeed, proficiency in this field and relevant approaches are needed to differentiate true sorting defects from other effects of mutations (such as ER-retention due to membrane-protein misfolding) that do not impact sorting signal recognition or the functionality of the trafficking machinery.

ADVANCES TOWARDS THE USE OF AN INTEGRATED AND UPDATED MULTIDISCIPLINARY TOOLBOX

The previous section enumerates some approaches and novel developments involving the different steps required for constructing hypotheses about the genetic disease mechanism and framework for therapy design. Although integration of such methods in single multidisciplinary studies constitute an ideal scientific strategy to test overarching hypotheses, published examples more commonly involve using only some of the above nominated approaches. These partially integrated views are, of course, valuable and can be composed to yield an enhanced overview of the disease.

For example, a recent study identified mutations in *VPS4A* associated with a new multisystem disorder (with the proposed name CIMDAG: Cerebellar hypoplasia and cataracts, Intellectual disability, Microcephaly, Dystonia and Anemia, Growth retardation) showing striking neurodevelopmental abnormalities (Rodger et al., 2020). *VPS4A* encodes an ATPase that plays a vital role in ESCRT-III regulation and subsequent vesicle budding into multi-vesicular bodies. Novel *de novo* patient

mutations were identified using WES/WGS approaches and their impact was analyzed using computational tools such as REVEL (Ioannidis et al., 2016) and CADD (Rentzsch et al., 2019). A thorough investigation of the pathogenesis mechanisms included assessing protein stability, localization in patient fibroblasts (Rodger et al., 2020). The authors demonstrated that while patient mutations did not impact Vps4a stability, they induced an increase in the fraction of enlarged endosomes (displaying different markers) and retention of the atypical ESCRT-III protein Ist1 (Increased Sodium Tolerance 1—involved in membrane fission) in the early endosomal compartment. Further, the study also characterized cellular phenotypes (including abnormalities in cell cycle and mitotic spindles, cilia and centrosome morphology) caused by a loss of Vps4a using CRISPRi in iPSC cell lines and neurons differentiated from these cell lines. These investigations provided clues into cellular and molecular mechanisms that may explain various clinical symptoms seen in patients. For example, numerous clinical features seen in ciliopathies have been observed in these patients and can be associated with defects in ciliogenesis while abnormalities in centrosome/mitotic spindle morphology may explain microencephaly observed in CIMDAG (Rodger et al., 2020).

Another example is the case of research applied to the study of Lowe syndrome (LS). This lethal genetic disease caused by abnormal function of the lipid phosphatase Ocrl1 (Oculo-Cerebro-Renal syndrome of Lowe) is characterized by mental retardation, bilateral congenital cataracts, and renal dysfunction (Recker et al., 2013).

The use of WES/WGS (Duran et al., 2016; Zheng et al., 2019) led to the discovery of novel LS causing mutations in the *OCRL1* gene, including several in non-coding regions, while molecular dynamics predicted structural consequences of such mutations (Ramadesikan et al., 2021). These studies were complemented by biochemical and cell biological assays to assess the mutational impact on protein function and availability (De Leo et al., 2016; Ramadesikan et al., 2021). Furthermore, models were prepared by various methods to gain mechanistic insight into LS such as KO/morpholino zebrafish lines (Coon et al., 2012; Ramirez et al., 2012; Gliozzi et al., 2020), humanized mouse models (Bothwell et al., 2011), Ocrl1-deficient cells by CRISPR (Madhivanan et al., 2020) along with stable lines expressing specific variants (Ramadesikan et al., 2021), and patient-derived iPSCs (Barnes et al., 2018; Hsieh et al., 2018; Liu et al., 2020; Akhtar et al., 2022). Taken together, LS research has greatly benefitted from the assimilation of a variety of techniques and is rapidly moving towards understanding LS in a patient mutation-specific manner to better guide the design novel therapeutic approaches.

FINAL REMARKS

In summary, we predict that the systematic utilization of an updated multidisciplinary toolbox including advanced approaches and multi-pronged strategies to study genetic disease will make possible the next quantum leaps in the understanding and treatment of genetic conditions.

Although we can accomplish these objectives by expanding/diversifying our own expertise, the more likely and efficient approach is to assemble multidisciplinary teams. For example, our (and others') research in Lowe syndrome greatly benefitted from successful collaborations with bioinformaticians, computer scientists, structural biologists, nephrologists, and clinicians. Nevertheless, the success of this team strategy ultimately depends on one's ability to face the challenge of providing proper and fair articulation of different disciplines and different scientific cultures (sometimes suffering serious communication issues and "cultural shocks").

Carefully written Collaboration plans where strategies for communication, management, conflict prevention, self-assessment, etc., are stated (Hall et al., 2019), along with a clear shared view of the project can substantially contribute to cohesiveness of the team and facilitate the pursuit of complex scientific goals (Bennett and Gadlin, 2012; Love et al., 2021). Indeed, collaboration plans are already being required and evaluated by several funding agencies and are likely to become widespread mandatory in the immediate future. Here is where the findings of team science and its emerging strategies (Bennett and Gadlin, 2012; Love et al., 2021) can move our research beyond the sum of disciplines to enter the realm of synergism.

AUTHOR CONTRIBUTIONS

SR, JL, and RA conceived and wrote the article.

REFERENCES

Adhya, D., Chennell, G., Crowe, J. A., Valencia-Alarcón, E. P., Seyforth, J., Hosny, N. A., et al. (2021). Application of Airy Beam Light Sheet Microscopy to Examine Early Neurodevelopmental Structures in 3d Hipsc-Derived Human Cortical Spheroids. *Mol. Autism* 12, 4. doi:10.1186/s13229-021-00413-1

Adolf-Bryfogle, J., and Dunbrack, R. L., Jr. (2013). The Pyrosetta Toolkit: A Graphical User Interface for the Rosetta Software Suite. *Plos One* 8, E66856. doi:10.1371/journal.pone.0066856

Adzhubei, I., Jordan, D. M., and Sunyaev, S. R. (2013). Predicting Functional Effect of Human Missense Mutations Using Polyphen-2. *Curr. Protoc. Hum. Genet* 76, 7.20.1–7.20.41. Chapter 7, Unit7.20. doi:10.1002/0471142905.hg0720s76

Aguilar, R. C. (2015). Introduction to the Analysis of the Intracellular Sorting Information in Protein Sequences: From Molecular Biology to Artificial Neural Networks. *Methods Mol. Biol.* 1260, 1–16. doi:10.1007/978-1-4939-2239-0_1

Akhtar, B. M., Bhatia, P., Acharya, S., Sharma, S., Sharma, Y., Bhuvanendran Nair Suseela Devi, A., et al. (2022). A Human Stem Cell Resource to Decipher the Biochemical and Cellular Basis of Neurodevelopmental Defects in Lowe Syndrome. *Biol. Open* 11. doi:10.1242/bio.059066

Aoki, Y., Manzano, R., Lee, Y., Dafinca, R., Aoki, M., Douglas, A. G. L., et al. (2017). C9orf72 and Rab7l1 Regulate Vesicle Trafficking in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Brain* 140, 887–897. doi:10.1093/brain/awx024

Arystarkhova, E., Haq, I. U., Luebbert, T., Mochel, F., Saunders-Pullman, R., Bressman, S. B., et al. (2019). Factors in the Disease Severity of Atp1a3

Mutations: Impairment, Misfolding, and Allele Competition. *Neurobiol. Dis.* 132, 104577. doi:10.1016/j.nbd.2019.104577

Arystarkhova, E., Ozelius, L. J., Brashear, A., and Sweadner, K. J. (2021). Misfolding, Altered Membrane Distributions, and the Unfolded Protein Response Contribute to Pathogenicity Differences in Na,K-Atpase Atp1a3 Mutations. *J. Biol. Chem.* 296, 100019. doi:10.1074/jbc.ra120.015271

Barnes, J., Salas, F., Mokhtari, R., Dolstra, H., Pedrosa, E., and Lachman, H. M. (2018). Modeling the Neuropsychiatric Manifestations of Lowe Syndrome Using Induced Pluripotent Stem Cells: Defective F-Actin Polymerization and Wave-1 Expression in Neuronal Cells. *Mol. Autism* 9, 44. doi:10.1186/s13229-018-0227-3

Bennett, L. M., and Gadlin, H. (2012). Collaboration and Team Science. *J. Investig. Med.* 60, 768–775. doi:10.2310/jim.0b013e318250871d

Bergamin, N., Dardis, A., Beltrami, A., Cesselli, D., Rigo, S., Zampieri, S., et al. (2013). A Human Neuronal Model of Niemann Pick C Disease Developed from Stem Cells Isolated from Patient's Skin. *Orphanet J. Rare Dis.* 8, 34. doi:10.1186/1750-1172-8-34

Betjes, M. A., Zheng, X., Kok, R. N. U., Van Zon, J. S., and Tans, S. J. (2021). Cell Tracking for Organoids: Lessons from Developmental Biology. *Front. Cel Dev. Biol.* 9, 675013. doi:10.3389/fcell.2021.675013

Bono, K., Hara-Miyauchi, C., Sumi, S., Oka, H., Iguchi, Y., and Okano, H. J. (2020). Endosomal Dysfunction in Ipsc-Derived Neural Cells from Parkinson's Disease Patients with Vps35 D620n. *Mol. Brain* 13, 137. doi:10.1186/s13041-020-00675-5

Bothwell, S. P., Chan, E., Bernardini, I. M., Kuo, Y.-M., Gahl, W. A., and Nussbaum, R. L. (2011). Mouse Model for Lowe Syndrome/Dent Disease 2 Renal Tubulopathy. *Jasn* 22, 443–448. doi:10.1681/asn.2010050565

Boutry, M., Branchu, J., Lustremant, C., Pujol, C., Pernelle, J., Matusiak, R., et al. (2018). Inhibition of Lysosome Membrane Recycling Causes Accumulation of Gangliosides that Contribute to Neurodegeneration. *Cel Rep.* 23, 3813–3826. doi:10.1016/j.celrep.2018.05.098

Braun, D., and Schweizer, U. (2017). The Chemical Chaperone Phenylbutyrate Rescues Mct8 Mutations Associated with Milder Phenotypes in Patients with Allan-Herndon-Dudley Syndrome. *Endocrinology* 158, 678–691. doi:10.1210/en.2016-1530

Carrion, M. D. P., Marsicano, S., Daniele, F., Marte, A., Pischedda, F., Di Cairano, E., et al. (2017). The Lrrk2 G2385r Variant Is A Partial Loss-Of-Function Mutation that Affects Synaptic Vesicle Trafficking through Altered Protein Interactions. *Sci. Rep.* 7, 5377. doi:10.1038/s41598-017-05760-9

Coon, B. G., Hernandez, V., Madhivanan, K., Mukherjee, D., Hanna, C. B., Barinaga-Rementeria Ramirez, I., et al. (2012). The Lowe Syndrome Protein Ocrl1 Is Involved in Primary Cilia Assembly. *Hum. Mol. Genet.* 21, 1835–1847. doi:10.1093/hmg/ddr615

Davies, C. W., Paul, L. N., and Das, C. (2013). Mechanism of Recruitment and Activation of the Endosome-Associated Deubiquitinase Amsh. *Biochemistry* 52, 7818–7829. doi:10.1021/bi401106b

De Leo, M. G., Staiano, L., Vicinanza, M., Luciani, A., Carissimo, A., Mutarelli, M., et al. (2016). Autophagosome-Lysosome Fusion Triggers A Lysosomal Response Mediated by Tlr9 and Controlled by Ocrl. *Nat. Cel Biol* 18, 839–850. doi:10.1038/ncb3386

De Rus Jacquet, A., Tancredi, J. L., Lemire, A. L., Desantis, M. C., Li, W. P., and O'shea, E. K. (2021). The Lrrk2 G2019s Mutation Alters Astrocyte-To-Neuron Communication via Extracellular Vesicles and Induces Neuron Atrophy in A Human Ipsc-Derived Model of Parkinson's Disease. *Elife* 10. doi:10.7554/eLife.73062

Dell'angelica, E. C., and Bonifacino, J. S. (2019). Coatopathies: Genetic Disorders of Protein Coats. *Annu. Rev. Cel Dev. Biol.* 35, 131–168. doi:10.1146/annurev-cellbio-100818-125234

Douguet, D., Patel, A., and Honoré, E. (2019). Structure and Function of Polycystins: Insights into Polycystic Kidney Disease. *Nat. Rev. Nephrol.* 15, 412–422. doi:10.1038/s41581-019-0143-6

Dufrêne, Y. F., and Pelling, A. E. (2013). Force Nanoscopy of Cell Mechanics and Cell Adhesion. *Nanoscale* 5, 4094–4104. doi:10.1039/c3nr00340j

Dunmore, B. J., Yang, X., Crosby, A., Moore, S., Long, L., Huang, C., et al. (2020). 4pba Restores Signalling of A Cysteine-Substituted Mutant Bmpr2 Receptor Found in Patients with Pah. *Am. J. Respir. Cel Mol Biol* 63 (2), 160–171. doi:10.1165/rcmb.2019-0321OC

Duran, D., Jin, S. C., Despenza, T., Nelson-Williams, C., Cogal, A. G., Abrash, E. W., et al. (2016). Digenic Mutations of Human OCRL Paralogs in Dent's Disease Type 2 Associated with Chiari I Malformation. *Hum. Genome* 3, 16042. doi:10.1038/hgv.2016.42

Dvela-Levitt, M., Kost-Alimova, M., Emani, M., Kohnert, E., Thompson, R., Sidhom, E.-H., et al. (2019). Small Molecule Targets Tmed9 and Promotes Lysosomal Degradation to Reverse Proteinopathy. *Cell* 178, 521–535. E23. doi:10.1016/j.cell.2019.07.002

Fausther, M., Lavoie, E. G., Goree, J. R., Baldini, G., and Dranoff, J. A. (2014). Nt5e Mutations That Cause Human Disease Are Associated With Intracellular Mistrafficking Of Nt5e Protein. *Plos One* 9, E98568. doi:10.1371/journal.pone.0098568

Flemming, J., Marczenke, M., Rudolph, I.-M., Nielsen, R., Storm, T., Erik, I. C., et al. (2020). Induced Pluripotent Stem Cell-Based Disease Modeling Identifies Ligand-Induced Decay of Megalin as A Cause of Donnai-Barrow Syndrome. *Kidney Int.* 98, 159–167. doi:10.1016/j.kint.2020.02.021

Frattolin, J., Watson, D. J., Bonneuil, W. V., Russell, M. J., Fasanella Masci, F., Bandara, M., et al. (2021). The Critical Importance of Spatial and Temporal Scales in Designing and Interpreting Immune Cell Migration Assays. *Cells* 10. doi:10.3390/cells10123439

Freedman, B. S., Brooks, C. R., Lam, A. Q., Fu, H., Morizane, R., Agrawal, V., et al. (2015). Modelling Kidney Disease with Crispr-Mutant Kidney Organoids Derived from Human Pluripotent Epiblast Spheroids. *Nat. Commun.* 6, 8715. doi:10.1038/ncomms9715

Ge, F., Zhu, Y.-H., Xu, J., Muhammad, A., Song, J., and Yu, D.-J. (2021). Muttmpredictor: Robust and Accurate Cascade Xgboost Classifier for Prediction of Mutations in Transmembrane Proteins. *Comput. Struct. Biotechnol. J.* 19, 6400–6416. doi:10.1016/j.csbj.2021.11.024

Gliozzi, M. L., Espiritu, E. B., Shipman, K. E., Rbaibi, Y., Long, K. R., Roy, N., et al. (2020). Effects of Proximal Tubule Shortening on Protein Excretion in A Lowe Syndrome Model. *Jasn* 31, 67–83. doi:10.1681/asn.2019020125

Guttmann, S., Bernick, F., Naorniakowska, M., Michgehl, U., Groba, S. R., Socha, P., et al. (2018). Functional Characterization of Novel Atp7b Variants for Diagnosis of Wilson Disease. *Front. Pediatr.* 6, 106. doi:10.3389/fped.2018.00106

Hall, K. L., Vogel, A. L., and Crowston, K. (2019). "'Comprehensive Collaboration Plans: Practical Considerations Spanning across Individual Collaborators to Institutional Supports," in *Strategies for Team Science Success*. Editor K. L. Hall. 1 Ed. (Cham: Springer). doi:10.1007/978-3-030-20992-6_45

Hsieh, W. C., Ramadesikan, S., Fekete, D., and Aguilar, R. C. (2018). Kidney-Differentiated Cells Derived from Lowe Syndrome Patient's Ipscs Show Ciliogenesis Defects and Six2 Retention at the Golgi Complex. *Plos One* 13, E0192635. doi:10.1371/journal.pone.0192635

Hung, C., Tuck, E., Stubbs, V., Van Der Lee, S. J., Aalfs, C., Van Spaendonk, R., et al. (2021). Sorl1 Deficiency in Human Excitatory Neurons Causes App-dependent Defects in the Endolysosome-Autophagy Network. *Cel Rep.* 35, 109259. doi:10.1016/j.celrep.2021.109259

Ioannidis, N. M., Rothstein, J. H., Pejaver, V., Middha, S., Mcdonnell, S. K., Baheti, S., et al. (2016). Revel: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am. J. Hum. Genet.* 99, 877–885. doi:10.1016/j.ajhg.2016.08.016

Jacoby, M., Cox, J. J., Gayral, S., Hampshire, D. J., Ayub, M., Blockmans, M., et al. (2009). Inpp5e Mutations Cause Primary Cilium Signaling Defects, Ciliary Instability And Ciliopathies In Human And Mouse. *Nat. Genet.* 41, 1027–1031. doi:10.1038/ng.427

Kellogg, E. H., Leaver-Fay, A., and Baker, D. (2011). Role of Conformational Sampling in Computing Mutation-Induced Changes in Protein Structure and Stability. *Proteins* 79, 830–838. doi:10.1002/prot.22921

Kwiatkowska, K., Marszałek–Sadowska, E., Traczyk, G., Koprowski, P., Musielak, M., Ługowska, A., et al. (2014). Visualization of Cholesterol Deposits in Lysosomes of Niemann-Pick Type C Fibroblasts Using Recombinant Perfringolysin O. *Orphanet J. Rare Dis.* 9, 64. doi:10.1186/1750-1172-9-64

Liu, H., Barnes, J., Pedrosa, E., Herman, N. S., Salas, F., Wang, P., et al. (2020). Transcriptome Analysis of Neural Progenitor Cells Derived from Lowe Syndrome Induced Pluripotent Stem Cells: Identification of Candidate Genes for the Neurodevelopmental and Eye Manifestations. *J. Neurodevelop Disord.* 12, 14. doi:10.1186/s11689-020-09317-2

Liu, S., Hoess, P., and Ries, J. (2022). Super-Resolution Microscopy for Structural Cell Biology. *Annu. Rev. Biophys* 51, 301–326. doi:10.1146/annurev-biophys-102521-112912

Lizarraga, S. B., Ma, L., Maguire, A. M., Van Dyck, L. I., Wu, Q., Ouyang, Q., et al. (2021). Human Neurons from Christianson Syndrome Ipscs Reveal Mutation-specific Responses to Rescue Strategies. *Sci. Transl Med.* 13. doi:10.1126/scitranslmed.aaw0682

Love, H. B., Cross, J. E., Fosdick, B., Crooks, K. R., Vandewoude, S., and Fisher, E. R. (2021). Interpersonal Relationships Drive Successful Team Science: An Exemplary Case-Based Study. *Humanities Soc. Sci. Commun.* 8. doi:10.1057/s41599-021-00789-8

Macken, W. L., Godwin, A., Wheway, G., Stals, K., Nazlamova, L., Ellard, S., et al. (2021). Biallelic Variants in Copb1 Cause A Novel, Severe Intellectual Disability Syndrome with Cataracts and Variable Microcephaly. *Genome Med.* 13, 34. doi:10.1186/s13073-021-00850-w

Madhivanan, K., Ramadesikan, S., Hsieh, W. C., Aguilar, M. C., Hanna, C. B., Bacallao, R. L., et al. (2020). Lowe Syndrome Patient Cells Display Mtor- and Rhogtpase-dependent Phenotypes Alleviated by Rapamycin and Statins. *Hum. Mol. Genet* 29, 1700–1715. doi:10.1093/hmg/ddaa086

Mamais, A., Kluss, J. H., Bonet-Ponce, L., Landeck, N., Langston, R. G., Smith, N., et al. (2021). Mutations in Lrrk2 Linked to Parkinson Disease Sequester Rab8a to Damaged Lysosomes and Regulate Transferrin-Mediated Iron Uptake in Microglia. *Plos Biol.* 19, E3001480. doi:10.1371/journal.pbio.3001480

Marom, R., Burrage, L. C., Venditti, R., Clément, A., Blanco-Sánchez, B., Jain, M., et al. (2021). Copb2 Loss of Function Causes A Coatopathy with Osteoporosis and Developmental Delay. *Am. J. Hum. Genet.* 108, 1710–1724. doi:10.1016/j.ajhg.2021.08.002

Min, Y.-L., Chemello, F., Li, H., Rodriguez-Caycedo, C., Sanchez-Ortiz, E., Mireault, A. A., et al. (2020). Correction of Three Prominent Mutations in Mouse and Human Models of Duchenne Muscular Dystrophy by Single-Cut Genome Editing. *Mol. Ther.* 28, 2044–2055. doi:10.1016/j.ymthe.2020.05.024

Mukherjee, D., Hanna, C. B., and Aguilar, R. C. (2012). Artificial Neural Network for the Prediction of Tyrosine-Based Sorting Signal Recognition by Adaptor Complexes. *J. Biomed. Biotechnol.* 2012, 498031. doi:10.1155/2012/498031

Murakami, H., Tamura, N., Enomoto, Y., Shimasaki, K., Kurosawa, K., and Hanada, K. (2020). Intellectual Disability-Associated Gain-Of-Function Mutations in Cert1 that Encodes the Ceramide Transport Protein Cert. *Plos One* 15, E0243980. doi:10.1371/journal.pone.0243980

Ng, P. C., and Henikoff, S. (2003). Sift: Predicting Amino Acid Changes that Affect Protein Function. *Nucleic Acids Res.* 31, 3812–3814. doi:10.1093/nar/gkg509

Ng, S. B., Buckingham, K. J., Lee, C., Bigham, A. W., Tabor, H. K., Dent, K. M., et al. (2010). Exome Sequencing Identifies the Cause of A Mendelian Disorder. *Nat. Genet.* 42, 30–35. doi:10.1038/ng.499

Ng, S. B., Turner, E. H., Robertson, P. D., Flygare, S. D., Bigham, A. W., Lee, C., et al. (2009). Targeted Capture and Massively Parallel Sequencing of 12 Human Exomes. *Nature* 461, 272–276. doi:10.1038/nature08250

Parfitt, D. A., Lane, A., Ramsden, C. M., Carr, A.-J. F., Munro, P. M., Jovanovic, K., et al. (2016). Identification and Correction of Mechanisms Underlying Inherited Blindness in Human Ipsc-Derived Optic Cups. *Cell Stem Cell* 18, 769–781. doi:10.1016/j.stem.2016.03.021

Ponomareva, O. Y., Eliceiri, K. W., and Halloran, M. C. (2016). Charcot-Marie-Tooth 2b Associated Rab7 Mutations Cause Axon Growth and Guidance Defects during Vertebrate Sensory Neuron Development. *Neural Dev.* 11, 2. doi:10.1186/s13064-016-0058-x

Ramadesikan, S., Skiba, L., Lee, J., Madhivanan, K., Sarkar, D., De La Fuente, A., et al. (2021). Genotype & Phenotype in Lowe Syndrome: Specific Ocrl1 Patient Mutations Differentially Impact Cellular Phenotypes. *Hum. Mol. Genet.* 30, 198–212. doi:10.1093/hmg/ddab025

Ramirez, I. B.-R., Pietka, G., Jones, D. R., Divecha, N., Alia, A., Baraban, S. C., et al. (2012). Impaired Neural Development in A Zebrafish Model for Lowe Syndrome. *Hum. Mol. Genet.* 21, 1744–1759. doi:10.1093/hmg/ddr608

Recker, F., Reutter, H., and Ludwig, M. (2013). Lowe Syndrome/Dent-2 Disease: A Comprehensive Review of Known and Novel Aspects. *J. Pediatr. Genet.* 2, 53–68. doi:10.3233/PGE-13049

Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., and Kircher, M. (2019). Cadd: Predicting the Deleteriousness of Variants throughout the Human Genome. *Nucleic Acids Res.* 47, D886–D894. doi:10.1093/nar/gky1016

Rodger, C., Flex, E., Allison, R. J., Sanchis-Juan, A., Hasenahuer, M. A., Cecchetti, S., et al. (2020). De Novo Vps4a Mutations Cause Multisystem Disease with Abnormal Neurodevelopment. *Am. J. Hum. Genet.* 107, 1129–1148. doi:10.1016/j.ajhg.2020.10.012

Romano, R., Rivellini, C., De Luca, M., Tonlorenzi, R., Beli, R., Manganelli, F., et al. (2021). Alteration of the Late Endocytic Pathway in Charcot-Marie-Tooth Type 2b Disease. *Cell. Mol. Life Sci.* 78, 351–372. doi:10.1007/s00018-020-03510-1

Saveri, P., De Luca, M., Nisi, V., Pisciotta, C., Romano, R., Piscosquito, G., et al. (2020). Charcot-Marie-Tooth Type 2b: A New Phenotype Associated with A Novel Rab7a Mutation and Inhibited Egfr Degradation. *Cells* 9. doi:10.3390/cells9041028

Schwarz, J. M., Rödelsperger, C., Schuelke, M., and Seelow, D. (2010). Mutationtaster Evaluates Disease-Causing Potential of Sequence Alterations. *Nat. Methods* 7, 575–576. doi:10.1038/nmeth0810-575

Sharma, S., Leclaire, M., and Gimzewski, J. K. (2018). Ascent of Atomic Force Microscopy as A Nanoanalytical Tool for Exosomes and Other Extracellular Vesicles. *Nanotechnology* 29, 132001. doi:10.1088/1361-6528/aaab06

Shoemark, A. (2017). Applications of Emerging Transmission Electron Microscopy Technology in Pcd Research and Diagnosis. *Ultrastructural Pathol.* 41, 408–414. doi:10.1080/01913123.2017.1365789

Sumya, F. T., Pokrovskaya, I. D., and Lupashin, V. (2021). Development and Initial Characterization of Cellular Models for Cog Complex-Related Cdg-Ii Diseases. *Front. Genet.* 12, 733048. doi:10.3389/fgene.2021.733048

Tucker, T. A., Fortenberry, J. A., Zsembery, A., Schwiebert, L. M., and Schwiebert, E. M. (2012). The ΔF508-CFTR Mutation Inhibits Wild-type CFTR Processing and Function when Co-expressed in Human Airway Epithelia and in Mouse Nasal Mucosa. *Bmc Physiol.* 12, 12. doi:10.1186/1472-6793-12-12

Usmani, M. A., Ahmed, Z. M., Magini, P., Pienkowski, V. M., Rasmussen, K. J., Hernan, R., et al. (2021). De Novo And Bi-allelic Variants in Ap1g1 Cause Neurodevelopmental Disorder with Developmental Delay, Intellectual Disability, and Epilepsy. *Am. J. Hum. Genet.* 108, 1330–1341. doi:10.1016/j.ajhg.2021.05.007

Vanier, M. T., and Latour, P. (2015). Laboratory Diagnosis of Niemann-Pick Disease Type C: The Filipin Staining Test. *Methods Cel Biol* 126, 357–375. doi:10.1016/bs.mcb.2014.10.028

Visentin, S., De Nuccio, C., Bernardo, A., Pepponi, R., Ferrante, A., Minghetti, L., et al. (2013). The Stimulation of Adenosine A2a Receptors Ameliorates the Pathological Phenotype of Fibroblasts from Niemann-Pick Type C Patients. *J. Neurosci.* 33, 15388–15393. doi:10.1523/jneurosci.0558-13.2013

Wanikawa, M., Nakamura, H., Emori, S., Hashimoto, N., and Murayama, T. (2020). Accumulation of Sphingomyelin in Niemann-Pick Disease Type C Cells Disrupts Rab9-dependent Vesicular Trafficking of Cholesterol. *J. Cel Physiol* 235, 2300–2309. doi:10.1002/jcp.29137

Wiegerinck, C. L., Janecke, A. R., Schneeberger, K., Vogel, G. F., van Haaften–Visser, D. Y., Escher, J. C., et al. (2014). Loss of Syntaxin 3 Causes Variant Microvillus Inclusion Disease. *Gastroenterology* 147, 65–68. E10. doi:10.1053/j.gastro.2014.04.002

Zheng, B., Chen, Q., Wang, C., Zhou, W., Chen, Y., Ding, G., et al. (2019). Whole-Genome Sequencing Revealed an Interstitial Deletion Encompassing Ocrl and Smarca1 Gene in A Patient with Lowe Syndrome. *Mol. Genet. Genomic Med.* 7, E876. doi:10.1002/mgg3.876

Zimmer, S. E., Takeichi, T., Conway, D. E., Kubo, A., Suga, Y., Akiyama, M., et al. (2022). Differential Pathomechanisms of Desmoglein 1 Transmembrane Domain Mutations in Skin Disease. *J. Invest. Dermatol.* 142, 323–332. E8. doi:10.1016/j.jid.2021.07.154

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