



Genetic Analysis of Children With Unexplained Developmental Delay and/or Intellectual Disability by Whole-Exome Sequencing

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Background: Whole-exome sequencing (WES) has been recommended as a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders (NDDs). We aimed to identify the genetic causes of 17 children with developmental delay (DD) and/or intellectual disability (ID).

Methods: WES and exome-based copy number variation (CNV) analysis were performed for 17 patients with unexplained DD/ID.

Results: Single-nucleotide variant (SNV)/small insertion or deletion (Indel) analysis and exome-based CNV calling yielded an overall diagnostic rate of 58.8% (10/17), of which diagnostic SNVs/Indels accounted for 41.2% (7/17) and diagnostic CNVs accounted for 17.6% (3/17).

Conclusion: Our findings expand the known mutation spectrum of genes related to DD/ID and indicate that exome-based CNV analysis could improve the diagnostic yield of patients with DD/ID.

Keywords: whole-exome sequencing, developmental delay, intellectual disability, exome-based CNV analysis, variants

BACKGROUND

Developmental delay (DD) and intellectual disability (ID) are major manifestations of neurodevelopmental disorders (NDDs) with a global prevalence of 1%–3% (Thapar et al., 2017; Ismail and Shapiro, 2019). DD/ID shows phenotypic pleiotropy, and the underlying cause of DD/ID is heterogeneous, in which genetic factors such as copy number variations (CNVs) and variants in single genes have been recognized as major reasons (Savatt and Myers, 2021). With the advent of next-generation sequencing (NGS), the field of genetics was transformed; and the number of genes known to be associated with DD/ID has increased significantly. For example, over 700 genes have been identified in X-linked, autosomal-dominant, and autosomal-recessive ID until 2015 (Vissers et al., 2016).

Chromosomal microarray has been recommended as a first-tier clinical test to identify chromosomal CNVs and regions of homozygosity in individuals with DD/ID, autism spectrum disorders (ASDs), or multiple congenital anomalies with a diagnostic yield of 15%–20% (Manning et al., 2010; Miller et al., 2010). Whole-exome sequencing (WES) could detect single-nucleotide

TABLE 1 | Summary of patients' clinical manifestations and molecular diagnoses.

Patient ID/ sex/ age	Phenotype	Gene/ locus	Diagnosis (OMIM phenotype)	Variants	Zygosity	Variant type	Inheritance	Classification (ACMG)	References (PMID)
P1/ male/ 2 years	Global developmental delay, intellectual disability	<i>MED13L</i>	Developmental delay and distinctive facial features with or without cardiac defects (#616789)	NM_015335.4: c.1284_1,285 insTTTAAGCTTTT (p.Lys429Phefs*7)	Heterozygous	Frameshift	AD; <i>de novo</i>	P	—
P2/ male/ 8 years	Perinatal polyhydramnios, intellectual disability, global developmental delay, ataxia, anemia	—	—	—	—	—	—	—	—
P3/ male/ 5 years	Spastic cerebral palsy, febrile seizure, global developmental delay, mild intellectual disability, muscular hypertonia	—	—	—	—	—	—	—	—
P4/ male/ 6 years	Bilateral dislocation of hip joints, clubfoot, strabismus, global developmental delay, intellectual disability	—	—	—	—	—	—	—	—
P5/ male/ 6 years	Cerebral atrophy, seizure, global developmental delay, intellectual disability, muscular hypertonia	<i>CNPY3</i>	Developmental and epileptic encephalopathy 60 (#617929)	NM_006586.4: c.283C > G (p.Arg95Gly); c.834del (p.Ter279Gluext*8)	Compound heterozygous	Missense, frameshift	AR maternally inherited, paternally inherited	VUS, VUS	—
P6/ female/ 9 years	Intellectual disability, global developmental delay, hypermetropia	—	—	—	—	—	—	—	—
P7/ male/ 3 years	Global developmental delay, intellectual disability	<i>SCN2A</i>	Developmental and epileptic encephalopathy 11 (#613721); episodic ataxia, type 9 (#618924); seizures, benign familial infantile, 3 (#607745)	NM_021007.3: c.1117del (p.Ala373Profs*9)	Heterozygous	Deletion	AD; <i>de novo</i>	P	—
P8/ male/ 4 years	Global developmental delay, agenesis of the corpus callosum, low anterior hairline, strabismus	<i>ARID1B</i>	Coffin-Siris syndrome 1 (#135900)	NM_001374828.1: c.4194T > G (p.Tyr1398*)	Heterozygous	Nonsense	AD; <i>de novo</i>	P	—
P9/ male/ 6 years	Intellectual disability, seizure, autism, cerebral	<i>PRRT2</i>	Convulsions, familial infantile, with paroxysmal	NM_145,239.2: c.649dup (p.Arg217Profs*8)	Heterozygous	Insertion	AD; maternally inherited	P	22101681, 22870186, 22877996, (Continued on following page)

TABLE 1 | (Continued) Summary of patients' clinical manifestations and molecular diagnoses.

Patient ID/ sex/ age	Phenotype	Gene/ locus	Diagnosis (OMIM phenotype)	Variants	Zygosity	Variant type	Inheritance	Classification (ACMG)	References (PMID)
	white matter atrophy		choreoathetosis (#602066); episodic kinesigenic dyskinesia 1 (#128200); seizures, benign familial infantile, 2 (#605751)						25667652, 22243967
P10/ female/ 5 years	Tetralogy of Fallot, autism, global developmental delay	7q11.23	Chromosome 7q11.23 duplication syndrome (#609757)	chr7:72649202-74191713dup, 1.54 Mb	Heterozygous	CNV	<i>De novo</i>	P	19249392, 26333794, 19752158
P11/ male/ 9 years	Moderate intellectual disability, global developmental delay	—	—	—	—	—	—	—	—
P12/ male/ 7 years	Mild intellectual disability, delayed speech and language development, low-set ears, downslanting palpebral fissures, short penis	<i>SETBP1</i>	Developmental delay, autosomal dominant 29 (#616078); Schinzel-Giedion midface retraction syndrome (#269150)	NM_015559.3: c.2311dup (p.Ser771Phefs*26)	Heterozygous	Frameshift	AD; unknown	LP	—
P13/ male/ 2 years	Premature birth, delayed speech and language development	—	—	—	—	—	—	—	—
P14/ male/ 7 years	Intellectual disability, global developmental delay	<i>GRIN2B</i>	Intellectual developmental disorder, autosomal dominant 6, with or without seizures (#613970); Developmental and epileptic encephalopathy 27 (#616139)	NM_000834.4: c.1711del (p.Ala571Profs*80)	Heterozygous	Deletion	AD; <i>de novo</i>	P	—
P15/ male/ 7 years	Prenatal hydrocephalus, global developmental delay, mild intellectual disability, retrognathia, strabismus	8p21.2p12	—	chr8:25184869-31641821del, 6.46 Mb	Heterozygous	CNV	<i>De novo</i>	P	—
P16/ male/ 6 years	Global developmental delay, moderate intellectual disability, neonatal feeding difficulties, strabismus	19p13.2	—	chr19:1,3044343-13227605del, 183.3 kb	Heterozygous	CNV	<i>De novo</i>	P	—

(Continued on following page)

TABLE 1 | (Continued) Summary of patients' clinical manifestations and molecular diagnoses.

Patient ID/ sex/ age	Phenotype	Gene/ locus	Diagnosis (OMIM phenotype)	Variants	Zygosity	Variant type	Inheritance	Classification (ACMG)	References (PMID)
P17/ male/ 4 years	Neonatal asphyxia, global developmental delay, muscular hypertonia	—	—	—	—	—	—	—	—

Abbreviations: CNV, copy number variation; AD, autosomal dominant; AR, autosomal recessive; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

variants (SNVs) and small insertions or deletions (Indels) by whole-exome capture and massively parallel DNA sequencing, which has a diagnostic advantage in situations of genetic heterogeneity or unknown causal genes compared with conventional tests of single gene or gene panels (Monroe et al., 2016). WES is also recommended as a first-tier clinical diagnostic test for individuals with NDDs with an overall diagnostic yield of 36%, including 31% for isolated NDD, and 53% for NDD plus associated conditions, which is greater than the diagnostic yield of CMA (15%–20%) (Srivastava et al., 2019). Recently, CNV calling has been performed by depth-of-sequence coverage analysis of WES data, which enables the detection of deletions and duplications at the exon level (Marchuk et al., 2018).

In this study, WES and exome-based CNV analysis were performed for 17 children with unexplained DD/ID, and a variety of diagnostic variants including SNVs/Indels and CNVs were identified, indicating that WES could help to identify their molecular etiology and the incorporation of CNV calling could improve the diagnostic rate.

METHODS

Patients

This study was approved by the institutional ethics committee of the Affiliated Suzhou Hospital of Nanjing Medical University. Written informed consent was obtained from each patient's parents. This study included 17 children with unexplained DD/ID referred to our center for reproduction and genetics, the affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu, China, from January 2018 to March 2021. The characteristics of each patient including age, gender, and main phenotypes are listed in **Table 1**, and their clinical details are listed in **Supplementary Table S1**. Global DD is defined as a delay in two or more developmental domains of cognition, speech/language, gross/fine motor, social/personal, and activities of daily living (Mithyantha et al., 2017). Severity of ID was determined based on the intelligence quotient (IQ): severe ID (IQ < 40), moderate ID (IQ range 40–60), and mild ID (IQ range 60–70). In the absence of IQ, ID was diagnosed by the pediatric neurologist or geneticist.

Whole-Exome Sequencing and Data Analysis

Genomic DNA was extracted from the whole blood of the patients and their parents. WES was performed using the SureSelect Human All Exon Kit (Agilent, Santa Clara, CA, USA) and Illumina NovaSeq 6,000 platform (Illumina, San Diego, CA, USA). The sequencing reads were aligned to the human reference genome (hg19/GRCh37) using Burrows-Wheeler Aligner tool, and PCR duplicates were removed by Picard v1.57 (<http://picard.sourceforge.net/>). The fraction of target bases covered at least 20× should be over 96%, with an average sequencing depth on target bases of over 100×. GATK (<https://software.broadinstitute.org/gatk/>) was employed for identifying the SNVs and Indels. Variant annotation and interpretation were conducted by ANNOVAR (Wang et al., 2010). The variants were searched in the dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>), 1000 Genomes Project database (<http://www.1000genomes.org/>), Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>), and the Genome Aggregation database (gnomAD) (<http://gnomad.broadinstitute.org/>). The pathogenicity of mutations was predicted by PROVEAN (<http://provean.jcvi.org/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), and MutationTaster (<http://www.mutationtaster.org/>). Disease and phenotype databases and published literature, such as OMIM (<http://www.omim.org/>), ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>), HGMD (<http://www.hgmd.org/>), and PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>), were also searched. The variants were classified according to the Standards and Guidelines for the Interpretation of Sequence Variants released by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (Richards et al., 2015). Finally, the variants with minor allele frequency <0.05 were selected for further interpretation considering ACMG category, evidence of pathogenicity, clinical synopsis, and inheritance mode of associated disease.

Sanger Sequencing

To validate the WES results, the identified candidate variants were amplified by PCR using genomic DNA from the patients and their parents. The primers used for PCR are listed in **Supplementary Table S2**. The PCR products were purified

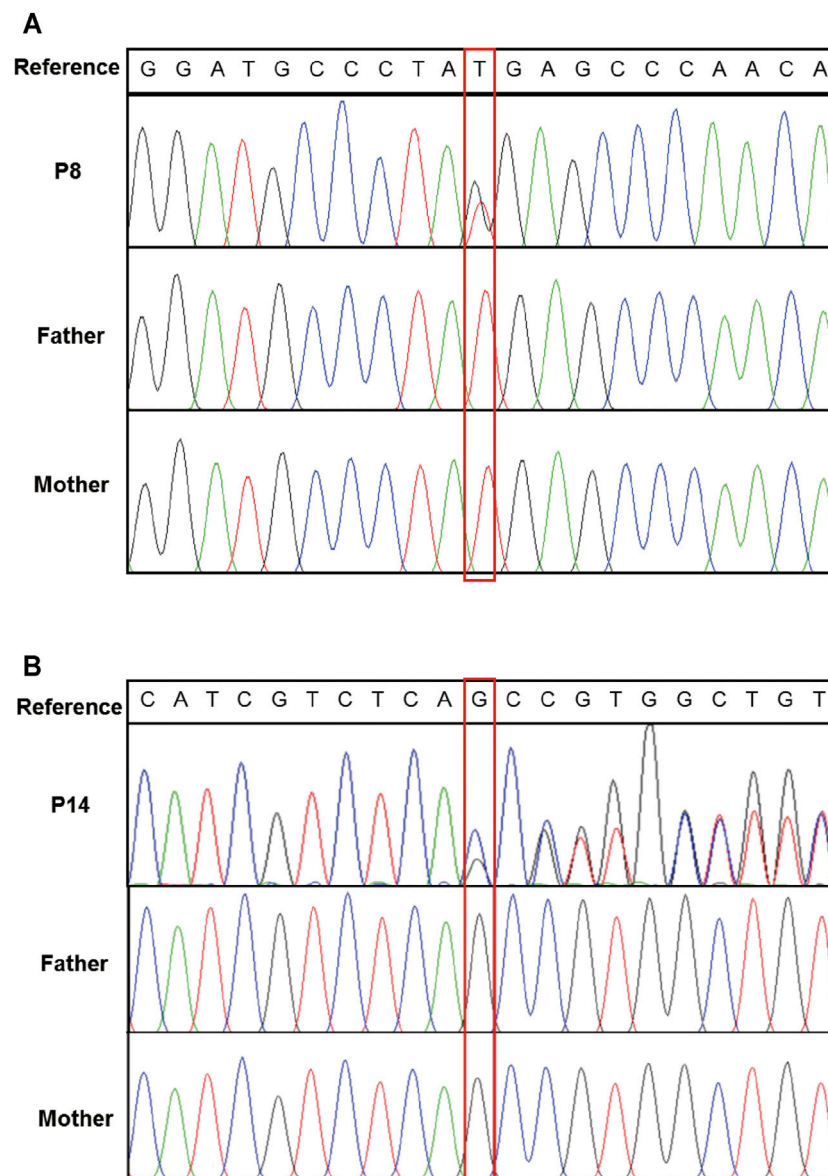


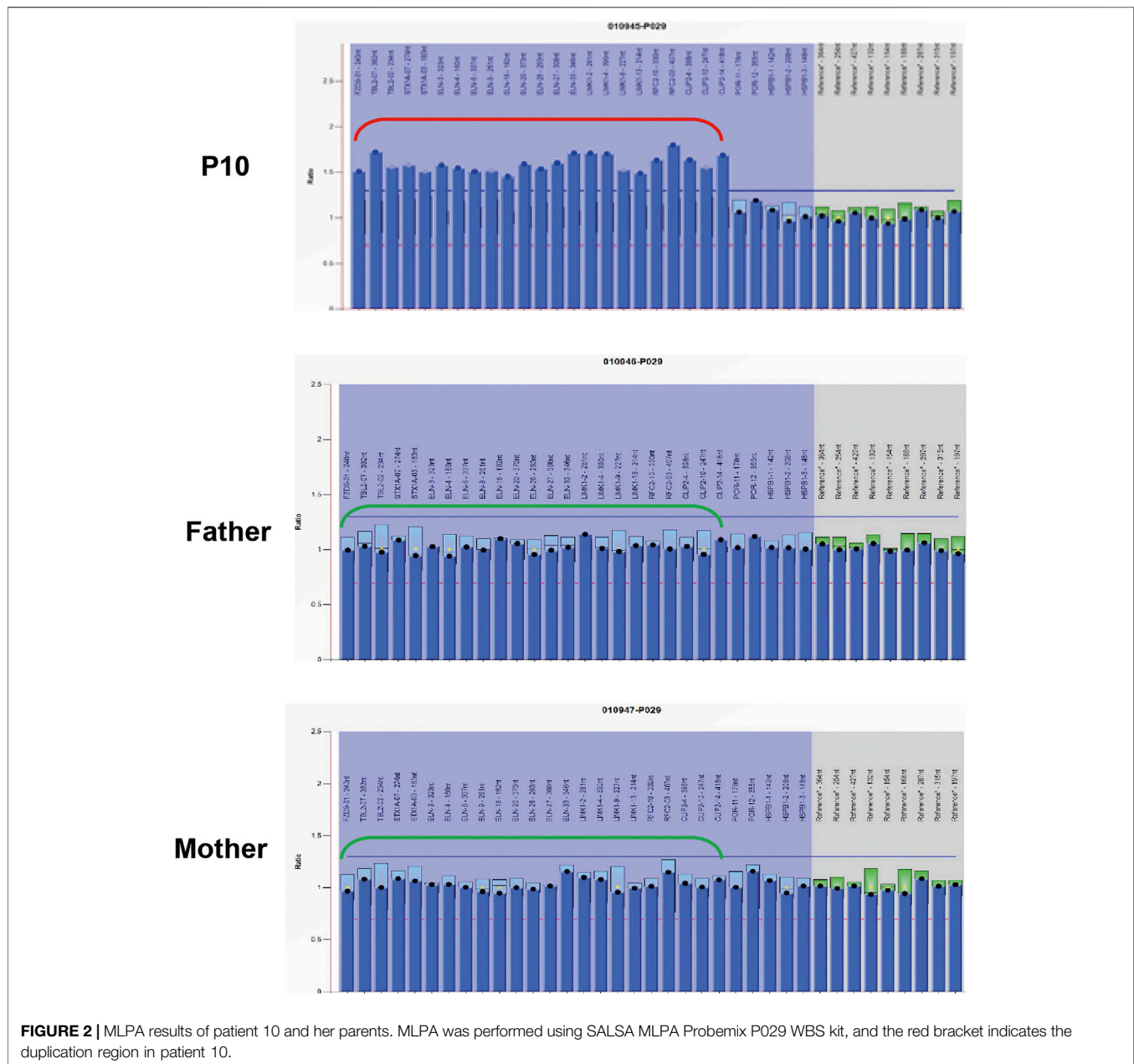
FIGURE 1 | Confirmation of *de novo* variants by Sanger sequencing. **(A)** Sanger sequencing of *ARID1B* in patient 8 and his parents. **(B)** Sanger sequencing of *GRIN2B* in patient 14 and his parents. Variants were indicated by red boxes.

and sequenced in two orientations using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The mutation sites were analyzed by comparison with the GenBank reference sequences of each candidate gene.

Exome-Based CNV Detection and Validation

A comprehensive tool was used for CNV calling. It included XHMM (<http://atgu.mgh.harvard.edu/xhmm>) and PCA method to remove sequencing noise and CNVkit (<https://github.com/etal/cnvkit>) fix module to perform GC and bias

correction; and then copy number calculation and CNV identification were performed in exons and long segment areas. The identified CNVs were interpreted according to the standards and guidelines for interpretation and reporting of postnatal constitutional CNVs released by the ACMG and the technical standards for the interpretation and reporting of constitutional copy-number variants recommended by the ACMG and the Clinical Genome Resource (ClinGen) (Kearney et al., 2011; Riggs et al., 2020). CNVs were further validated by multiplex ligation-dependent probe amplification (MLPA) or quantitative PCR (qPCR) or CNV sequencing (CNV-seq).



RESULTS

A total of 17 children with DD/ID were enrolled and analyzed by WES. As shown in **Table 1**, the age of 17 probands in this study ranged from 2 to 9 years, with a mean age of 5.6 years. Male-to-female ratio was 15:2. The clinical characteristics of 17 probands include global DD (14/17) and ID (13/17) (**Table 1**, **Supplementary Table S1**).

Diagnostic Yields

Among the 17 probands, WES in family trios (Trio-WES) was performed for 14 patients (patients 1–11 and 15–17). Patient 12 and his father underwent WES as a father–proband duo, for the

sample of the patient’s mother was unavailable. And patients 13 and 14 had singleton WES. Analysis of WES data revealed that the coverage for over 97% of the targeted bases were over 20×, with an average sequencing depth of over 100×. An overall diagnostic rate of 58.8% (10/17) was achieved after analysis of SNV/Indel and CNV, of which diagnostic SNVs/Indels accounted for 41.2% (7/17) and diagnostic CNVs accounted for 17.6% (3/17) (**Table 1**).

A total of eight variants in seven genes were identified in seven probands by SNV/Indel analysis and confirmed by Sanger sequencing, including six variants in six genes associated with autosomal dominant disorders and two compound heterozygous variants in *CNPY3* gene related to an autosomal recessive

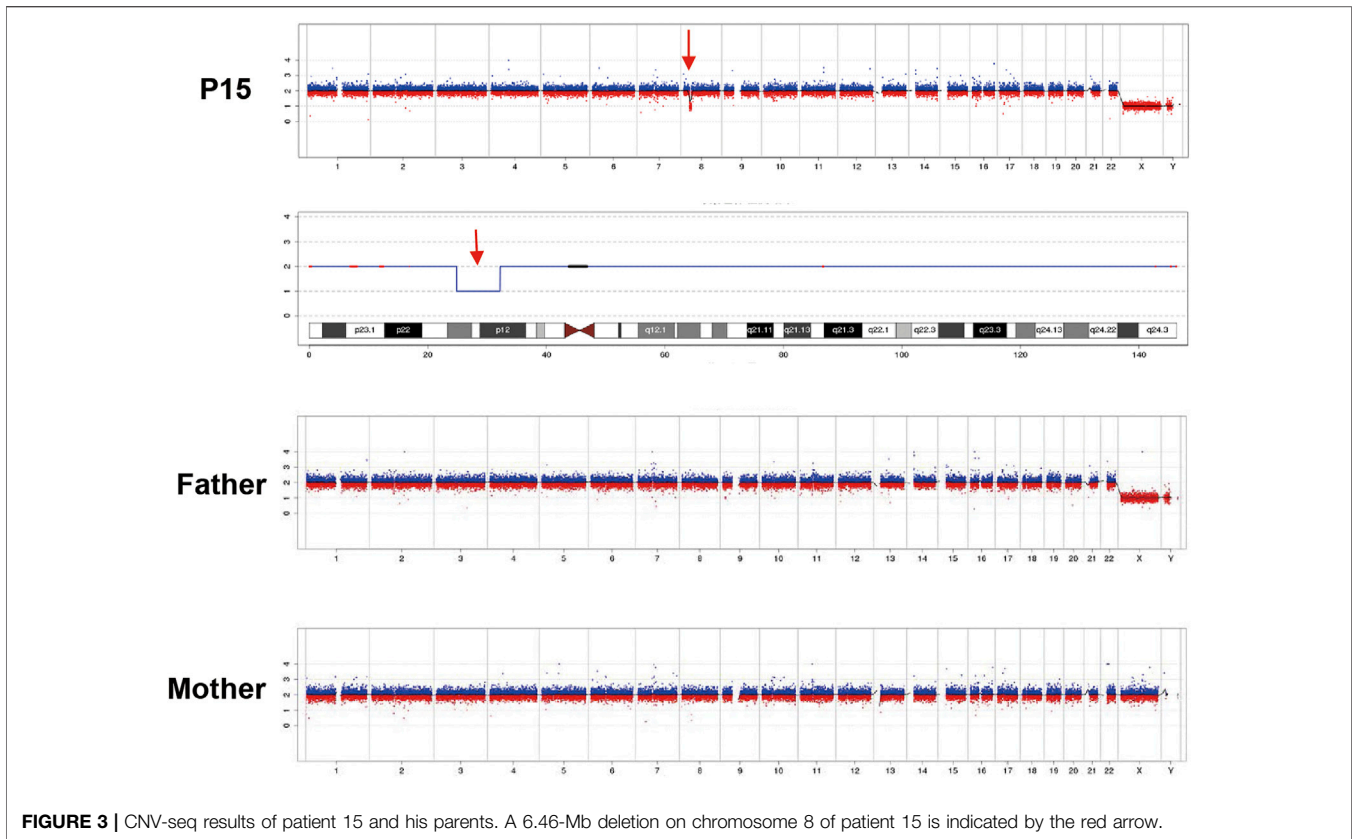


FIGURE 3 | CNV-seq results of patient 15 and his parents. A 6.46-Mb deletion on chromosome 8 of patient 15 is indicated by the red arrow.

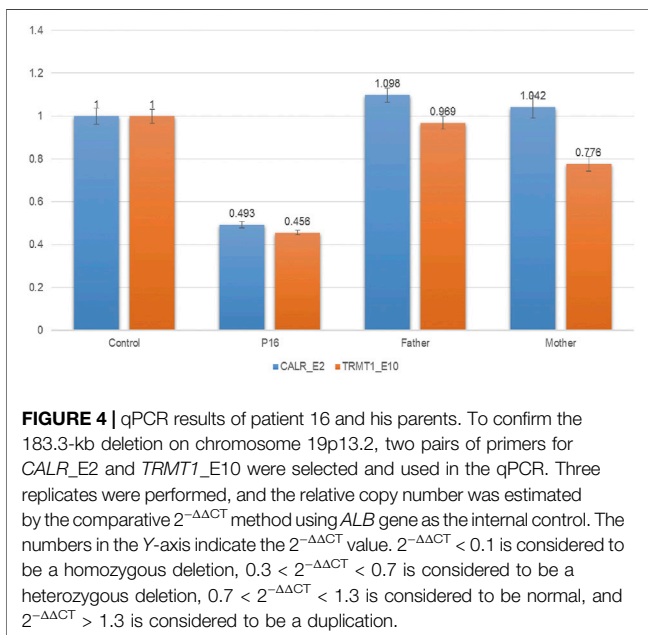


FIGURE 4 | qPCR results of patient 16 and his parents. To confirm the 183.3-kb deletion on chromosome 19p13.2, two pairs of primers for *CALR_E2* and *TRMT1_E10* were selected and used in the qPCR. Three replicates were performed, and the relative copy number was estimated by the comparative $2^{-\Delta\Delta CT}$ method using *ALB* gene as the internal control. The numbers in the Y-axis indicate the $2^{-\Delta\Delta CT}$ value. $2^{-\Delta\Delta CT} < 0.1$ is considered to be a homozygous deletion, $0.3 < 2^{-\Delta\Delta CT} < 0.7$ is considered to be a heterozygous deletion, $0.7 < 2^{-\Delta\Delta CT} < 1.3$ is considered to be normal, and $2^{-\Delta\Delta CT} > 1.3$ is considered to be a duplication.

disorder early infantile epileptic encephalopathy 60 (EIEE60). In autosomal dominant disorders, four variants detected in patients 1, 7, 8, and 14 occurred *de novo* (Figure 1); the origin of a variant in *SETBP1* gene of patient 12 is unknown, for his mother's sample

is unavailable; and a variant in *PRRT2* gene of patient 9 was inherited from his unaffected mother. The two compound heterozygous variants in *CNPY3* gene of patient 5 were inherited from his mother and father (Table 1).

Exome-based CNV analysis revealed three *de novo* pathogenic CNVs. A 1.54-Mb duplication on chromosome 7q11.23 related to chromosome 7q11.23 duplication syndrome (MIM #609757) was identified in patient 10 and confirmed by MLPA (Figure 2). In patient 15, a 6.46-Mb deletion on chromosome 8p21.2p12 was detected and validated by CNV-seq (Figure 3). A 183.3-kb deletion on chromosome 19p13.2 was found in patient 16 and verified by qPCR (Figure 4).

Case Example

Patient 5 is a 6-year-old boy whose parents are healthy and non-consanguineous. He was born full term by natural delivery without abnormalities, weighing 2,900 g. He was bed ridden and presented with global DD, spastic quadriplegia, ID, and intractable seizure. He had multiple admissions for pneumonia and seizure. His first seizure started at age 6 months, and his electroencephalography (EEG) at 2 years of age showed diffuse sharp waves, spike waves, and multiple spike and slow wave complex. MRI of his brain at 6 years old revealed enlargement of the lateral ventricles and cerebral atrophy (Figure 5A).

WES in family trios (Trio-WES) was performed with DNA from patient 5 and his parents. For patient 5, the coverage for 97.87% of the targeted bases was over 20×. Ultimately, two compound heterozygous variants in *CNPY3* gene (c.283C > G

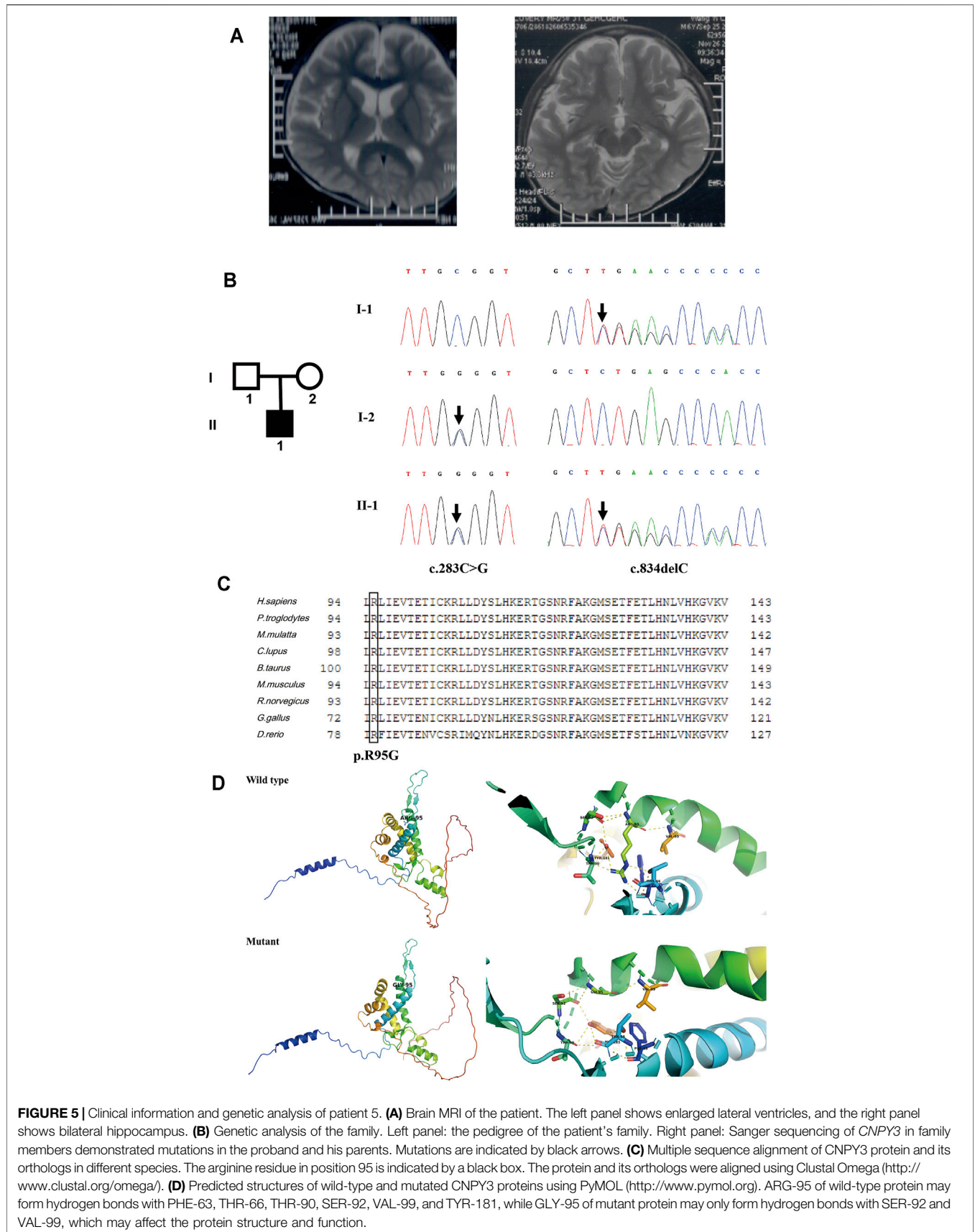


FIGURE 5 | Clinical information and genetic analysis of patient 5. **(A)** Brain MRI of the patient. The left panel shows enlarged lateral ventricles, and the right panel shows bilateral hippocampus. **(B)** Genetic analysis of the family. Left panel: the pedigree of the patient’s family. Right panel: Sanger sequencing of *CNPY3* in family members demonstrated mutations in the proband and his parents. Mutations are indicated by black arrows. **(C)** Multiple sequence alignment of *CNPY3* protein and its orthologs in different species. The arginine residue in position 95 is indicated by a black box. The protein and its orthologs were aligned using Clustal Omega (<http://www.clustal.org/omega/>). **(D)** Predicted structures of wild-type and mutated *CNPY3* proteins using PyMOL (<http://www.pymol.org>). ARG-95 of wild-type protein may form hydrogen bonds with PHE-63, THR-66, THR-90, SER-92, VAL-99, and TYR-181, while GLY-95 of mutant protein may only form hydrogen bonds with SER-92 and VAL-99, which may affect the protein structure and function.

and c.834del) were detected. Sanger sequencing validated the two compound heterozygous variants in the patient. His mother was heterozygous for the c.283C > G variant in exon 3 of *CNPY3* gene, and his father was heterozygous for the c.834del variant in exon six of *CNPY3* gene (Figure 5B). These two variants were not recorded in the dbSNP, 1000 Genomes Project database, ExAC, or gnomAD. The c.283C > G variant was a novel missense mutation causing a substitution from arginine to glycine of *CNPY3* protein (p.Arg95Gly), which is predicted to be deleterious by PROVEAN with a score of -6.62, probably damaging by PolyPhen-2 with a score of 1.0, and disease causing by MutationTaster with a probability value of 0.999. Furthermore, sequence alignment of human *CNPY3* protein and its orthologs in different species revealed that the R95 residue is highly conserved among species (Figure 5C). And protein structural analysis revealed that substitution from arginine to glycine of *CNPY3* protein at position 95 may reduce the formation of hydrogen bonds, thus affecting the protein structure and function (Figure 5D). The c.834del variant is a novel frameshift deletion mutation, which results in a prolonged protein with the addition of eight amino acid residues (EPTQHPLS) to the C-terminal of *CNPY3* protein (p.Ter279Gluext*8). According to the ACMG variant classification guideline (Richards et al., 2015), the c.283C > G variant could be classified as uncertain significance with two supporting (PM2_Supporting and PP3) evidences, and the c.834del variant could be classified as uncertain significance with one moderate (PM4) and one supporting (PM2_Supporting) evidences.

DISCUSSION

In this study, WES was performed for 17 probands with DD/ID with an overall diagnostic yield of 58.8% (10/17), which is consistent with a recent study that WES identified pathogenic variants in 53.5% (54/101) of patients with DD/ID (Hiraide et al., 2021). A total of eight *de novo* variants were detected, including five SNVs/Indels and three CNVs, corroborating the burden of *de novo* variants in DD/ID (Brunet et al., 2021). And further functional studies are needed to elucidate the effect of the identified variants at the transcriptional or translational level. In addition, exome-based CNV analysis revealed three pathogenic CNVs, increasing the diagnostic yield by 17.6% (3/17), which is consistent with a recent study that incorporation of exome-based CNV calling improved the diagnostic rate of trio-WES by 18.92% (14/74) in patients with NDDs (Zhai et al., 2021). However, WES analysis yielded negative results for seven patients in this study, possibly due to technical limitations of WES (e.g., deep intron mutations and structural variants).

SNV/Indel analysis identified eight variants of seven genes, including five novel heterozygous variants in patients 1, 7, 8, 12, and 14; one reported heterozygous variant in patient 9; and two compound heterozygous variants in patient 5. A novel *de novo* heterozygous variant c.1284_1285insTTTAAGCTTTT of *MED13L* gene was detected in patient 1, resulting in a frameshift and a premature stop codon (p.Lys429Phefs*7). The

mediator complex subunit 13-like (MED13L) gene encodes a subunit of the mediator complex that functions as transcriptional regulation by physically linking DNA-binding transcription factors and RNA polymerase II in early development of the heart and brain (Utami et al., 2014). *MED13L* haploinsufficiency is involved in DD and distinctive facial features with or without cardiac defects (MIM #616789). In addition to DD/ID, patient 1 had mild dysmorphic facial features, but cardiac malformations were not observed, which is consistent with previous reports on variable penetrance of cardiac malformations (Adegbola et al., 2015; Cafiero et al., 2015).

For patient 7, a novel *de novo* heterozygous variant c.1117del of *SCN2A* gene was identified, leading to a frameshift and a premature termination (p.Ala373Profs*9). *SCN2A* encodes the voltage-gated sodium channel Nav1.2, which plays a role in the initiation and conduction of action potentials (Wolff et al., 2017). Mutations in *SCN2A* were related to a spectrum of epilepsies and NDDs with phenotypic heterogeneity, including developmental and epileptic encephalopathy 11 (MIM #613721); episodic ataxia type 9 (MIM #618924); and benign familial infantile seizures 3 (MIM #607745). Patient 7 is 3 years old with DD and ID, but he is seizure-free until now. He may have later-onset epilepsy, or he may show ID and/or autism without epilepsy as 16% (32/201) of previously reported cases with *SCN2A* mutations (Wolff et al., 2017).

WES identified a novel *de novo* heterozygous nonsense mutation (c.4194T > G, p. Tyr1398*) of *ARID1B* gene in patient 8. To date, nine genes have been reported to be related to Coffin-Siris syndrome, and mutations of *ARID1B* gene were the most common reason for Coffin-Siris syndrome. *ARID1B* encodes a small subset of SWI/SNF (SWI/SNF/Sucrose Non-Fermentable) complexes that play an important role in chromatin remodeling (Sekiguchi et al., 2019). Agenesis of corpus callosum was detected prenatally and confirmed after birth in patient 8, but hypoplasia of the fifth digits/nails was not observed, which is consistent with a previous report that most patients with corpus callosum anomalies and *ARID1B* mutations (n = 8/11) had normal fingers and toes (Mignot et al., 2016).

For patient 9, a heterozygous variant (c.649dup, p. Arg217Profs*8) of *PRRT2* gene was identified, which was inherited from his mother. *PRRT2* encodes the proline-rich transmembrane protein 2 (PRRT2) and is highly expressed in the brain and spinal cord (Chen et al., 2011). *PRRT2* is associated with familial infantile convulsions with paroxysmal choreoathetosis (MIM #602066), episodic kinesigenic dyskinesia 1 (MIM #128200), and benign familial infantile seizures 2 (MIM #605751); and the penetrance of episodic kinesigenic dyskinesia 1 is estimated to be 60%–90% (van Vliet et al., 2012). The c.649dup variant was a mutation hotspot in families of different origins (Wang et al., 2011; Ono et al., 2012; Schubert et al., 2012), and a homozygous c.649dup mutation was detected in five individuals of an Iranian family with severe non-syndromic ID (Najmabadi et al., 2011). However, it was reported that only 0.6% (8/1,423) of individuals with heterozygous *PRRT2* mutations present ID (Ebrahimi-Fakhari et al., 2015), and *PRRT2* mutations are not related to increased susceptibility to ASD (Huguet et al., 2014),

suggesting that the maternally inherited c.649dup variant of *PRRT2* may not completely explain the phenotypes of ID and autism observed in patient 9.

A novel heterozygous variant c.2311dup of *SETBP1* gene was detected in patient 12, resulting in a frameshift and a premature termination (p.Ser771Phefs*26). *SETBP1* encodes the SET binding protein 1 expressed ubiquitously, but little is known about the function of *SETBP1* (Hoischen et al., 2010). *De novo* gain-of-function variants of *SETBP1* are associated with Schinzel-Giedion midface retraction syndrome (MIM #269150), while haploinsufficiency of *SETBP1* caused by loss-of-function (LoF) variant or a heterozygous gene deletion is related to DD, autosomal dominant 29 (MIM #616078). Patient 12 exhibited mild ID (IQ 65–70), consistent with a recent study that ID of various levels was observed in 77% (23/30) of patients with *SETBP1* haploinsufficiency disorder (Jansen et al., 2021). In addition, another study indicated that aberrant speech and language development are central to *SETBP1* haploinsufficiency disorder (Morgan et al., 2021), and patient 12 also showed impaired speech and language development.

For patient 14, a novel heterozygous variant c.1711del of *GRIN2B* gene was identified, leading to a frameshift and a premature stop codon (p.Ala571Profs*80). *GRIN2B* encodes the subunit NR2B of *N*-methyl-D-aspartate (NMDA) receptors, which are neurotransmitter-gated ion channels involved in the regulation of synaptic function in the central nervous system (Endele et al., 2010). *GRIN2B* is related to intellectual developmental disorder, autosomal dominant 6, with or without seizures (MIM #613970), and developmental and epileptic encephalopathy 27 (MIM #616139). A previous study of 58 individuals with *GRIN2B* encephalopathy revealed that 52% (30/58) of the patients have had seizures with a variable age of onset (0–9 years) (Platzer et al., 2017). Patient 14 is 7 years old, and he is seizure-free until now.

EIEE is a group of neurological disorders characterized by frequent epileptic seizures and development delay beginning in infancy (McTague et al., 2016). In 2018, biallelic variants in *CNPY3* gene (MIM #610774) have been identified to cause EIEE60 (MIM #617929) (Mutoh et al., 2018). *CNPY3* gene is located on chromosome 6p21.1, comprises six exons and five introns, and encodes a co-chaperone of 278 amino acid residues in the endoplasmic reticulum. In this study, two compound heterozygous variants of *CNPY3* gene were identified in patient 5. To date, three individuals with biallelic *CNPY3* variants have been reported, and their MRI showed diffuse brain atrophy and hippocampal malrotation (Mutoh et al., 2018). Brain MRI of patient 5 also showed cerebral atrophy, but hippocampal malrotation was not observed. And the other phenotypes of patient 5 were consistent with the three reported patients in the literature.

Three pathogenic CNVs were detected by exome-based CNV analysis in patients 10, 15, and 16. A 1.54-Mb heterozygous duplication on chromosome 7q11.23 was identified in patient 10; hence, he was diagnosed with chromosome 7q11.23 duplication syndrome (MIM #609757). 7q11.23 duplication syndrome is characterized by DD, speech delay, and congenital anomalies (Morris et al., 2015). To date, more than 150 individuals with

7q11.23 duplication syndrome have been reported (Mervis et al., 2015). Patient 10 was diagnosed with tetralogy of Fallot after birth and underwent surgical repair. And he showed delayed motor, speech, and autistic features at 3 years old, which are similar to the characteristics of 7q11.23 duplication patients reported in the literature.

For patient 15, a 6.46-Mb heterozygous deletion on chromosome 8p21.2p12 was detected, which overlaps with the deletion region of four cases recorded in the DECIPHER database (<https://www.deciphergenomics.org>). DECIPHER patient #390234 had attention deficit hyperactivity disorder, depressivity, expressive language delay, and mild ID. Patient #1557 had abnormality of the skin, blepharophimosis, fine hair, hearing impairment, hypothyroidism, ID, microtia, obesity, and short palm. Patient #415150 had mild ID, seizure. And patient #372147 had delayed speech and language development, generalized hypotonia, hypermetropia, short stature, and strabismus. A previous study reviewed 21 patients with deletions overlapping 8p21p12 region; and growth retardation, psychomotor retardation, and postnatal microcephaly were characteristics of most patients (Willemsen et al., 2009). Patient 15 also showed mild ID and DD, and he had hydrocephalus diagnosed prenatally, which was not reported in patients with deletion on 8p21.2p12. He underwent neurosurgical cerebrospinal fluid diversion after birth; afterwards, his follow-up CT scan was normal.

A 183.3-kb deletion on chromosome 19p13.2 encompassing *NFIX* gene was identified in patient 16. Haploinsufficiency of *NFIX* causes Sotos syndrome 2 (MIM #614753), which is characterized by postnatal overgrowth, macrocephaly, DD, and intellectual impairment (Malan et al., 2010). Patients with Sotos syndrome 2 will develop marfanoid habitus with age. The birth weight of patient 16 is 3.45 kg, and he had neonatal feeding difficulties. Now he is 6 years old with a weight of 21 kg (normal range) and height of 128 cm (+2 SD), consistent with a previous report that the median height of patients with Sotos syndrome 2 is 2.0 SD above the mean (range –0.5 to +3.8 SD) (Klaassens et al., 2015). He also had strabismus, DD, and moderate ID, similar to reported patients with 19p13.2 deletion encompassing *NFIX* gene (Klaassens et al., 2015; Jezela-Stanek et al., 2016).

In conclusion, WES could identify the underlying genetic causes for patients with unexplained DD/ID, and exome-based CNV analysis could detect clinically significant submicroscopic CNVs, thus improving the diagnostic yield. Our findings not only broaden the known mutation spectrum of genes associated with DD/ID but also indicate the potential of WES and exome-based CNV analysis in clinical diagnosis and discovery of disease-causing mutations and CNVs.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to ethical concerns regarding patient privacy and consent. Requests to access the datasets should be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional ethics committee of the Affiliated Suzhou Hospital of Nanjing Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minors' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JX, QZ, and TW are responsible for testing strategy design and manuscript preparation. Data analysis and interpretation were performed by JX, HT, JM, and QH. Genetic counselling was conducted by QZ, YD, FY, AG, and WZ. All authors read and approved the final 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/fgene.2021.738561/full#supplementary-material>

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