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Trio-whole exome sequencing reveals the importance of *de novo* variants in children with intellectual disability and developmental delay

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Understanding the genetic basis of developmental delay (DD) and intellectual disability (ID) remains a considerable clinical challenge. This study evaluated the clinical application of trio whole exome sequencing (WES) in children diagnosed with DD/ID. The study comprised 173 children with unexplained DD/ID. The participants underwent trio-WES and their demographic, clinical, and genetic characteristics were evaluated. Based on their clinical features, the participants were classified into two groups for further analysis: a syndromic DD/ID group and a non-syndromic DD/ID group. The genetic diagnostic yield of the 173 children diagnosed with DD/ID was 49.7% (86/173). This included 58 pathogenic or likely pathogenic single nucleotide variants (SNVs) in 41 genes identified across 54 individuals (31.2%) through trio-WES. Among these, 22 SNVs had not been previously reported. Additionally, 30 copy number variations (CNVs) were detected in 36 individuals (20.8%). The diagnostic yield in the syndromic DD/ID group was higher than that in the non-syndromic DD/ID group (57.8% vs. 47.2%, $P < 0.001$). Within the syndromic DD/ID subgroup, the diagnostic yield of the DD/ID with epilepsy subgroup (83.9%) was significantly higher than those of the other subgroups ($P < 0.001$). Based on the analysis of the individuals' clinical phenotypes, the individuals with facial dysmorphism shown a higher diagnostic yield (68.2%, $P < 0.001$). The diagnostic yield of SNVs was higher in the individuals with DD/ID accompanied by epilepsy, whereas the diagnostic yield of CNVs was higher in the DD/ID without epilepsy group. Similarly, the diagnostic yield of *de novo* SNVs was higher in the DD/ID with epilepsy group, while the diagnostic yield of *de novo* CNVs was higher in the DD/ID without epilepsy group (all $P < 0.001$). Trio-WES is a crucial tool for the genetic diagnosis of DD/ID, demonstrating a diagnostic yield of up to 49.7%. *De novo* variants in autosomal dominant genes are significant contributors to DD/ID, particularly in non-consanguineous families.

Keywords Trio-whole exome sequencing, *De novo* variant, Intellectual disability, Developmental delay

Intellectual disability (ID), affecting 1–3% of the general population, is characterized by significant limitations in both intellectual and adaptive functioning that manifest during developmental periods[1,2]. Global developmental delay (DD) refers to delayed cognitive and physical development in children under the age of five[3]. The prevalence of DD/ID presents a public health challenge, imposing a substantial burden on the affected families and society at large[4]. The identification of the underlying causes of DD/ID is crucial for tailored clinical follow-up, improved genetic counselling of families, and reduced treatment costs.

Genetic abnormalities at the chromosomal or gene level account for 25–50% of individuals with DD/ID[5,6]. More than 2000 genes, implicated in various pathways and biological processes, are listed in the Online Mendelian Inheritance in Man (OMIM) database[7]. Single nucleotide variants (SNVs) and copy number variations (CNVs) have been identified as key mutations associated with DD/ID[8,9]. CNVs are considered significant contributors to DD/ID. They have been utilized as primary tools for the initial diagnostic screening of DD/ID since 2010[10]. Notably, the diagnostic yield of CNVs is reported to be less than 25%[11–13]. Multiple studies indicate that SNVs are also important genetic factors in the aetiology of DD/ID[14,15].

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The advent of next-generation sequencing (NGS) technology, particularly whole exome sequencing (WES), has significantly enhanced the discovery and diagnosis of genes associated with DD/ID[16,17]. In 2021, the American Society for Medical Genetics and Genomics (ACMG) strongly endorsed WES as a primary diagnostic tool for DD/ID[18]. The estimated diagnostic yield of targeted NGS for ID is approximately 21%[19]. This diagnostic yield can be further increased to between 34 and 55% through trio-based WES[20–22]. However, the diagnostic yields and positive test results have demonstrated considerable variability across different studies and populations, underscoring the genetic complexity of DD/ID. Given the high genetic heterogeneity of DD/ID, additional studies are essential to elucidate the extensive genetic spectrum of these conditions. In this study, a sample of 173 children diagnosed with DD/ID was utilized to further investigate the genetic spectrum associated with these conditions.

Materials and methods

Subjects

173 children diagnosed with DD/ID at the Children's Medical Center of the Affiliated Hospital of Guangdong Medical University between January 2020 and December 2022, along with their parents, were recruited for this study. The diagnosis of DD/ID was clinically confirmed and classified by two trained paediatric neurologists according to the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[23]. The diagnosis of DD/ID was based on the assessment of each individual's intelligence quotient (IQ), using the Wechsler Intelligence Scale for Children (for those over five years of age), or development quotient (DQ), assessed through the Gesell Development Diagnosis Scale (for children under five years of age). The DQ is used to evaluate developmental progress across five domains. Children under five years of age who score below 75 in at least two domains were diagnosed with DD, while those over five years of age with an IQ score below 70 were diagnosed with ID. Additionally, the criteria set forth in the International Classification of Diseases, 10th Edition (ICD-10), were employed to assess the severity of DD/ID[24]. Children with DD whose DQ scores ranged from 75 to 55 in at least two domains were classified as mild DD; those with scores between 54 and 40 were classified as moderate DD; and those with scores below 35 were classified as severe DD. Individuals with IQ scores between 69 and 50 were classified as mild ID; those with scores between 49 and 35 were classified as moderate ID; and those with scores below 34 were classified as severe ID.

The diagnostic criteria and classification of epilepsy were based on the norms of the International League against Epilepsy[25,26]. The clinical diagnosis of autistic spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) was performed according to the diagnostic criteria in the DSM-5[23]. Head circumference abnormalities (including microcephaly and macrocephaly) and facial abnormalities, as well as various other types of organ abnormalities, were defined according to the Human Phenotypic Ontology Guidelines[27]. This study was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University (PJ2021-097) and was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to their enrolment in the study.

Next-generation sequencing

Genomic DNA was extracted from peripheral blood cells using an automated nucleic acid extractor (NP968-S, Xi'an China). A high-throughput gene sequencer (MGISEQ-2000RS, Guada, China) was selected as the WES platform, achieving an average sequencing depth of 150×, with 20× coverage at 98% and 30× coverage at 99%. FastQC software was utilized for quality assessment and the removal of adapter sequences and low-quality data. The raw WES sequencing data were obtained in FastQ format, using the GRCh37 reference genome. Trio-WES was performed for all children diagnosed with DD/ID and their parents.

Screening and validation of SNVs

FastQC software was used to process the raw data. Adapter sequences and low-quality data after sequencing were removed, thereby generating high-quality data for subsequent analysis. The Burrows-Wheeler Alignment tool was utilized to align the sequenced samples with variant sequences reported in the human reference genome (GRCh37/hg19, http://genome.ucsc.edu/). GATK Unified Genotyper and SAMtools were applied to identify SNVs and small insertions and deletions (InDes). Variants located within genes and transcripts were annotated using ANNOVAR. The impact of genetic variants on protein function was predicted using various computational tools, including SIFT, Polyphen2, LRT, MutationTaster, and FATHMM. The frequency of these variants in the population was compared against established databases, such as Thousand Genomes, the Exome Sequencing Project (ESP), and gnomAD.

Screening and verification of CNVs

NGS can be utilized to identify all aneuploid pathogenic/possibly pathogenic (P/LP) CNVs (> 100 kb in size). The Weaver algorithm was employed to detect alterations in the DNA copy number at the exon level. This algorithm calculates the statistical likelihood of each exon's copy number by comparing the sequence coverage depth of the proband with the baseline depth distribution derived from a reference sample set.

Variation filtering and classification

1. Filtration based on allele frequency. Variants with a minimum allele frequency (MAF) ≤ 0.01 in any population group of gnomAD (https://gnomad.broadinstitute.org/) were selected, while all others were discarded.
2. Filtration based on protein-level predictions. DNA variants, located in the upstream or downstream regions of 5'-UTR and 3'-UTR, as well as intronic variants situated more than five base pairs away from the exon-intron junction, were excluded. Additionally, missense variants classified as benign or possibly benign

in the InterVar (http://wintervar.wglab.org/) or ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases were also discarded. Missense, nonsense, frameshift (insertions or deletions), non-frameshift (insertions or deletions), synonymous (potentially deleterious effects on splicing), and splice site-affecting variants were selected.

3. Filtration based on genetic patterns. The variant genetic pattern of the proband was inferred through trio-WES analysis. Variants that aligned with the altered gene were selected based on common genetic patterns, including *de novo* variants associated with autosomal or X-linked dominant genes, homozygous or compound heterozygous variants in autosomal recessive genes, and heterozygous variants in the X-linked genes of males. We assumed full penetrance and consistent expression of these variants in children with unaffected parents and without a family history.

The pathogenicity of a variant was determined based on the criteria set forth by the American Society of Medical Genetics and Genomics (ACMG)[28]. A variant was classified as pathogenic if either partial or complete testing, or clinical manifestations, were indicated.

Validation of identified variants

After amplifying the DNA from the probands and their parents, Sanger sequencing was employed to design primer sequences that encompassed the variant exon site of the suspected pathogenic gene, along with flanking sequences. An ABI BigDye Terminator v3.1 kit was used for sequencing, according to the manufacturer's instructions (4337457, ABI Company USA). The sequencing results were subsequently validated with an automatic sequencer (3730XL, ABI Company USA).

Statistical analysis

The data were analysed using Graph Prism 8.0.2 statistical software. The variables were expressed as frequency counts and percentages. The Chi-square test was used for comparisons of categorical variables, with the significance level set at $P < 0.05$.

Results

Demographic and clinical characteristics

The cohort comprised 169 unrelated non-consanguineous families, including 165 families with three members (the proband and his/her parents; Trio) and four families with four members (the proband, his/her siblings and parents; Quadro). A total of 173 children diagnosed with DD/ID were included in this cohort. The specific demographic and clinical features of the participants are listed in Table 1. The cohort included 70 females (40.5%) and 103 males (59.5%), with a mean age of 3.8 ± 5.2 years. Overall, 98.3% (170/173) of the children with DD/ID were born at term, while 95.4% (165/173) had no documented family history of epilepsy or DD/ID. Epilepsies were identified in 53.8% (96/173) of the cohort, including 78.1% (75/96) who experienced seizures during infancy and 54.2% (52/96) who exhibited focal seizures. Among the 168 children with available neuroimaging data, 70.2% (118/168) had abnormal neuroimaging findings, predominantly characterized by brain dysplasia (86.4%, 102/118). Electroencephalogram (EEG) data were available for 171 children (98.8%, 171/173), revealing that 65.5% (112/171) had abnormal EEG results, particularly multifocal epileptiform discharges (44.6%, 51/112). Further analysis of the clinical features revealed that over half of the individuals exhibited dystonia (101/173, 61.8%) and epilepsy (96/173, 55.5%). Other prevalent clinical features included speech delay (59/173, 34.1%), facial dysmorphism (44/173, 25.4%), and dysphagia (32/173, 18.5%).

Based on their clinical features, the children were divided into two groups: a non-syndromic DD/ID group (73/173, 42.2%) and a syndromic DD/ID group (100/173, 57.8%). The syndromic DD/ID group was further subdivided into six subgroups: (1) DD/ID with epilepsy (56/100, 56.0%); (2) DD/ID with dystonia (83/100, 83.0%); (3) DD/ID with hearing impairment (20/100, 20.0%); (4) DD/ID with multiple malformations (55/100, 55.0%); (5) DD/ID with behavioural disorders (40/100, 40.0%); and (6) DD/ID with abnormal head circumference (25/100, 25.0%). Given that children in this cohort may present with multiple clinical manifestations, an individual child may be classified into several subgroups.

Diagnostic yield and genetic discovery

Overall, P/LP variants were identified in 86 children with DD/ID, representing a diagnostic yield of 49.7% (86/173). Among these, 54 children (54/173, 31.2%) had P/LP SNVs, 36 children (36/173, 20.8%) had CNVs, and four children (4/173, 2.3%) had both P/LP SNVs and CNVs. The diagnostic yield in the syndromic DD/ID group was 75.0%, which was significantly higher than in the non-syndromic DD/ID group (15.1%; $P < 0.001$). Within the six syndromic DD/ID subgroups, the diagnostic yield ranged from 20.0 to 83.0%, which significant differences noted among the groups ($P < 0.001$). The DD/ID with epilepsy subgroup displayed the highest diagnostic yield. Furthermore, the diagnostic yield associated with abnormal clinical examinations varied from 30.0 to 62.5%, which no significant differences were found across all groups (all $P > 0.05$). Notably, the diagnostic yields differed among various clinical features. The facial dysmorphism associated with an increased diagnostic yield of 68.2% ($P < 0.001$).

Based on the high prevalence of epilepsy among individuals in this cohort and the highest diagnostic yield observed in the syndromic DD/ID group, we further analysed the children by categorizing them into two groups: a DD/ID with epilepsy group and a DD/ID without epilepsy group. There were 96 children (96/173, 55.5%) in the DD/ID with epilepsy group and 77 children (77/173, 44.5%) in the DD/ID without epilepsy group (Table 2). No significant difference was found in the diagnostic yield between the two groups. Notably, the diagnostic yield of P/LP SNVs was higher in the DD/ID with epilepsy group, whereas the diagnostic yield of CNVs was higher in the DD/ID without epilepsy group. Similarly, the diagnostic yield of *de novo* P/LP SNVs was higher in

Characteristics	Number (%)	Diagnostic yield (*n*/%)	χ^2 value	*P* value
Gender				
Male	103 (59.5)	46 (44.7)	2.598	0.107
Female	70 (40.5)	40 (57.1)		
Mean of age	3.8 ± 5.2 years (6 months to 11 years)			
History of DD/ID	8 (4.6%)	6 (75.0)	-	-
DD/ID level				
mild	41 (23.7)	17 (41.5)	2.385	0.303
moderate	56 (32.4)	32 (57.1)		
Severe	76 (43.9)	37 (48.7)		
Classification				
Non-sydromic DD/ID	73 (42.2)	11 (15.1)	**60.626**	**0.000**
Sydromic DD/ID	100 (57.8)	75 (75.0)		
Sydromic DD/ID subgroup				
DD/ID + Epilepsy	56 (56.0)	47 (83.9)	**49.239**	**0.000**
DD/ID + Dystonia	83 (83.0)	47 (56.6)		
DD/ID + Hearing loss	20 (20.0)	4 (20.0)		
DD/ID + MCA	55 (55.0)	33 (60.0)		
DD/ID + Behavioural troubles	40 (40.0)	8 (20.0)		
DD/ID + Abnormal head circumference	25 (25.0)	13 (52.0)		
Clinical examinations				
Abnormal brain MRI	118/168 (70.2)	63 (53.3)	2.009	0.156
Abnormal EEG	112/171 (65.5)	53 (47.3)	0.726	0.394
Abnormal metabolic test	8/95 (8.4)	5 (62.5)	0.549	0.459
Abnormal BAEP	10/125 (8.0)	3 (30.0)	1.649	0.199
Clinical features				
Speech delay	59/173 (34.1)	28 (47.5)	0.182	0.670
Hearing loss	10/173 (5.8)	4 (40.0)	0.400	0.527
Developmental regression	11/173 (6.4)	7 (63.6)	0.911	0.340
Epilepsy	96/173 (55.5)	53 (55.2)	2.607	0.106
Dystonia	101/173 (61.8)	54 (53.5)	1.368	0.242
Visual loss	6/173 (3.5)	4 (66.7)	0.715	0.398
Somnipathy	15/173 (8.7)	5 (33.3)	1.762	0.184
Facial dysmorphism	44/173 (25.4)	30 (68.2)	**23.869**	**0.000**
Congenital heart disease	10/173 (5.8)	4 (40.0)	0.400	0.527
Short stature	11/173 (6.4)	5 (45.5)	0.085	0.770
Limb/spinal defects	19/173 (11.0)	10 (52.6)	0.073	0.787
Congenital anomalies of Urogenital system	3/173 (1.7)	2 (66.7)	0.351	0.553
Metabolic disorder	8/173 (4.6)	5 (62.5)	0.549	0.459
Stereotyped movement	16/173 (9.2)	6 (37.5)	1.052	0.305
ASD	11/173 (6.4)	8 (72.7)	2.489	0.115
Dysphagia	32/173 (18.5)	16 (3.1)	0.001	0.971
ADHD	21/173 (12.1)	3 (14.3)	**11.998**	**0.001**
Macrocephaly	15/173 (8.7)	9 (60.0)	0.696	0.404
Microcephaly	8/173 (4.6)	4 (50.0)	0.000	0.987
Hemiplegic paralysis	4/173 (2.3)	1 (25.0)	1.000	0.317
Abnormal skin/hair	12/173 (6.9)	7 (58.3)	0.383	0.536
Ataxia	3/173 (1.7)	1 (33.3)	0.328	0.567

Table 1. Clinical characteristics of 173 individuals and diagnostic yields for diagnostic trio-whole exome sequencing. *DD* developmental delay, *ID* intellectual disability, *MRI* magnetic resonance imaging, *EEG* electroencephalo-graph, *BAEP* brainstem auditory evoked potential, *MCA* multiple congenital deformity, *ASD* autistic spectrum disorder, *ADHD* attention deficit hyperactivity disorder. Significant values are in bold.

the DD/ID with epilepsy group, while the diagnostic yield of *de novo* CNVs was higher in the DD/ID without epilepsy group. The diagnostic yield of *de novo* P/LP SNVs was higher in the DD/ID with epilepsy group, while the diagnostic yield of *de novo* CNVs was higher in the DD/ID without epilepsy group. The diagnostic yield among children with mild DD/ID in the DD/ID with epilepsy group was higher than that in the DD/ID without

Characteristics	ID/DD plus epilepsy group		ID/DD group		χ^2 value	*P* value
	Number of patients	Number (%)	Number of patients	Number (%)		
Diagnostic rate	96	53 (55.2)	77	37 (48.1)	0.877	0.363
SNV	96	39 (40.6)	77	15 (19.5)	8.897	**0.003**
AD	39	21 (53.8)	15	5 (33.3)	1.826	0.230
AR	39	9 (23.1)	15	6 (40.0)	1.547	0.309
XL	39	9 (23.1)	15	4 (26.7)	0.076	1.000
CNV	96	14 (14.6)	77	22 (28.6)	5.073	**0.037**
De novo variants	96	34 (35.4)	77	24 (31.2)	0.346	0.628
SNV	96	27 (28.1)	77	8 (10.4)	8.328	**0.004**
CNV	96	7 (7.3)	77	16 (20.8)	6.743	**0.013**
Male	96	24 (25.0)	77	22 (28.6)	0.279	0.597
DD/ID level						
Mild	96	30 (31.2)	77	11 (13.0)	6.800	**0.009**
Moderate	96	24 (25.0)	77	32 (40.3)	4.588	**0.035**
Severe	96	42 (45.8)	77	34 (44.2)	0.049	0.878
MRI abnormality						
Normal	96	28 (29.2)	77	22 (28.6)	0.007	1.000
Abnormal	96	68 (70.8)	77	50 (64.9)	0.038	0.866
Not found	96	3 (3.1)	77	2 (2.6)	0.042	1.000
Other symptom						
Language delay	96	86 (89.6)	77	70 (90.9)	0.085	0.804
Motor delay	96	79 (82.3)	77	67 (87.0)	1.022	0.442
Autism spectrum disorder	96	9 (9.4)	77	6 (7.8)	0.135	0.791

Table 2. Comparison of clinical characteristicss between the two groups. *SNV* single-nucleotide variant, *AD* autosomal dominant, *AR* autosomal recessive *XL* X-linked, *CNV* copy number variation, *DD*, developmental delay, *ID* intellectual disability, *MRI* magnetic resonance imaging. Significant values are in bold.

epilepsy group. In cases with moderate-to-severe DD/ID, the diagnostic yield in the DD/ID without epilepsy group was higher than that in the DD/ID with epilepsy group [68.8% (66/96) vs. 87.0% (66/77), $\chi^2 = 6.800$, $P = 0.009$]. No significant differences were observed between the two groups regarding the diagnostic yields of other clinical features, including speech delay, ASD, and abnormal cerebral magnetic resonance imaging (MRI).

Gene distribution of detected SNVs

A total of 58 P/LP SNVs across 41 genes were identified in 54 children (54/173, 31.2%), including 21 novel P/LP SNVs (Table 3). Among the 58 detected P/LP SNVs (Supplementary Table 1), 34 *de novo* variants (34/58, 58.6%) were found, encompassing four mosaic variants. Eighteen compound heterozygous variants inherited from either parent (18/58, 31.0%) were identified, including four children with both compound heterozygous SNVs and CNVs. Additionally, three individuals carried homozygous variants inherited from their parents (3/58, 5.2%), while another three individuals exhibited maternal-inherited variants (3/58, 5.2%). The inherited patterns included autosomal dominant (AD) inheritance (24/58, 41.4%), autosomal recessive (AR) inheritance (22/58, 37.9%), X-linked dominant (XLD) inheritance (7/58, 12.1%), X-linked inheritance (7/58, 5.2%), and X-linked recessive (XLR) inheritance (2/58, 3.4%).

Gene distribution of detected CNVs

Thirty P/LP CNVs (Supplementary Table 2) were identified in 36 children (36/173, 20.8%), including 21 deletions (21/30, 70.0%) and nine duplications (9/30, 30.0%). These P/LP CNVs included 19 *de novo* P/LP CNVs, nine maternal-inherited P/LP CNVs (including two children with concurrent P/LP SNVs), and two paternal-inherited CNVs (including two children with concurrent P/LP SNVs). Seven children showed 15q11.2-15q13.1 deletion, including five children diagnosed with Angelman syndrome and two children diagnosed with Prader-Willi Syndrome. Additionally, three children presented with a deletion in the 7q11.23 region. CNVs were observed in two or more children as follows: seven individuals on chromosome 15; three individuals on chromosome 5, chromosome 7, chromosome 8, chromosome 9, and chromosome 16; and two individuals on chromosomes 1 and 2, respectively (Supplementary Fig. 1).

In this cohort, Case 78 (Xp21.1-Xp11.23 duplication)[29] and Case 165 (20q11.22- 20q12 duplication) exhibited overlapping CNVs previously associated with DD[30]. Additionally, Case 130 carried two mosaic duplicate CNVs that have been reported to be associated with severe DD (DECIPHER database, https://decipher.sanger.ac.uk/patient/279178/genotype/52947/browser; https://decipher.sanger.ac.uk/patient/277310/genotype/131973/browser).

No.	Sex	Age	Gene	Inheritance mode	Variant	Zygosity	Inherited form	Evidence of pathogenicity	Pathogenicity
9	F	4y	*KCNB1*	AD	NM_004975.4:c.962G > A(p.Gly321Asp)	Het	*De novo*	PS2, PM2, PP3	LP
13	M	2.5y	*ATP6V0A1*	AD	NM_001130021.2:c.2401 C > T(p.His801Tyr)	Het	*De novo*	PS2, PM2, PV3	LP
28	M	5y	*KCTD7*	AR	NM_153033.4:c.679G > T(p.Glu227*)	Com. het	Paternal	PVS1, PM2	LP
43, 44	F, F	6y, 6 m	*PCDH19*	XL	NM_001184880.2:c.2774dup(p.Tyr926Valfs*3)	Het	Maternal	PVS1, PM2, PP1	LP
46	F	4.5y	*CACNA1A*	AD	NM_001127222.1:c.4043G > A(p.Arg1348Gln)	Het	*De novo*	PS1, PS2, PM2, P3	P
48	M	3y	*SCN1A*	AD	NM_001165963.3:c.1234T > C(p.Phe412Leu)	Het	*De novo*	PS2, PM2, PP3	LP
58	M	5y	*TSC2*	AD	NM_000548.5:c.5332_5387del(p.Ala1778Hisfs*89)	Het	Maternal	PVS1, PM2, PP1	LP
60	F	5y	*PCDH19*	XL	NM_001184880.2:c.2005 C > T(p.Gln669*)	Het	*De novo*	PVS1, PS2, PM2	P
61	F	1.5y	*GNAO1*	AD	NM_020988.3:c.142 A > C(p.Thr48Pro)	Het	*De novo*	PS2, PM2, PP3	LP
76	M	6y	*SLC2A1*	AD	NM_006516.3:c.1097_1100del(p.Tyr366*)	Het	*De novo*	PVS1, PS2, PS4, PM2	P
86	F	3.5y	*VPS13B*	AR	NM_017890.4:c.5230 C > T(p.Gln1744*)	Com. het	Maternal	PVS1, PM2	LP
99	F	4.5y	*MYO5A*	AR	NM_000259.3:c.4667del(p.Phe1556Serfs*13)	Hom	Paternal, Maternal	PVS1, PM2, PM3	P
106	F	2.5y	*FGFR3*	AD	NM_000142.4:c.1138G > A(p.Gly380Arg)	Het	*De novo*	PS2, PS4, PM2, PP3	P
123	M	3.5y	*GBA*	AR	NM_000157.3:c.1448T > C(p.Leu483Pro)	Com. het	Maternal	PS3, PS4, PP3	P
125	M	3.5Y	*NEB*	AR	NM_001271208.1:c.13338del(p.Gln4446Hisfs*23)	Com. het	Paternal	PVS1, PM2	LP
126	F	3.5Y	*CHD8*	AD	NM_001170629.1:c.2868dup(p.Asn957*)	Het	*De novo*	PVS1, PS2, PM2	P
133	F	8y	*MECP2*	XLD	NM_004992.3:c.1225_1226del(p.Ser409Glnfs*26)	Het	*De novo*	PVS1, PS2, PM2	P
143	F	1 m	*SLC25A20*	AR	NM_000387.6:c.476T > C(p.Leu159Pro)	Com. het	Paternal	PM2, PM3, PP3, PP4	LP
152	F	1.8y	*MECP2*	XLD	NM_001110792.2:c.538 C > T(p.Arg180*)	Het	*De novo*	PVS1, PS2, PM2	P
168	F	1y	*SMC1A*	XLD	NM_006306.4:c.138_139insA(p.Phe47Ilefs*5)	Het	*De novo*	PS2, PM2,	P
171	M	1 m	*SCN4A*	AR	NM_000334.4:c.4137del(p.Gln1379Hisfs*12)	Com. het	Paternal	PVS1, PM2	LP

Table 3. The novel P/LP single-nucleotide variants in our cohort. *F* female, *M* male, *y* year, *m* month, *DD* developmental delay, *ID* intellectual disability, *Mi* mild, *Mo* moderate, *S* severe, *AD* autosomal dominant, *AR* autosomal recessive, *XL* X-linked, *XLD* X-linked dominant, *Het* heterozygote, *Com. het* compound heterozygote, *Hom* homozygote, *P* pathogenic, *LP* likely pathogenic.

Impact on medical management

The clinical effects following genetic testing are listed in Table 4. Twenty-four families were advised to undergo genetic counselling. Twelve children (12/54, 22.2%) were effectively treated based on the results of the genetic testing, including drugs specific for the *KCNQ2*, *SCN1A*, *CACNA1A*, and *TSC2* variants, as well as replacement therapies for *ATP7A* variants. According to the available data, pyridoxine treatment improved seizures in Case 19 and Case 20 with *PIGS* variants. Perampanel improved seizures in Case 26 with *GRIA2* variants. A ketogenic diet improved seizures in Case 65 with *ATAD3A* variants.

Discussion

Technological advancements over the past decade have resulted in the identification of many new genes associated with DD/ID. This progress signals a shift towards genetic diagnostics grounded in fundamental biological mechanisms[31]. However, the high clinical and genetic heterogeneity of DD/ID presents a considerable challenge in determining the aetiologies of these conditions[32]. A meta-analysis indicated that the overall diagnostic yield of WES in individuals with neurodevelopmental disorders (NDDs) was 36%, with a yield of 31% in individuals with isolated NDDs and 53% in those with NDDs accompanied by additional conditions[33]. In this study, the overall diagnostic yield of WES in 173 children was 49.7%. The diagnostic yield in the syndromic DD/ID group was 75.0%, while the diagnostic yield in the non-syndromic DD/ID group was 15.1%. In our cohort, the diagnostic yield was relatively high for the following reasons: (1) All participants in our cohort underwent trio-WES, and numerous studies have demonstrated that this approach enhances the diagnostic yield[34]. (2) The participants in the syndromic DD/ID group accounted for 57.8% of our cohort, while a significant proportion of participants with epilepsy had moderate or severe DD/ID (70.8%), suggesting potential selection bias. Given that specific clinical features can enhance the diagnostic yield, the relatively severe clinical features in the children within our study may have contributed to the increased diagnostic yield.

Notably, 58 P/LP SNVs were identified in our cohort, of which *de novo* variants comprised 58.6% (34/58), particularly in the DD/ID with epilepsy group. Twenty-one P/LP SNVs were identified for the first time, expanding the known spectrum of related genes. Among the cases with P/LP SNVs, AD inheritance was predominant (26/54, 48.1%), while recessive inheritance (including AR and XLR) represented 31.5% (17/54) of all cases. Soden suggested a higher level of AR inheritance[35]. However, the population in his study included individuals with inherited metabolic diseases and those who died in the neonatal period. It is noteworthy that AR variants occur in more than 50% of the population with consanguineous marriages[36,37]. However, such individuals were rare in our cohort.

Clinical benefits	Gene (Disease)	Effects (Case details)
Management implications	*SCN1A* (DS)	Remitted and avoiding OXC (Case 2: VPA, TPM, CZP, seizure-free for 6 months; Case 27: VPA, CZP, seizure-free for 6 months; Case 45: VPA, CZP, TPM, seizure-remitted for 2–3/year; Case 82: VPA, CZP, seizure-free for 1 year)
	MMACHC (MMA)	Remitted (Case 18: vitamin B12
	PIGS (DEE 95)	Remitted (Case 19, Case 20: VPA, pyridoxine, seizure-remitted for 0–1/month)
	KCNQ2 (DEE 7)	Remitted (Case 24, Case 25: OXC, seizure-free for 1 year)
	GRIA2 (NEDLIB)	Remitted (Case 26: LEV, PER, seizure-remitted for 0–1/month)
	SCN1A (GEFS+)	Remitted and avoiding OXC (Case 48: VPA, seizure-free for 1.5 year)
	ATP7A (MNK)	Remitted (Case 11, Case 12, Case 52: VPA, histidine-copper)
	ATAD3A (HAYOS)	Remitted (Case 65: VPA, TPM, CZP, seizure-remitted after KD for 1–2/month)
	SLC2A1 (GLUT1DS2)	Remitted (Case 76: KD, seizure-free for 2 years)
	GBA (GD)	Remitted (Case 123: ERT)
	SCN8A (DEE 13)	Remitted (Case 171: VPA, LEV, seizure-free for 8 months)
	SMN1 exon7 Deletion (SMA)	Remitted (Case 142: nusinersen)
Long-term follow up	*NF1*	Case 22: follow-up the onset of pacinian neurofibroma
	TSC2 (tuberous sclerosis)	Case 58: LEV, VPA, seizure-remitted for 0–1/year
	15q11.2-15q13.1 Deletion (PWS)	Case 2, Case 155: management of eating behavior, nutrition, and growth and development
Reproductive planning	AR: *MFSD8*, *MMACHC*, *PIGS*, *KCTD7*, *ACTL6B*, *ATAD3A*, *VPS13B*, *PLA2G6*, *MYO5A*, *ARSA*, *GBA*, *NEB*, *SLC25A20*, *SCN8A*, SMN1 exon7 Deletion	Suggesting the family conduct genetic counseling (Case 7, Case 18, Case 19, Case 20, Case 28, Case 30, Case 65, Case 86, Case 94, Case 99, Case 110, Case 123, Case 125, Case 143, Case 171, Case 142)
	AD: *TSC2*	Suggesting the family conduct genetic counseling (Case 58)
	XLR: *ABCD1*, Xq21.1 Deletion, Xq28 Duplication, Xp22.12 Deletion, Xq28 Duplication, Xp11.23 Deletion, Xq28 Deletion	Suggesting the family conduct genetic counseling (Case 149, Case 11, Case 12, Case 67, Case 90, Case 117, Case 118, Case 147)
	XL: *PCDH19*	Suggesting the family conduct genetic counseling (Case 43, Case 44)

Table 4. Clinical effects after genetic test. *DS* Dravet syndrome, *MMA* methvlmalonic acidemia, *DEE* Developmental and epileptic encephalopathy, *NEDLIB* Neurodevelopmental disorder with language impairment and behavioral abnormalities, *GEFS+* Generalized epilepsy with febrile seizures plus, *MNK* Menkes disease, *GLUT1DS2* Glucose transporter-1 deficiency syndrome 2, *GD* Gaucher disease, *SMA* Spinal muscular atrophy, *PWS* Prader-Willi Syndrome, *HAYOS* Harel-Yoon syndrome, *VPA* valproate, *OXC* oxcarbazepine, *LEV* levetiracetam, *TPM* topiramate, *PER* perampanel, *CZP* clonazapam, *KD* ketogenic diet, *ERT* enzyme replacement therapy.

The associations between current clinical features and the identified pathogenic variants associated with DD/ID were inconsistent across different study cohorts. Previous studies have indicated that the diagnostic yield of WES is not comparable between syndromic DD/ID and non-syndromic DD/ID[38–40]. A meta-analysis reported a diagnostic yield of 54% in syndromic DD, compared to 31% in non-syndromic DD[33]. Our study demonstrated that the diagnostic yield in the syndromic DD/ID group was significantly higher than that in the non-syndromic DD/ID group, in partial agreement with previous findings[33,41]. Furthermore, notable differences were present among the syndromic DD subgroups[38,42]. For example, the diagnostic yield in the behavioural disorder subgroup was significantly lower than that in the other subgroups[38]. In our study, we found significant differences in the diagnostic yield among the syndromic DD/ID subgroups, with the highest yield observed in the DD/ID with epilepsy subgroup. Epilepsy is a common clinical feature in individuals with DD/ID. The pathogenesis of epilepsy, particularly epileptic encephalopathy, is widely thought to have a genetic basis[43]. The prevalence of DD/ID in individuals with inherited epilepsies was found to be higher than among those with non-inherited epilepsy[43]. Additionally, some studies have shown that specific clinical features, such as craniofacial abnormalities and head circumference abnormalities, can increase the WES diagnostic yield in individuals with DD/ID, although these effects were not statistically significant[39,40]. In our cohort, the facial dysmorphism associated with an increased diagnostic yield.

Trio-WES provides genotype information derived from parents, facilitating precise and immediate differentiation of the *de novo* origin[6,44]. *De novo* variants account for 42% of severe DD cases, even in consanguineous populations[14]. Current studies have demonstrated a high diagnostic yield of *de novo* variants in individuals with sporadic ID by trio-WES analyses[45,46]. The overall diagnostic yield of DD/ID in Pode-Shakked's study was 38.9%, with *de novo* variants identified in 32.5% of the probands, representing 83.5% of all molecular diagnoses. This underscores the significant role of *de novo* variants in the aetiology of DD/ID[47]. In our study, the overall diagnostic yield of DD/ID was 49.7%, which was similar to previous studies. Our study revealed that the diagnostic yield of P/LP SNVs was significantly higher in the DD/ID with epilepsy group. Twenty-two children (22.9%) in the DD/ID with epilepsy group were diagnosed with developmental epileptic encephalopathy (DEE). Some studies have reported high diagnostic yields within DEE cohorts[39,48]. In many cases of DEE, developmental disabilities arise not only due to frequent epileptic activity but also from the direct effects of genetic mutations[49].

The genes associated with DEE in our study included *SCN1A*, *PCDH19*, *SMC1A*, *PIGS*, *KCNQ2*, *KCNB1*, *KCNT1*, *ATP6V0A1*, *ACTL6B*, *CACNA1A*, and *GNAO1*. Most of these genes encode proteins that play critical biological roles, such as ion channels, neurogenesis, synaptogenesis, and synaptic plasticity. Notably, genes

encoding ion channels are particularly significant in children with DD/ID and epilepsy (10/96, 10.4%), especially in those with *SCN1A* variants. However, P/LP *SCN1A* variants are extremely rare in individuals with DD/ID without epilepsy.

Different clinical phenotypes may be associated with various locations of pathogenic variants in genes[50]. For example, there are different clinical phenotypes associated with the position of variants in *ATP6V0A1*[51,52]. Biallelic variants of *ATP6V0A1*, locating in the topological domain, are associated with NDDs, including cerebellar atrophy and early-onset progressive myoclonic epilepsy with ataxia. Heterozygous variants of *ATP6V0A1*, locating in the transmembrane domain, are associated with DEE 104, including intractable epilepsy beginning in infancy, severe DD, but without specific observable changes on cerebral MRI. By employing reverse phenotyping, clinicians can validate patients' clinical phenotypes based on genetic analysis results. As such, the expanded phenotypic spectrum of diseases with known genes can facilitate the identification of the genetic aetiologies of extremely rare diseases presenting with atypical phenotypes.

Hundreds of CNVs have been identified as causative factors in DD/ID, with most of these being *de novo* CNVs[53]. In this study, CNVs were detected in 36 individuals (36/173, 20.8%). Consistent with previous studies, the majority of these CNVs were *de novo* and predominantly included deletions and duplications. Notably, the diagnostic yield of CNVs in the DD/ID without epilepsy group was significantly higher than that in the DD/ID with epilepsy group (28.6% vs. 14.6%). We also identified recurrent pathogenic CNVs, with chromosome 15 frequently harbouring pathogenic CNVs, notably, deletions in the 15q11.2-15q13.1 region. Interestingly, our study revealed that four children had two alleles (*ATAD3A*, *VPS13B*, *GBA*, *SLC25A20*) with both SNVs and CNVs contributing to the corresponding clinical phenotypes. This underscores the importance of performing CNV analysis and WES to elucidate the aetiology of extremely rare diseases. Currently, the findings regarding the cognitive abilities of individuals with spinal muscular atrophy (SMA) related to the *SMN1* gene are inconsistent. Compared to healthy controls, individuals with SMA demonstrate poorer visuospatial abilities, executive function, and language performance. Cognitive impairment was observed in 11 of the 20 individuals with SMA in this previous study[54]. Notably, male individuals exhibit significantly lower cognitive scores[55]. In another study, two individuals (3%) presented with mild ID, while the remaining individuals fell within the normal cognitive range, irrespective of SMA type, gender, or functional status[56]. In addition to delays in motor development, cognitive and language delays were also noted in the individual with SMA (Case 142) in our study.

The CNVs identified in three children (Case 78, Case130, and Case165) have not been previously reported. These CNVs overlapped with those documented in the DECIPHER database. Case 78, a 10-month-old infant, presented with a phenotype similar to those reported in a previous study, including severe DD, an enlarged head circumference (+2 SD), and facial dysmorphism (such as a prominent forehead, wide palpebral fissures, and anteverted nares)[29]. Additionally, the patient presented with other clinical features, including epilepsy, strephexopodia, and hypotonia. The clinical features of Case 130 became apparent during the neonatal period, including poor response, dysphagia, and hypotonia, but no physical deformity or seizures were noted. The brain MRI revealed callosal hypoplasia. Unfortunately, due to treatment discontinuation, this child succumbed to recurrent respiratory infections at the age of two months. Case 165 exhibited clinical features similar to those previously reported in the neonatal period[30], including craniofacial malformations (such as telecanthus, low-set ears, micrognathia, and microcephaly), hypotonia, and conductive hearing loss. He exhibited frequent poor response and dysphagia. Additionally, a palmar crease was observed on the right hand, together with left toe deformities and bilateral breast lymphangiomas. He succumbed to recurrent respiratory infections at the age of three months. Previous studies have indicated that many CNVs involving a single gene are associated with DD/ID, and these are prone to duplication or deletion. To date, CNVs frequently encompassing one or more genes have yet to be associated with specific phenotypes[57]. The roles of these CNVs in the pathogenesis of DD/ID remain to be elucidated.

Notably, 87 children (50.3%) in the current study were not genetically diagnosed. A limitation of this study is that the ID genes analysed were based solely on identified variants. The Deciphering Developmental Disorders (DDD) study achieved an initial diagnostic yield of 27%[14,58]. In 2018, the data were re-analysed in light of new molecular and clinical findings, resulting in an increased diagnostic yield of 40% [59]. Therefore, systematic re-analysis of non-diagnostic WES data may improve the diagnostic yield[60,61]. In our study, we included one or more probands per family, which has been shown to enhance the diagnostic yield of genetic testing. The inclusion of only one proband per family is common in certain studies, while other studies include one or more probands per family[13,42,62], making it inappropriate to compare them directly with the former. Careful genetic counselling, both pre- and post-testing, is essential in these cases. Epigenetic mechanisms, including DNA methylation and DNA imprinting, which do not adhere to Mendelian inheritance patterns, have been associated with ID and growth disorders[63,64]. If the diagnostic efficacy of WES cannot be confirmed after re-analysis, alternative diagnostic tools, such as DNA methylation testing and whole genome sequencing (WGS), may be employed. WGS offers greater sensitivity for detecting CNVs by sequencing non-coding regions, resulting in a higher diagnostic yield than WES[65,66]. Nevertheless, the complexity of the data analysis process and the high cost limit the use of WGS in ID diagnosis.

Conclusions

In conclusion, trio-WES serves as a vital tool for the genetic diagnosis of ID, demonstrating a diagnostic yield of 31.2% in our cohort. CNV analysis is also an essential component of genetic diagnosis, contributing to a combined diagnostic yield of 49.7%. This study identified 21 novel P/LP SNVs and three CNVs, thereby enriching our understanding of the molecular spectrum associated with DD/ID. Re-analysis of WES may further improve the diagnostic yield. Ongoing updates to the primary database, along with the development of new bioinformatics tools, are necessary for obtaining comprehensive clinical histories to facilitate variant identification and interpretation.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by W Y, Z CZ, H BL, C YH, X CP, L L, and R SW. The first draft of the manuscript was written by L CY and L YW. All authors commented on previous versions of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics declaration
This study was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University (PJ2021-097) and was performed in line with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants before enrolment. All reported and stored individual data were de-identified.

Consent to participate
Written informed consent was obtained from the parents.

Additional information
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