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Investigation of Genetic Aetiology in Intellectual Developmental Disorder with Trio-Whole Exome Sequencing Approach

Isik GORKER[1], Engin ATLI[2], Leyla BOZATLI[1], Selma DEMİR[2] , Sinem YALCINTEPE[2], Emine Ikbal ATLI[2], Can KOSUKCU[3], Hakan GURKAN[2]

[1]Department of Child & Adolescent Psychiatry, Trakya University Faculty of Medicine, Edirne, Türkiye
[2]Department of Medical Genetics, Trakya University Faculty of Medicine, Edirne, Türkiye
[3]Department of Bioinformatics, Hacettepe University, Institute of Health Sciences, Ankara, Türkiye

ABSTRACT

Introduction: Intellectual development disorder (IDD) is a heterogeneous condition, and genetic studies are essential to unravel the underlying cellular pathway for brain development and functioning in its etiology. This study aimed to investigate the possible genetic alterations contributing to IDD by performing next generation sequencing (NGS) in affected individuals and their parents, with a particular focus on the discovery of novel disease-associated genes.

Methods: Cases diagnosed with IDD according to DSM-5 criteria, who had normal results in conventional cytogenetic analyses, array comparative genomic hybridization and Fragile X (*FMR1*) testing, were analyzed by using Trio-Whole Exome Sequencing (Trio-WES). Genomic DNA was extracted, amplicon libraries were generated, and sequencing was conducted on a NGS platform.

Results: We detected pathogenic and/or likely pathogenic variations in *MANBA*, *TLK2*, *NAA15*, *CSF1R*, *DRD4*, *TRIO* genes in 5 of 7 cases included in the study. Protein stability prediction analysis were performed for the p.(Arg339Gln) variant in *TLK2* and p.(Asn1406Ser) variant in TRIO gene. Both variants were predicted to reduce protein stability and classified as "destabilizing."

Conclusion: Trio-WES provided a substantial contribution to the molecular diagnosis of IDD. These findings highlight the utility of whole exome sequencing as a powerful tool for uncovering novel disease-associated genes.

Keywords: Intellectual developmental disorder, next generation sequencing, novel variation, Trio-WES, whole exome sequencing

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INTRODUCTION

Intellectual Developmental Disorder (IDD) is a neurodevelopmental disorder characterized by substantial impairments in conceptual, social, and practical domains of intellectual and adaptive functioning. As defined by the DSM-5, IDD diagnosis requires deficits in both intellectual functioning and adaptive behavior with onset during the developmental period. Global prevalence is estimated at 1–3%, and severe forms are predominantly monogenic in origin (1-3.)

Although non-genetic prenatal, perinatal, and neonatal risk factors contribute to IDD (~30–50% of cases), genetic causes account for the majority of severe cases (4). Cytogenetic abnormalities and copy number variants (CNVs) explain approximately 30% of diagnoses, leaving ~60% of IDD cases with unexplained etiology (3, 5).

In the past decade, the application of whole exome sequencing (WES), especially trio-whole exome sequencing (trio-WES), has revolutionized the molecular diagnosis of IDD. Systematic reviews report diagnostic yields ranging from 30% to 50%, with de novo variants in autosomal dominant genes playing a key role, particularly in non-consanguineous families. Clinical exome-sequencing cohorts report diagnostic rates of approximately 34–36%, often leading to changes in case management and genetic counseling (6-9).

This study aims to apply trio-WES in individuals with unexplained developmental delay and IDD, where conventional cytogenetics, Fragile-X molecular testing, and chromosomal microarray were negative. We seek to elucidate the molecular etiology, investigate genotype–phenotype correlations, and assess the clinical utility of molecular diagnosis in guiding management and genetic counseling.

METHODS

Determination of the study cases

Our cases that applied to the Child and Adolescent Psychiatry Clinic and were diagnosed with IDD according to DSM-5 were included in the study. Given the high prevalence of attention deficit hyperactivity disorder (ADHD) among individuals with mild intellectual disability (IDD), cases with ADHD comorbidity were also included in the study. According to a recent clinical study by Yilmaz et al. (2021), 64.9% of children and adolescents with mild IDD exhibited comorbid ADHD symptoms(10). This high comorbidity rate reflects the importance of considering ADHD as a representative comorbid condition in studies investigating the etiology of developmental disorders. Therefore, we included ADHD as the primary psychiatric comorbidity for analysis while excluding other

Correspondence Address: Işık GORKER Trakya University, Faculty of Medicine, Department of Child & Adolescent Psychiatry, Edirne, Türkiye • **E-mail:** isikgorker@gmail.com
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Highlights

- **Trio-WES and bioinformatics used to identify genetic causes in IDD.**
- **WES, is widely used for non-syndromic intellectual disabilities.**
- **Two novel variants found that have not yet been reported in the literature.**

psychiatric diagnoses to maintain cohort homogeneity. The exclusion factors were the presence of another disease or genetic diagnosis to explain IDD, a diagnosis of epilepsy, and the presence of a psychiatric disease other than ADHD.

In this study, a total of 21 pediatric cases diagnosed with developmental delay and IDD were evaluated. Case selection was based on specific exclusion criteria within the framework of a clinical and genetic evaluation protocol. These criteria included: (i) individuals with chromosomal abnormalities diagnosed by karyotype analysis, (ii) cases with molecularly confirmed Fragile X syndrome, (iii) cases with pathogenic or likely pathogenic copy number variants detected by microarray-based comparative genomic hybridisation (array CGH). Following these exclusion criteria, Trio-WES was applied to seven case whose etiologies remained unexplained by conventional diagnostic methods to identify potential monogenic causes.

In the next-generation DNA sequencing analysis method used in this study, three steps were taken:

1) DNA isolation from peripheral blood samples and quality/quantity control of isolated samples

2) Creation of libraries by amplifying the targeted regions and reading the sequences using the NGS analysis platform

3) Bioinformatics analysis of data

DNA extraction from peripheral blood and concentration measurement

Genomic DNA isolation was performed using the EZ1 Advanced XL automated nucleic acid isolation system (Qiagen, Hilden, Germany) with the EZ1 DNA Blood isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol from 2 mL of peripheral blood samples obtained from cases and their parents who applied to the Department of Child and Adolescent Psychiatry. Cases were diagnosed with IDD according to the The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) diagnostic criteria as a result of clinical examination. The final volume of isolated genomic DNA (gDNA) was adjusted to 100 µL. The concentrations and purity values of the DNA, in terms of quality measurements, were carried out using the NanoDrop device. The isolation process was repeated for those with a 260/280 ratio of greater than 2 and concentrations below 20 ng/µL in terms of the purity of the DNA. To be used in other steps after isolation, special laboratory codes were given to each DNA, considering the cases rights and ethical values. The DNA was stored in a deep freezer at −20°C under appropriate conditions.

Construction of libraries through the amplification of the targeted regions and sequence analysis using the NGS analysis platform

The stage of constructing libraries of 37 Mb exonic regions of the human genome, which we aimed to sequence in our study, was carried out with the QIAseq Human Exome Kit (Qiagen, Hilden, Germany) using isolated genomic DNAs. The concentrations of genomic DNA obtained from the peripheral blood samples of cases were re-measured on a Qubit 4 fluorometer (Thermo Fisher Scientific, USA) using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA) before the DNA library construction.

A minimum coverage depth of 20x and above was accepted as reliable in about 99% of the target regions. Following a wet laboratory, sequencing was performed using the Illumina Novaseq 6000 instrument. The main steps of library preparation and sequencing were performed according to the QIAseq Human Exome Kit protocol (i.e. fragmentation, adapter ligation, amplification of DNA libraries, creation of library pools, hybridisation capture and binding to streptavidin beads, post-capture amplification and sequencing). In the next step, bioinformatics processes (i.e. BCL to FastQ conversion, quality assessments, mapping to reference genome, filtering of low-quality reads and variants, and VCF file generation) were performed using the Qiagen CLC Genomics Workbench 12.0.3 (Qiagen, Hilden, Germany).

Bioinformatics analysis of data

FastQ raw data files in a text format containing the targeted sequences of the genes and the quality scores were obtained after NGS. Qiagen Clinical Insight (QCI) software was used for the bioinformatics analysis of BAM files converted from FastQ files aligned to the ready-for-analysis human genome (hg19) reference sequence. Using the QCI programme, genetic variations that successfully passed the quality scoring and certain filters were identified, and their pathogenicity was determined. Datas were visualised by the Integrative Genomics Viewer programme (IGV) was used to visually examine the targeted nucleotide sequences (11).

In filtering the variants, synonymous variants were filtered, and variants with a minor allele frequency greater than 1% in open access databases and population knowledge banks, such as ExAC database, GenomAD database and the single nucleotide polymorphism database (dbSNP) were filtered. The following open access online databases were used to evaluate whether the detected genetic variations and their pathogenicity were related to the clinics of the cases: Human Genome Mutation Database (HGMD), National Centre for Biotechnology Information (NCBI) from: https://www.ncbi.nlm.nih.gov), dbSNP, VarSome (the Human Genomics Community) and Franklin by Genoox(12-15). As in silico prediction algorithms, Mutation Taster (16), Polymorphism Phenotyping v2 (PolyPhen) (17), Sorting Intolerant From Tolerant (18), DANN scoring (19), genomic evolutionary rate profiling (GERP) score (20) and CADD (21) scoring data were taken into account.

Protein modelling method

Four *in silico* tools, which estimate the impact of missense variants on protein stability, and calculate the changes of unfolding Gibbs free energy, ΔΔG were employed. Protein stability predictions were calculated with DynaMut2 (22), INPS-3D (23), PremPS (24), and FoldX (25) using the AlphaFold) structure of TLK2/Serine/threonine-protein kinase tousled-like 2(AF-Q86UE8-F1-model_v4.pdb) and RCSB Protein Data Bank structure of TRIO/N-terminal DHPH cassette of Trio in complex with nucleotide-free Rac1 (2nz8.pdb) as input PDB files, since protein domains containing disease-associated variants are only represented by these pdb structures.

The ΔΔG value between the wild type protein and results of altered amino acid substitution were calculated for the p.(Arg339Gln) variant in TLK2 and p.(Asn1406Ser) variant in TRIO. Finally, 3D structures of the wild type and altered TLK2 and TRIO protein structures were generated with DynaMut2. The ΔΔG values (kcal/mol) for the related substitutions

were interpreted as follows: Predicted ΔΔG<0 is destabilizing in the case of DynaMut2 and INPS-3D. Predicted ΔΔG>0 is destabilizing in the case of FoldX 5.0 and PremPS.

RESULTS

In the study, cases who were diagnosed with IDD (cases whose conventional cytogenetic, arrayCGH and Fragile X analysis results were found to be normal) and their parents (trio) were analyzed by WES analyzes to identify new (novel) pathogenic variations associated with IDD, de-novo pathogenic variations, new genes and/or chromosome loci. Seven case with IDD with their non-consanguineous parents (trio) were included in the study (Table 1). Three of the cases were girls, and four were boys. In the bioinformatics analysis, pathogenic and likely pathogenic variants associated with their clinics were detected in three cases. Two of these variants were *de novo*. No pathogenic or likely pathogenic variants were detected in two cases. In these two cases, two likely pathogenic variants related to their clinics were detected, but segregation analysis revealed that they were inherited from their parents (Table 2). Genotype, family segregation results, *in silico* prediction scores, dbSNP records, ACMG scores, ClinVar data and pathogenicity information of the variations detected in the cases were given in Table 3.

Case 1: As a result of the trio-WES analysis performed on the case diagnosed with IDD and ADHD who applied with learning disability, low academic achievement and hyperactivity symptoms, no variant that could be associated with the IDD clinic was detected.

Case 2: As a result of the trio-WES analysis performed on the case diagnosed with IDD and ADHD who applied with learning disability and inattentiveness symptoms, no variant that could be associated with the IDD clinic was detected.

Case 3: In the case diagnosed with IDD and ADHD who applied with speech delay and hyperactivity symptoms, the variant NM_005908.4(MANBA):c.2158-2A>G was detected as homozygos was reported as pathogenic in the Clinvar database. The NM_005908.4(MANBA):c.2158-2A>G variant was registered in the HGMD database, with accession number CS982260, and was associated with the mannosidosis, beta, lysosomal phenotype. The NM_005908.4(MANBA):c.2158-2A>G variant was determined as pathogenic (PVS1, PM2, PP5) in *in silico* analysis performed considering the ACMG-2015 criteria. The pathogenic and likely pathogenic variants of the MANBA gene were associated with the autosomal recessive mannosidosis, beta A phenotype. The parents of Case 3 were found to be heterozygous for the NM_005908.4(MANBA):c.2158-2A>G variant.

Case 4: In the case diagnosed with IDD and ADHD who applied with speech delay symptoms, the variant NM_001330418.2(TLK2):c.569G>A (p.Arg190Gln) was detected as heterozygous and was reported as a

Table 1. Clinical features and psychiatric diagnoses of the case

	Age	Gender	First admission complaints	Psychiatric Diagnosis
Case 1	15	Girl	aggressive behaviour, agitation, incompatibility with peers	Mild IDD
Case 2	15	Boy	inability to adapt to school	Mild IDD
Case 3	11	Boy	speech development failure, hyperactivity, impatience	IDD
Case 4	8	Boy	speech development failure	Mild IDD
Case 5	15	Boy	speech development failure, irritability, peer discord	Mild IDD
Case 6	17	Boy	irritability, inattentiveness	Mild IDD
Case 7	14	Girl	speech development failure, irritability, restlessness	Mild IDD

ADHD:Attention deficit and hyperactivity disorder; IDD:Intellectual and developmental disabilites

Table 2. Pathogenic, likely pathogenic variants detected in Cases 3, 4, 5, 6, and 7

Variant detected in **Case 3** and its parents			
Variant (HGVS)	**Case 3**	**Mother**	**Father**
NM_005908.4(MANBA):c.2158-2A>G	GG	AG	AG
Variant detected in **Case 4** and its parents			
Variant (HGVS)	**Case 4**	**Mother**	**Father**
NM_001330418.2(TLK2):c.569G>A (p.Arg190Gln)	GA	GG	GG
Variant detected in **Case 5** and its parents			
Variant (HGVS)	**Case 5**	**Mother**	**Father**
NM_057175.5(NAA15):c.691+1G>A	GA	GA	GG
Variants detected in **Case 6** and its parents			
Variant (HGVS)	**Case 6**	**Mother**	**Father**
NM_005211.3(CSF1R):c.1349G>A (p.Trp450Ter)	GA	GG	GA
NM_000797.4(DRD4):c.435_436insTG (p.Gln146CysfsTer201)	GG/ GTGG	GG/GG	GG/ GTGG
Variants detected in **Case 7** and its parents			
Variant (HGVS)	**Case 7**	**Mother**	**Father**
NM_007118.4(TRIO):c.4394A>G (p.Asn1465Ser)	AG	AA	AA

HGVS: Human genome variation society

Table 3. Genotype, family segregation results, in silico prediction scores, dbSNP records, ACMG scores, ClinVar data and pathogenicity information of the variations detected in the cases

Case	Gene	Genotype	Segregation	Inheritance	Transcript	HGVSc	HGVSp	dbSNP	ClinVar	ACMG	Pathogenicity
3	MANBA	Homozygous	Maternal, paternal	A.R	NM_005908.4	c.2158-2A>G	--	rs772852668	Pathogenic	PVS1, PM2, PP5	Pathogenic
4	TLK2	Homozygous	de novo	A.D	NM_001330418.2	c.569G>A	p.Arg190Gln	rs1567948287	VUS	PS2, PM2, PP2, PP3	Likely pathogenic
5	NAA15	Homozygous	Maternal	A.D	NM_057175.5	c.691+1G>A	--	rs1579109565	Likely pathogenic	PVS1, PM2, PP5	VUS
6	CSF1R	Homozygous	de novo	A.R	NM_005211.3	c.1349G>A	p.Trp450Ter	--	--	PVS1, PM2	VUS
6	DRD4	Homozygous	de novo	A.D	NM_000797.4	c.435_436insTG	p.Gln146CysfsTer201	--	--	PVS1, PM2	Likely pathogenic
7	TRIO	Homozygous	de novo	A.D	NM_007118.4	c.4394A>G	p.Asn1465Ser	--	--	PS2, PM1, PM2, PP3, PP5	Pathogenic

ACMG: American College of Medical Genetics, A.D: Autosomal dominant, A.R: Autosomal recessive, dbSNP: Single nucleotide polymorphism database, HGVS: Human genome variation society, VUS: Variant of unknown significance

variant of 'clinically unknown' in the Clinvar database. The variant NM_001330418.2(TLK2):c.569G>A (p.Arg190Gln) has not been reported in the HGMD database. The NM_001330418.2(TLK2):c.569G>A (p.Arg190Gln) variant was determined as a variant of unknown clinical significance (PM2, PP2, PP3) in the *in silico* analysis performed considering the ACMG-2015 criteria. The result of the segregation analysis showed that the NM_001330418.2(TLK2):c.569G>A (p.Arg190Gln) variant was found to be a homozygous wild type in the parents of Case 4. According to this result, the NM_001330418.2(TLK2):c.569G>A (p.Arg190Gln) variant was evaluated as 'likely pathogenic' and '*de novo*' based on the 'PS2' score according to the ACMG-2015 criteria.

Case 5: In the case diagnosed with IDD and ADHD who applied with speech delay, irritability and peer problems symptoms, the variant NM_057175.5(NAA15):c.691+1G>A was detected as heterozygous and was reported as 'pathogenic' in the Clinvar database. The NM_057175.5(NAA15):c.691+1G>A variant has not been reported in the HGMD database. The NM_057175.5(NAA15):c.691+1G>A variant was determined as pathogenic (PVS1, PM2, PP5) in the *in silico* analysis performed considering the ACMG-2015 criteria. The segregation analysis showed that the NM_057175.5(NAA15):c.691+1G>A variant was not detected in the father of Case 5 and that the mother was heterozygous for the variant. The pathogenic and likely pathogenic variants of the NAA15 gene were associated with the autosomal dominant phenotype IDD with behavioural abnormalities.

Case 6: In the case diagnosed with IDD and ADHD who applied with irritability and inattentiveness symptoms, the variants NM_005211.3(CSF1R):c.1349G>A (p.Trp450Ter) and NM_000797.4(DRD4):c.435_436insTG (p.Gln146CysfsTer201) were detected as heterozygous and were not reported in the ClinVar and HGMD databases. The NM_005211.3(CSF1R):c.1349G>A(p.Trp450Ter) and NM_000797.4(DRD4):c.435_436insTG (p.Gln146CysfsTer201) variants were determined as likely pathogenic (PVS1, PM2) in the in silico analysis performed considering the ACMG-2015 criteria. As a result of the segregation analysis, the NM_005211.3(CSF1R):c.1349G>A(p.Trp450Ter) and NM_000797.4(DRD4): c.435_436insTG(p.Gln146CysfsTer201) variants were not determined in the parents. The pathogenic and likely pathogenic variants of the CSF1R gene are autosomal recessive inherited brain abnormalities, neurodegeneration and dysosteosclerosis and autosomal dominant inherited leukoencephalopathy, hereditary diffuse, with spheroids phenotypes. The pathogenic and likely pathogenic variants of the DRD4 gene show an autosomal dominant inheritance attention deficit hyperactivity disorder; associated with the ADHD phenotype.

Case 7: In the case diagnosed with IDD who applied with speech delay and irritability symptoms, the variant NM_007118.4(TRIO):c.4394A>G (p.Asn1465Ser) was detected as heterozygous and was not reported in the ClinVar and HGMD databases. The NM_007118.4(TRIO):c.4394A>G (p.Asn1465Ser) variant was determined as likely pathogenic (PM1, PM2, PP3, PP5) in the in silico analysis performed considering the ACMG-2015 criteria. The segregation analysis showed that the NM_007118.4(TRIO):c.4394A>G (p.Asn1465Ser) variant was not found in the parents of Case 7. According to this result, the NM_007118.4(TRIO):c.4394A>G (p.Asn1465Ser) variant was evaluated as pathogenic and de novo based on the PS2 score according to the ACMG-2015 criteria. The TRIO gene pathogenic and likely pathogenic variants inherited the autosomal dominant IDD with macrocephaly; associated with the phenotypes.

Gibbs Free Energy Calculation and Disruption of 3D Protein Structures for Missense Variants; *TLK2*:p.(Arg339Gln) and *TRIO*:p.(Asn1465Ser)

Three clashes, two van der Waals, and six hydrogen bond interactions were reduced to two clashes, one van der Walls, and four hydrogen bond interactions after the Arginine at position 339 turned into Glutamine in TLK2. The two ionic bonds were lost, four hydrophobic interactions with seven polar bonds on protein structure remained the same after p.Arg339Gln alteration in TLK2.

Six clashes, and three van der Waals interactions were reduced to three clashes, and one van der Walls interaction after the Asparagine at position 1465 turned into Serine in TRIO. The two hydrophobic bonds were lost, seven polar bonds on protein structure remained the same, and four hydrogen bonds were increased to six, after the p.(Asn1465Ser) alteration in TRIO (Figure 1). Both p.(Arg339Gln) in TLK2 and p.(Asn1465Ser) variant in TRIO decreased protein stability and predicted as "Destabilizing" with all four different protein stability prediction tools upon point mutations. The ΔΔG scores calculated for the variant are displayed in Table 4.

DISCUSSION

In approximately 50% of IDD cases, the aetiology is unknown. Considering the large number of genes associated with IDD, genetic factors are important (26). Recent advances in human genetics have facilitated the identification of causative mutations for various genetic diseases, which may provide the possibility of individually tailored treatments for cases with particular mutations. WES analysis allowed de novo mutations to be identified in IDD studies (27). The most widely used NGS approaches are targeted panel and WES, which have lower cost and more manageable data volumes compared with whole genome sequencing (28).

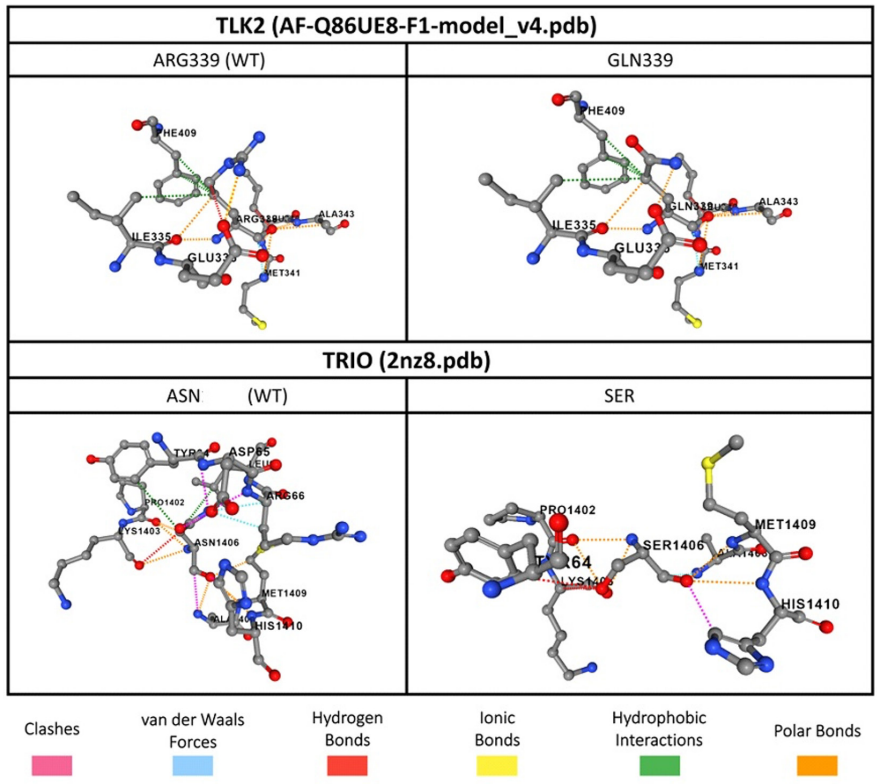

Figure 1. Calculated protein stability prediction models with DynaMut2.

Table 4. The ΔΔG scores calculated for the *TRIO:c.4394A>G* and *TLK2:c.569G>A* variants in protein modelling

Tool	Variant	ΔΔG Score (kcal/mol)	Prediction
DynaMut2	*TLK2*; R339Q	- 0.78	Destabilizing
	TRIO; N1406S	- 1.3	
INPS-3D	*TLK2*; R339Q	- 0.76	
	TRIO; N1406S	- 0.36	
PremPS	*TLK2*; R339Q	0.83	
	TRIO; N1406S	0.69	
FoldX 5.0	*TLK2*; R339Q	0.48	
	TRIO; N1406S	3.15	

Predicted ΔΔG<0 is destabilizing in the case of DynaMut2 and INPS-3D, Predicted ΔΔG>0 is destabilizing in the case of PremPS and FoldX 5.0.

We performed trio-WES and bioinformatics analyses to identify potential genetic causes in seven cases diagnosed with IDD. While pathogenic and likely pathogenic variants associated with their clinics were detected in three cases, no pathogenic or likely pathogenic variants were detected in two cases. In two cases, two likely pathogenic variants that were related to their clinics but showed parental inheritance were detected.

Among the individuals analyzed, one case with IDD and ADHD carried a pathogenic variant in the MANBA gene, which is known to be associated with β-mannosidosis, a lysosomal storage disorder. MANBA encodes the lysosomal enzyme β-mannosidase, which plays a critical role in the degradation of N-linked oligosaccharides. Functional deficiency due to homozygous or compound heterozygous mutations in MANBA leads to the accumulation of undegraded glycoproteins, resulting in progressive neurodegeneration. Clinical features observed in this individual—developmental delay, speech impairment, hyperactivity, and aggression—are consistent with previously reported MANBA-related phenotypes (29,30). The c.2158-2A>G variant in the MANBA gene affects the canonical acceptor splice site of intron 15 and has been classified as pathogenic based on its disruption of mRNA splicing, which leads to aberrant transcripts and loss of β-mannosidase function (31). ClinVar classifications from multiple diagnostic laboratories, unanimously support its pathogenic nature (32). In this context, the c.2158-2A>G mutation supports a genotype-phenotype correlation at both the molecular level; robust bioinformatic and clinical expression data have clearly identified this mutation as an etiological cause of β-mannosidosis. However, functional analysis data for this mutation are limited in the literature; therefore, experimental studies confirming the splicing effects and lack of enzyme activity at the mRNA level are needed.

Another individual exhibiting features of IDD and ADHD was found to harbor a likely pathogenic de novo variant in the TLK2 gene. This gene encodes a serine/threonine kinase essential for chromatin remodeling and genomic stability through its interaction with histone chaperones ASF1A/B. Structural protein modeling revealed destabilization of the encoded protein due to the mutation. Disruption in TLK2-mediated

chromatin regulation has been linked to neurodevelopmental phenotypes, and the clinical presentation in this case was consistent with the MRD57 syndrome (33). p.Arg190 is located in the N-terminal region of TLK2; this region may be involved in the nuclear localization signal and is critical for protein dimerization, nuclear targeting, and activation(34). Given that Arg190 is a positively charged amino acid and its location, the Arg→Gln substitution may affect the protein's conformation, localization, or interaction network; it should be evaluated specifically for DNA damage response, chromatin maintenance, and interactions with ASF1A/B chaperones (35). The clinical effects of TLK2 missense variants generally include loss of kinase activity, impaired intracellular localization, and reduced protein-protein interactions (e.g., with CHD7, CHD8, RAD50, and BRD4) (33). In conclusion, although the TLK2 p.Arg190Gln variant has not been reported in the literature, the potential functional role in the N-terminal region of the protein structure suggests a possible pathogenic factor based on in silico predictions and defined mechanisms for TLK2 variants.

In a separate case, a likely pathogenic variant in NAA15 was identified. NAA15 encodes a core component of the NatA complex, responsible for co-translational N-terminal acetylation of nascent proteins, a modification essential for protein localization and function. Haploinsufficiency of NAA15 has been shown to impair neuronal development and synaptic regulation. The clinical profile—including developmental and speech delay, behavioral disturbances, and epilepsy—closely mirrored previously described NAA15-related neurodevelopmental disorders (36-38). This variant, which we identified in our case, was not considered a sufficient finding to explain the child's clinical picture because it was inherited from the mother in the segregation analysis.

Another individual presented with novel likely pathogenic variants in both CSF1R and DRD4. The CSF1R gene encodes a receptor tyrosine kinase critical for microglial proliferation and homeostasis, and mutations in this gene can lead to white matter abnormalities due to impaired microglial function. Meanwhile, DRD4 encodes a dopamine receptor involved in modulating synaptic transmission. Variants in DRD4, particularly tandem repeats polymorphisms in exon 3, have been associated with altered dopaminergic signaling and are implicated in attention-deficit phenotypes such as ADHD (39-42). Since both the CSF1R and DRD4 gene variants detected in the case were inherited from the father in segregation analysis, they were not considered sufficient findings to explain their pathogenic impact on the child's clinical picture.

Lastly, a de novo likely pathogenic variant in the TRIO gene was detected in one of the case. TRIO encodes a Rho guanine nucleotide exchange factor that regulates actin cytoskeleton remodeling through activation of Rac1 and RhoA. Pathogenic variants have been shown to disrupt Rac1-mediated dendritic spine development, leading to impaired synapse formation and neurodevelopmental deficits. The phenotypic features observed aligned with this known mechanism of TRIO-related disorders (43-45). The mutation, NM_007118.4(TRIO):c.4394A>G, is de novo and corresponds to the p.Asn1465Ser amino acid change located in the Dbl homology 1 (DH1) subdomain of TRIO. This variant is classified as likely pathogenic in ClinVar and pathogenic in some sources, and most studies have confirmed this mutation in TRIO-associated genetic disorders (46). Turning to the clinical context, large cohort studies and case reports in the literature indicate that de novo mutations in the TRIO gene generally lead to a wide phenotypic spectrum, including developmental delay, intellectual disability, macrocephaly or microcephaly, behavioral problems (ADHD, obsessive-compulsive behavior, etc.), epilepsy, skeletal anomalies, and dental malformations).

Trio WES demonstrated a significant contribution to the clinical diagnosis of IDD. In this study, we identified one *novel* and two *de novo* variants that have not yet been reported in the literature. Notably, variants in the *TLK2* and *TRIO* genes were predicted to destabilize protein structure based on protein modeling analyses. These findings are consistent with previous reports suggesting that novel and/or de novo mutations represent a major cause of IDD. Furthermore, our results emphasize that family-based WES is not only a valuable diagnostic approach for sporadic cases but also an effective strategy for the discovery of novel disease-associated genes.

Overall, our findings underscore the pathogenic relevance of de novo variants and highlight the involvement of disrupted intracellular signaling pathways, chromatin remodeling, and protein modification in the etiology of IDD. A key limitation of the study is the lack of functional assays to validate variant pathogenicity. Although in silico predictions and structural modeling provide supportive evidence, experimental validation will be essential to establish definitive mechanistic insights.

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Informed Consent: The patients in this study were recruited in accordance with the Declaration of Helsinki's ethical principles and Good Clinical Practice guidelines.

Peer-review: Externally peer-reviewed.

Availability of Data and Materials: All data generated or analysed during this study are included in this article [and/or] its supplementary material files. Further enquiries can be directed to the corresponding author.

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REFERENCES

1. Maia N, Nabais Sá MJ, Melo-Pires M, de Brouwer APM, Jorge P. Intellectual disability genomics: current state, pitfalls and future challenges. BMC Genomics. 2021;22:909. [Crossref]
2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: 2013. [Crossref]
3. Topper S, Ober C, Das S. Exome sequencing and the genetics of intellectual disability. Clin Genet. 2011;80:117–126. [Crossref]
4. Huang J, Zhu T, Qu Y, Mu D. Prenatal, perinatal and neonatal risk factors for intellectual disability: a systemic review and meta-analysis. PLoS One. 2016;25:11(4):e0153655. [Crossref]
5. Anazi S, Maddirevula S, Faqeih E, Alsedairy H, Alzahrani F, Shamseldin HE, et al. Clinical genomics expands the morbid genome of intellectual disability and offers a high diagnostic yield. Mol Psychiatry. 2017;22(4):615–624. [Crossref]
6. Xu J, Su W, Wang Y, Luo Y, Ye F, Xu Y, et al. Genetic analysis of 280 children with unexplained developmental delay or intellectual disability using whole exome sequencing. BMC Pediatr. 2024;24(1):766. [Crossref]
7. Ballesta-Martínez MJ, Pérez-Fernández V, López-González V, Sánchez-Soler MJ, Serrano-Antón AT, Rodríguez-Peña LI, et al. Validation of clinical exome sequencing in the diagnostic procedure of patients with intellectual disability in clinical practice. Orphanet J Rare Dis. 2023;18(1):201. [Crossref]
8. Boyarchuk O, Volianska L, Smashna O, Makukh H. Exome sequencing in 90 children with developmental delay: a single-center experience. Front Genet. 2024;15:15052024. [Crossref]
9. Carvill GL, Mefford HC. Next-generation sequencing in intellectual disability. J Pediatr Genet. 2015;4(3):128–135. [Crossref]
10. Hesapcioglu ST, Ceylan MT, Kasak M, Yavas CP. Psychiatric comorbidities of mild intellectual disability in children and adolescents in a clinical setting. Int J Dev Disabil. 2021;67(2):151–157. [Crossref]

11. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol. 2011;29(1):24–26. [Crossref]
12. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285–291. [Crossref]
13. Sherry ST, Ward M, Sirotkin K. dbSNP: database for single nucleotide polymorphisms and other classes of minor genetic variation. Genome Res. 1999;9(8):677–679. [Crossref]
14. Stenson PD, Mort M, Ball EV, Evans K, Hayden M, Heywood S, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Hum Genet. 2017;136(6):665–677. [Crossref]
15. National Center for Biotechnology Information (NCBI). Welcome to NCBI. https://www.ncbi.nlm.nih.gov
16. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11(4):361–362. [Crossref]
17. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248–249. [Crossref]
18. Ng PC, Henikoff S. SIFT. Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31(13):3812–3814. [Crossref]
19. Quang D, Chen Y, Xie X. DANN. a deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics. 2015;31(5):761–763. [Crossref]
20. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol. 2010;6(12):e1001025. [Crossref]
21. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46(3):310–315. [Crossref]
22. Rodrigues CHM, Pires DEV, Ascher DB. DynaMut2: assessing changes in stability and flexibility upon single and multiple point missense mutations. Protein Sci. 2021;30(1):60–69. [Crossref]
23. Fariselli P, Martelli PL, Savojardo C, Casadio R. INPS. predicting the impact of non-synonymous variations on protein stability from sequence. Bioinformatics. 2015;31(17):2816–2821. [Crossref]
24. Chen Y, Lu H, Zhang N, Zhu Z, Wang S, Li M. PremPS: predicting the impact of missense mutations on protein stability. PLoS Comput Biol. 2020;16(12):e1008543. [Crossref]
25. Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L. The FoldX web server: an online force field. Nucleic Acids Res. 2005;33(Web Server issue):W382-W388. [Crossref]
26. Gu Y, Xiang B, Zhu L, Ma X, Chen X, Cai T. Three intellectual disability-associated de novo mutations in MECP2 identified by trio-WES analysis. BMC Med Genet. 2020;21:99. [Crossref]
27. Murakami H, Tamura N, Enomoto Y, Shimasaki K, Kurosawa K, Hanada K. Intellectual disability-associated gain-of-function mutations in CERT1 that encodes the ceramide transport protein CERT. PLoS One. 2020;15(12):0243980. [Crossref]
28. Gao C, Wang X, Mei S, Li D, Duan J, Zhang P, et al. Diagnostic yields of Trio-WES accompanied by CNVseq for rare neurodevelopmental disorders. Front Genet. 2019;10:485. [Crossref]
29. Alkhayat AH, Kraemer SA, Leipprandt JR, Macek M, Kleijer WJ, Friderici KH. Human β-mannosidase cDNA characterization and first identification of a mutation associated with human β-mannosidosis. Hum Mol Genet. 1998;7:75–83. [Crossref]
30. Bedilu R, Nummy KA, Cooper A, Wevers R, Smeitink J, Kleijer WJ, et al. Variable clinical presentation of lysosomal beta-mannosidosis in patients with null mutations. Mol Genet Metab. 2002;77(4):282–290. [Crossref]
31. Brozkova SD, Varga L, Meszarosova AU, Slobodova Z, Skopkova M, Soltysova A, et al. Variant c.2158-2A>G in MANBA is an important and frequent cause of hereditary hearing loss and beta-mannosidosis among the Czech and Slovak Roma population-evidence for a new ethnic-specific variant. Orphanet J Rare Dis. 2020;26:222. [Crossref]
32. National Center for Biotechnology Information (NCBI). ClinVar. NM_005908.4(MANBA):c.2158-2A>G. https://www.ncbi.nlm.nih.gov/clinvar/variation/590928
33. Pavinato L, Villamor-Payà M, Sanchiz-Calvo M, Andreoli C, Gay M, Vilaseca M, et al. Functional analysis of TLK2 variants and their proximal interactomes implicates impaired kinase activity and chromatin maintenance defects in their pathogenesis. J Med Genet. 2022;59(2):170–179. [Crossref]
34. Töpf A, Oktay Y, Balaraju S, et al. Severe neurodevelopmental disease caused by a homozygous TLK2 variant. Eur J Hum Genet. 2020;28(3):383–387. [Crossref]
35. Simon B, Lou HJ, Huet-Calderwood C, Shi G, Boggon TJ, Turk BE, et al. Tousled-like kinase 2 targets ASF1 histone chaperones through client mimicry. Nat Commun. 2022;13(1):749. [Crossref]
36. Stessman HA, Xiong B, Coe BP, Wang T, Hoekzema K, Fenckova M, et al. Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. Nat Genet. 2017;49(4):515–526. [Crossref]
37. Sugiura N, Patel RG, Corriveau RA. N-methyl-D-aspartate receptors regulate a group of transiently expressed genes in the developing brain. J Biol Chem. 2001;276:14257–14263. [Crossref]
38. Cheng H, Gottlieb L, Marchi E, Kleyner R, Bhardwaj P, Rope AF, et al. Phenotypic and biochemical analysis of an international cohort of individuals with variants in NAA10 and NAA15. Hum Mol Genet. 2019;28(17):2900–2919. [Crossref]
39. Manor I, Tyano S, Eisenberg J, Bachner-Melman R, Kotler M, Ebstein RP. The short DRD4 repeats confer risk to attention deficit hyperactivity disorder in a family-based design and impair performance on a continuous performance test (TOVA). Mol Psychiatry. 2002;7(7):790–794. [Crossref]
40. Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW. Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS One. 2011;6(10):e26317. [Crossref]
41. Ridge SA, Worwood M, Oscier D, Jacobs A, Padua RA. FMS mutations in myelodysplastic, leukemic, and normal subjects. Proc Natl Acad Sci USA. 1990;87:1377–1380. [Crossref]
42. Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, Soto-Ortolaza A, et al. Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. Nat Genet. 2011;44(2):200–205. [Crossref]
43. Pengelly RJ, Greville-Heygate S, Schmidt S, Seaby EG, Jabalameli MR, Mehta SG, et al. Mutations specific to the Rac-GEF domain of TRIO cause intellectual disability and microcephaly. J Med Genet. 2016;53(11):735–742. [Crossref]
44. Ba W, Yan Y, Reijnders MR, Schuurs-Hoeijmakers JH, Feenstra I, Bongers EM, et al. TRIO loss of function is associated with mild intellectual disability and affects dendritic branching and synapse function. Hum Mol Genet. 2016;25(5):892–902. [Crossref]
45. Barbosa S, Greville-Heygate S, Bonnet M, Godwin A, Fagotto-Kaufmann C, Kajava AV. et al. Opposite modulation of RAC1 by mutations in TRIO is associated with distinct, domain-specific neurodevelopmental disorders. Am J Hum Genet. 2020;106(3):338–355. [Crossref]
46. Gazdagh G, Hunt D, Gonzalez AMC, Rodriguez MP, Chaudhry A, Madruga M, et al. Extending the phenotypes associated with TRIO gene variants in a cohort of 25 patients and review of the literature. Am J Med Genet A. 2023;191(7):1722–1740. [Crossref]