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Clinical and Genetic Characteristics of Patients with Unexplained Intellectual Disability/Developmental Delay without Epilepsy

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Keywords

Intellectual disability · Autism spectrum disorder · Chromosomal microarray analysis · Clinical exome sequencing · Whole-exome sequencing

Abstract

Introduction: Global developmental delay (DD), intellectual disability (ID), and autism spectrum disorder (ASD) are mainly evaluated under the neurodevelopmental disorder framework. In this study, we aimed to determine the genetic diagnosis yield using step-by-step genetic analysis in 38 patients with unexplained ID/DD and/or ASD. ***Methods:*** In 38 cases (27 male, 11 female) with unexplained ID/DD and/or ASD, chromosomal microarray (CMA) analysis, clinical exome sequencing (CES), and whole-exome sequencing (WES) analysis were applied, respectively. ***Results:*** We found a diagnostic rate of only CMA analysis as 21% (8/38) presenting 8 pathogenic and likely pathogenic CNVs. The rate of patients diagnosed with CES/WES methods was 32.2% (10/31). When all pathogenic and likely pathogenic variants were evaluated, the diagnosis rate was 44.7% (17/38). A dual diagnosis was obtained in a case with 16p11.2 microduplication and de novo SNV. We identified eight novel variants: *TUBA1A* (c.787C>G), *TMEM63A* (c.334-2A>G), *YY1AP1* (c.2051_2052del), *ABCA13* (c.12064C>T), *ABCA13* (c.13187G>A), *USP9X* (c.1189T>C), *ANKRD17* (c.328_330dup), and *GRIA4* (c.17G>A). ***Conclusion:*** We present diagnostic rates of a complementary approach to genetic analysis (CMA, CES, and WES). The combined use of genetic analysis methods in unexplained ID/DD and/or ASD cases has contributed significantly to diagnosis rates. Also, we present detailed clinical characteristics to improve genotype-phenotype correlation in the literature for rare and novel variants.

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Introduction

Neurodevelopmental disorder (NDD), decreased motor functions of the brain, impaired cognitive functions, delay in speech, and/or inadequacy in social skills are defined [Mithyantha et al., 2017]. NDDs are categorized as intellectual disability/developmental delay (ID/DD),

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communication disorders, autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD), specific learning disorders, motor disorders, and other NDDs according to the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5) [Blesson and Cohen, 2020]. ID/DD and ASD are the most common NDDs in children [Liu et al., 2022]. In the text revision of the DSM-5 (DSM-5-TR), ID has been added in parentheses next to the equivalent term, intellectual developmental disorder. ID is characterized by a decrease in cognitive functioning and adaptive functioning beginning during childhood [Schalock et al., 2007]. ASD is known as limited social communication and interaction, repetitive behavior patterns. Although ID/DD and ASD have separate definitions, they are a large and heterogeneous group of diseases that affect brain functions with overlapping etiologies and clinical findings [Shan et al., 2022].

The American Academy of Pediatrics and the American College of Medical Genetics and Genomics recommends that children with NDD be evaluated primarily by chromosomal microarray (CMA) analysis [Miller et al., 2010; Huang et al., 2021; Liu et al., 2022]. According to recent studies, the rate of determination of NDD etiology by CMA is around 20% on average [Miller et al., 2010; Chaves et al., 2019; Maia et al., 2022]. Next-generation sequencing (NGS) analysis is recommended in the next step in patients without pathology with CMA [Huang et al., 2021]. NGS is very important in elucidating the molecular etiologies of multisystemic and heterogeneous diseases where it is difficult to make a definitive diagnosis with clinical findings such as NDD [Maia et al., 2022]. Detection of genetic variants associated with ID/DD has increased with the help of NGS. The genetic cause of approximately 35–50% of patients with NDD is explained by exome sequencing [Srivastava et al., 2019; Hiraide et al., 2021].

In this study, we aimed to investigate the genetic etiology of 38 patients with unexplained ID/DD and/or ASD. The genetic diagnostic yield of CMA, clinical exome sequencing (CES), and whole-exome sequencing (WES) analyses were determined in these patients, respectively.

Materials and Methods

Patients

Between the year of 2019 and 2021, a total of 140 patients were referred to the Department of Medical Genetics from the Pediatric Neurology Clinic. Thirty-eight patients (a mean age of 5.5 years) were evaluated with unexplained ID/DD ± ASD. The exclusion criteria for this study were as follows:

- patients with known cause of ID/DD ± ASD
- epilepsy
- abnormal karyotype (numerical and structural chromosomal abnormalities)
- genetic diagnosis of Rett syndrome and fragile X (shown in Fig. 1)

Molecular etiologies of patients with unexplained ID/DD and/or ASD were evaluated by CES/WES following CMA. Family segregation analysis was done by Sanger sequencing and/or CMA.

DNA Isolation

Genomic DNA was obtained from peripheral venous blood samples of the patients according to the QIAamp Blood & Tissue (Qiagen, Hilden, Germany) kit protocol.

Chromosomal Microarray Analysis

DNA isolated from the patient's sample was analyzed using the Illumina CytoSNP-12 v2.1 (300K) chip. It was studied using HumanCytoSNP-12v2.1_LM.bpm SNP manifest file and HumanCytoSNP-12v2.1_LM.egt SNP cluster file. BlueFuse Multi v4.5 (32,178) analysis program and BeadArray v2 standard algorithm; BG_Annotation_Ens74_20160909.db and Ensembl version 74; GRCh37 Genome build name were used. The data obtained as a result of the analysis performed on the patient were searched in Databases of Genomic Variants, DECIPHER, Online Mendelian Inheritance in Man (OMIM), and other relevant databases with the methods recommended in the literature, and the analysis of the data was made based on the recommendations of the American College of Medical Genetics (ACMG) – ClinGen guideline. The logR value of the microarray data obtained from the study was determined as 0.15 (<0.2) and the median call rate value was 0.98 (0.98–1). Loss and gain copy number variations (CNVs) of 1 Mb and/or above are detected. These detected variants were reported by using the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home), DECIPHER (https://www.deciphergenomics.org/), Simons Foundation Autism Research Initiative Gene (https://gene.sfari.org/) databases, and OMIM.

Clinical Exome Sequencing

Genomic DNA was extracted from peripheral blood and CES was performed by capturing the coding regions and splice sites of targeted genes using the Twist CES kit (South San Francisco, USA). After library enrichment and quality control, the samples were sequenced using the DNBSEQ-G400 (MGI, China) instrument with 100 bp paired-end reads at an average sequencing depth of 100×. The patients' genome coding regions sequenced with the platform using the raw data were evaluated using the Genomize® (https://seq.genomize.com) data analysis platform. Pathogenic variants associated with clinical features were filtered by following steps, in order: (1) all missense, nonsense, frameshift, frame, and synonymous variants, (2) variants with a 1.0% minor allele frequency in population studies (1000 Genomes [1000 G], Genome Aggregation Database [gnomAD]). The reference genome hg19 was used. Genome Integrative Viewer was used to view sequence data. New variants in the HGMD® and ClinVar (http://ncbi.nlm.nih.gov/clinvar) databases were checked. Pathogenicity of new variants was interpreted by using in-silico variant prediction programs (Mutation Taster, Combined Annotation Dependent Depletion [CADD]). Pathogenicity classification was made according to the ACMG criteria [Richards et al., 2015].

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Whole-Exome Sequencing

Genomic DNA was extracted from peripheral blood and WES was performed by capturing of the coding regions and splice sites of targeted genes using the SureSelect v6 Exome kit (Agilent, Inc.). After library enrichment and quality control, the samples were sequenced using the HiSeq4000 (Illumina, Inc.) instrument with 100 bp paired-end reads at an average sequencing depth of 100×. Raw reads were quality trimmed with Trimmomatic (version 0.40) [Bolger et al., 2014]. Surviving high-quality reads were mapped to reference human genome (hg19) (https://www.ncbi.nlm.nih.gov/grc/human/issues/HG-19) using the Burrows-Wheeler Alignment Tool (http://bio-bwa.sourceforge.net) [Li and Durbin, 2009]. The obtained SAM file was merged with the unmapped and paired reads file with PICARD to add metadata and to convert hard-clipped bases to soft clips. Genome Analysis Toolkit (version 4.2.3.0) (https://gatk.broadinstitute.org/hc), a software package to analyze high-throughput sequencing data, was used to call single-nucleotide polymorphisms and indels. The following modules of Genome Analysis Toolkit were used in this order: RealignerTargetCreator; IndelRealigner; BaseRecalibrator; PrintReads; Haplotypecaller; SelectVariants; VariantFiltration; and CombineVariants to call and filter single-nucleotide polymorphisms and indels. This produced an average of 11.4 Gb of mappable sequences per sample, with >79% of the exome covering >50×, enabling high-confidence variant detection (average coverage was 106× after raw data process). Annotation of detected variants was performed using VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM, and PubMed. Rare variants were classified according to the ACMG/AMP variant interpretation framework [Richards et al., 2015]. The amount of human genome covered for the Twist CES kit is 34.9 Mb (in mega base pair DNA), compared to 60 Mb for the SureSelect WES kit.

Sanger Sequencing

In Sanger confirmation, specific PCR primer sets were designed for each candidate variant as a first step. PCR was done for each sample in accordance with the appropriate protocol. The PCR was checked using 2% agarose gel electrophoresis to determine whether the product could be obtained or not. Before sequencing, the PCR products were purified using NucleoFast 96 PCR kit (MACHEREY-NAGEL, Düren, Germany). After completion of the thermal cycle step, the sequence reactions were purified according to the protocol of the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research Corp., USA). Capillary electrophoresis of the purified sequence products was sequenced by ABI 3130 (Applied Biosystems Inc.). Then variants were analyzed using SeqScape 2.5.0 (Applied Biosystems Inc.) software.

Results

The mean age of the 38 patients from 37 different families in this study was 5.5 years (6 months–17 years). The ratio of men to women was 2.45 (27/11). Most of the patients presented dysmorphic facial features (25/38; 65.7%). ASD was present in 14 of 38 cases with a rate of 36.8% (Table 1). A total diagnostic yield of 44.7% (17/38) was obtained. All patients performed CMA analysis as a first-tier genetic test. A total of 11 different CNVs were detected, 4 pathogenic, 4 likely pathogenic, and 3 of uncertain significance, in 11 (11/38 = 28.9%) patients (Table 2). These CNVs included 7 microdeletions (2 pathogenic, 3 likely pathogenic, 2 of uncertain significance) and 4 microduplications (2 pathogenic, 1 likely pathogenic, 1 of uncertain significance).

In the second tier, CES was performed in 31 patients. In these 31 patients, we included patients that were still genetically undiagnosed following CMA analysis (a patient with pathogenic CNV that did not fully overlap with clinical and cranial MR findings, 3 patients with CNV of uncertain significance, and 27 other patients). We determined a total of 11 variants as 6 pathogenic variants, 4 likely pathogenic variants, and 1 variant of uncertain significance in 9 (9/31; 29%) patients (Table 3). Pathogenic mutations were detected in the *TUBA1A*, *TUBB4A*, *DHCR7*, *YY1AP1*, and *NIPBL* genes, and likely pathogenic mutations were in the *DHCR7*, *YY1AP1*, *ABCA13*, and *SPR* genes. These variants were associated with lissencephaly 3 (OMIM #611603), leukodystrophy, hypomyelinating, 6 (OMIM #612438), Smith-Lemli-Opitz syndrome (OMIM #270400), Grange syndrome (GRNG; OMIM #602531), Cornelia de Lange syndrome 1 (OMIM #122470), dystonia, dopa responsive, due to sepiapterin reductase deficiency (OMIM #612716), and ASD. In addition, one uncertain significance variant was detected in the *USP9X* gene (Table 3).

In the third tier, WES analysis was performed in patients that presented no genetic variants explaining the clinical phenotype following CMA and CES analysis. In this step, 7 patients preferred not to continue their clinical follow-up and we performed WES analysis in 15 patients. WES analysis revealed 4 different variants (2 pathogenic and 2 of uncertain significance) in 4 (4/15; 26.6%) patients (Table 3). These pathogenic variants were associated with leukodystrophy, hypomyelinating, 19, transient infantile (HLD19; OMIM #618688) and mental retardation, autosomal dominant 35 (OMIM #616355). Furthermore, when the cases in which we performed NGS were evaluated separately, the diagnostic yield of CES analysis alone was 25.8% (8/31) and the diagnostic yield of WES analysis was 13.3% (2/15). We identified 8 novel rare variants in the *TUBA1A* (c.787C>G), *TMEM63A* (c.334-2A>G), *YY1AP1* (c.2051_2052del), *ABCA13* (c.12064C>T), *ABCA13* (c.13187G>A), *USP9X* (c.1189T>C), *ANKRD17* (c.328_330dup), and *GRIA4* (c.17G>A) genes. Flow in the application of genetic testing, patient participation rates, and diagnostic rates is shown in Figure 1.

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Table 1. Clinical features of all patients

Case	Age	Sex	Birth weight, g	Birth type	Consanguinity	Clinical features	Dysmorphic features	Brain magnetic resonance imaging
1	1 year	M	3,600	39 w, C/S	No	DD/ID	Dysmorphic facial features	Normal
2	5 years	M	3,800	38 w, C/S	Yes	DD/ID, ASD	–	Normal
3	12 years	F	3,600	38 w, NSVD	No	DD/ID	Dysmorphic facial features, scoliosis	Normal
4	2 years	F	2,250	38 w, C/S	No	DD/ID	Microcephaly, dysmorphic facial features, bilateral iris coloboma, laryngomalacia, recurrent respiratory infections	Thin corpus callosum, periventricular leukomalacia
5	1 year 10 months	M	3,500	38 w, NSVD	Yes	DD/ID, ASD	–	No
6	17 years	F	NA	NA	Adopted child	DD/ID, ASD	Atrial septal defect	No
7	9.5 years	F	3,000	38 w, C/S	No	DD/ID	Microcephaly, enuresis nocturna	Ventricular dilatation and thin corpus callosum, moderate atrophy of the cerebral sulci
8	1.5 years	M	2,800	37 w, C/S	No	DD/ID	Dysmorphic facial features, hypotonicity	Normal
9	7 years	M	3,150	38 w, C/S	Yes	DD/ID	Microcephaly, dysmorphic facial features, pectus carinatum	Normal
10	1 years	M	2,300	39 w, C/S	No	DD/ID	Intrauterine growth retardation, dysmorphic facial features, atrial septal defect, patent ductus arteriosus	NA
11	3 years	M	3,950	38 w, C/S	No	DD/ID	Dysmorphic facial features, inverted nipples, pes planus	Cavum septum pellucidum, ventricular asymmetry
12	13 years	F	3,250	38 w, C/S	No	DD/ID	Dysmorphic facial features, irregularly spaced teeth, truncal hypotonia, congenital hip dislocation, flexion contracture of digit, gait impairment	Cerebellar atrophy, gliosis in periventricular white matter
13	1 year	F	2,850	38 w, NSVD	No	DD/ID	Dysmorphic facial features, nystagmus, hypotonia	Hypomyelination of the brain white matter
14	8 years	M	3,150	39 w, C/S	No	DD/ID	Dysmorphic facial features, short stature, microurethra, syndactyly of 2nd and 3rd toes	Normal
15	15 years	F	2,600	38 w, C/S	No	DD/ID	Dysmorphic facial features, clinodactyly, operation scar on bilateral third and fourth fingers, syndactyly of the feet, intentional tremor, hyperpigmented area in the right gluteal region, cerebellar tests are clumsy, recurrent abdominal pain	Mega cisterna magna
16	4 years	M	3,500	38 w, NSVD	Yes	DD/ID, ASD	Dysmorphic facial features, short stature, neonatal hypotonia, lower limb muscle weakness, pes planus	Normal
17	8 years	M	3,300	42 w, C/S	No	DD/ID, ASD	–	–
18	1.5 years	M	3,800	39 w, C/S	No	DD/ID, ASD	Dysmorphic facial features, atrial septal defect	Thin corpus callosum
19	1 years	M	2,450	38 w, na	No	DD/ID	Microcephaly, dysmorphic facial features, lower limb muscle weakness, brachydactyly	Normal
20	3 years	M	2,965	32 w, C/S	No	DD/ID, ASD	Hypotonia, tip-toe gait, 1 × 1 cm cafe au lait in the umbilical region, pes planus	Gliotic areas in the bilateral parietal region (left > right)

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Table 1 (continued)

Case	Age	Sex	Birth weight, g	Birth type	Consanguinity	Clinical features	Dysmorphic features	Brain magnetic resonance imaging
21	5 years	M	3,650	39 w, C/S	No	DD/ID, ASD	–	Normal
22	8 years	F	3,800	38 w, C/S	No	DD/ID, ASD	–	Right hippocampal sulcus remnant cyst
23	6 months	F	1,355	32 w, C/S	Yes	DD/ID	Microcephaly, dysmorphic facial features, short neck, bilateral brachydactyly, clinodactyly in fifth fingers, pes planus and prominent calcaneus, atrial septal defect, tight filum terminale	Ventricular dilatation and thin corpus callosum
24	7 years	M	3,250	38 w, C/S	Yes	DD/ID, ASD	–	Normal
25	10 years	M	2,900	39 w, C/S	No	DD/ID	Thin and long fingers, kyphoscoliosis, uveitis	Periventricular white matter hyperintensities
26	8.5 years	F	NA	28 w	Adopted child	DD/ID	Dysmorphic facial features, clinodactyly, pes planus, tip-toe gait	Normal
27	7 years	M	3,700	38 w, C/S	Yes	DD/ID	Dysmorphic facial features, lower limb muscle weakness	Thin corpus callosum, periventricular leukomalacia, mild ventricular dilatation
28	4.5 years	M	3,700	38 w, C/S	Yes	DD/ID	Dysmorphic facial features, patent ductus arteriosus	Normal
29	3 years	M	3,000	38 w, C/S	No	DD/ID, ASD	Dysmorphic facial features, tip-toe gait	Normal
30	3 years	F	3,350	39 w, C/S	No	DD/ID, ASD	Dysmorphic facial features	Normal
31	1 year	M	2,610	37 w, C/S	No	DD/ID	–	Mild hypomyelination
32	4 years	M	2,500	39 w, NSVD	Yes	DD/ID	–	Normal
33	1 year	M	4,060	39 w, C/S	No	DD/ID	Dysmorphic facial features	N/A
34	4 years	M	3,400	39 w, NSVD	No	DD/ID, ASD	–	Normal
35	11 years	M	3,100	38 w, C/S	No	DD/ID, ASD	Dysmorphic facial features, obesity	Normal
36	3 years 10 months	M	NA	31 w, C/S	No	DD/ID	Dysmorphic facial features, short stature, hypotonia	Thin corpus callosum, periventricular leukomalacia
37	4 years	M	1,100	29 w, C/S	No	DD/ID	Unsteady gait, dysmorphic facial features	Cortical atrophy
38	5 years	M	850	24 w, NSVD	No	DD/ID	Dysmorphic facial features	Normal

M, male; F, female; C/S, cesarean section; NSVD, normal spontaneous vaginal delivery; DD/ID, developmental delay/intellectual disability; ASD, autism spectrum disorder.

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Table 2. CNV data detected in 11 patients

Case	Chromosomal location	Genomic coordinates (NCBI 37/hg19)	Type	Copy number	Size, bp	Inheritance	Clinical significance in DECIPHER database	OMIM genes	DD/ID and/or ASD associated OMIM genes
1	2p16.3	Chr 2: 51909753–523846668	Deletion	1	474,000	De novo	Uncertain significance	-	-
2	2q37.3	Chr 2: 242917734–243029573	Deletion	1	111,839	De novo	Uncertain significance	–	–
3	5q14.2	Chr 5: 82334790–82579437	Deletion	1	244,648	De novo	Likely pathogenic	*XRCC4, SCARNA18*	*XRCC4*
4	6p21.31p21.1	Chr 6: 35836705–41361584	Deletion	1	5,524,000	De novo	Pathogenic	*SLC26A8, PNPLA1, PPIL1, DNAH8, DAAM2, MOCS1, TREM2, MAPK13, BRPF3, KCTD20, SRSF3, CPNE5, C6orf89, TBC1D22B, CMTR1, MDGA1, ZFAND3, GLO1, GLP1R, LRFN2, OARD1, TSPO2, APOBEC2*	*PPIL1, LRFN2*
5	6q16.3	Chr 6: 102204406–102595588	Deletion	1	391,000	De novo	Likely pathogenic	*GRIK2*	*GRIK2*
6	15q11.1q11.2	Chr 15: 20071673–23226254	Duplication	3	3,100,000	Adopted child	Likely pathogenic	*NBEAP1, POTEB, TUBGCP5, CYFIP1, NIPA2, NIPA1*	*TUBGCP5, CYFIP1*
7*	16p11.2	Chr 16: 29287978–30347731	Duplication	3	1,059,753	Paternal	Pathogenic	*KIF22, PRRT2, TLCD3B, ALDOA, TBX6, CORO1A, BOLA2, SLX1B, SPN, ZG16, SEZ6L2, TLCD3B, C16ORF92, CDIPT, PPP4C, SLX1A*	*KIF22, ALDOA*
8	17q25.3	Chr 17: 79973528–80801745	Deletion	1	828,218	De novo	Likely pathogenic	*RAC3, DCXR, CSNK1D, CYBC1, WDR45B, TBCD, ZNF750, CENPX, FASN, SLC16A3, CD7, SECTM1, TEX19, UTS2R, HEXD, CYBC1, FOXK2, RAB40B, FN3KRP, FN3K*	*RAC3*
9	22q13.2q13.33	Chr 22: 42688724–51169045	Duplication	3	8,480	De novo	Pathogenic	*RRP7A, CYB5R3, FBLN1, A4GALT, ATXN10, PPARA, TRMU, CELSR1, MLC1, MOV10L1, TUBGCP6, SCO2, ARSA, SERHL, NFAM1, RRP7A, SERHL2, RRP7B, POLDIP3, ARFGAP3, PACSIN2, RNU12, TTLL1, BIK, MCAT, TTLL12, SCUBE1, TSPO, MPPED1, EFCAB6, SULT4A1, PRR5, ARHGAP8, NUP50, KIAA0930, UPK3A, SMC1B, WNT7B, MIRLET7BHG, GRAMD4, PKDREJ, MIRLET7B, GTSE1, TBC1D22A, TAFA5, TTLL8, SBF1, PP6R2,HDAC10, BRD1, PNAX2, ZBED4, MAPKA12, ALG12, PLXNB2, CRELD2, MAPKA11, IL17REL, NCAPH2, MOV10L1, MAPKA8IP2, CHKB, SELENOO, TUBGCP6, CPT1B, SHANK3*	*SHANK3*
10	7q11.23	Chr7: 72283565–74145064	Deletion	1	1,861,500	De novo	Pathogenic	*NSUN5, FZD9, BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, DNAJC30, BUD23, STX1A, ABHD11-AS1, CLDN3, CLDN4, METTL27, TMEM270, ELN, LIMK1, EIF4H, MIR59, LAT20, RFC2, CLIP2, GTF2IRD1, GTF2I*	–
11	9p22.1p21.3	Chr 9: 19579427–20617114	Duplication	3	1,037,688	Paternal	Uncertain significance	*MLLT3, SLC24A2*	*SLC24A2*

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Table 3. All variants detected by CES/WES analysis

Case	Gene	Exon	Inheritance	Zygosity	Nucleotide variation	Amino acid variation	ACMG classification	Associated phenotype
7*	*TUBA1A* (NM_001270399.1)	4	AD	Heterozygous	c.787C >G (de novo)	p.Pro263Ala	Pathogenic	LIS3
12	*TUBB4A* (NM_006087.4)	4	AD	Heterozygous	c.763G>A (de novo)	p.Val255Ile	Pathogenic	HLD6
13	*TMEM63A* (NM_014698.3)	Intron 5	AD	Heterozygous	c.33-2A>G (pat)	–	Pathogenic	HLD19
14	*DHCR7* (NM_001360.3)	Intron 8	AR	Heterozygous	c.964-1G>C (pat)	–	Pathogenic	SLOS
		9		Heterozygous	c.1295A>G (mat)	p.Tyr432Cys	Likely pathogenic	SLOS
15	*YY1AP1* (NM_001198903.1)	10	AR	Heterozygous	c.1903_1906del (pat)	p.Glu636ProfsTer13	Pathogenic	GRNG
		10		Heterozygous	c.2051_2052del (mat)	p.Pro684ArgfsTer26	Pathogenic	
16	*ABCA13* (NM_152701.5)	39	AD	Heterozygous	c.12064C>T (pat)	p.Arg4022Ter	Likely pathogenic	ASD
17	*ABCA13* (NM_152701.5)	48	AD	Heterozygous	c.13187G>A (mat)	p.Trp4396Ter	Likely pathogenic	ASD
18	*PPP2R5D (NM_006245.4)*	5	AD	Heterozygous	c.632A>C (de novo)	p.Gln211Pro	Pathogenic	MRD35
19	*NIPBL* (NM_133433.4)	45	AD	Heterozygous	c.7834dup	p.Arg2612LysfsTer20	Pathogenic	CDLS1
20	*SPR* (NM_003124.5)	1	AD	Heterozygous	c.1A>T (mat)	p.Met1Leu	Likely pathogenic	SRD
21	*USP9X* (NM_001039590.3)	10	XLR	Hemizygous	c.1189T>C (mat)	p.Ser397Pro	Uncertain significance	XLID99
22	*ANKRD17* (NM_032217.5)	1	AD	Heterozygous	c.328_330dup (inheritance not known)	p.Gly110dup	Uncertain significance	CAGS
23	*GRIA4* (NM_001077243.2)	2	AD	Heterozygous	c.17G>A (mat)	p.Arg6Lys	Uncertain significance	NEDSGA

LIS3, lissencephaly 3; HLD6, leukodystrophy, hypomyelinating, 6; HLD19, leukodystrophy, hypomyelinating, 19, transient infantile; SLOS, Smith-Lemli-Opitz syndrome; GRNG, Grange syndrome; ASD, autism spectrum disorder; MRD35, mental retardation, autosomal dominant 35; CDLS1, Cornelia de Lange syndrome 1; SRD, sepiapterin reductase deficiency; XLID99, intellectual developmental disorder, X-linked 99; CAGS, Chopra-Amiel-Gordon syndrome; NEDSGA, neurodevelopmental disorder with or without seizures and gait abnormalities.

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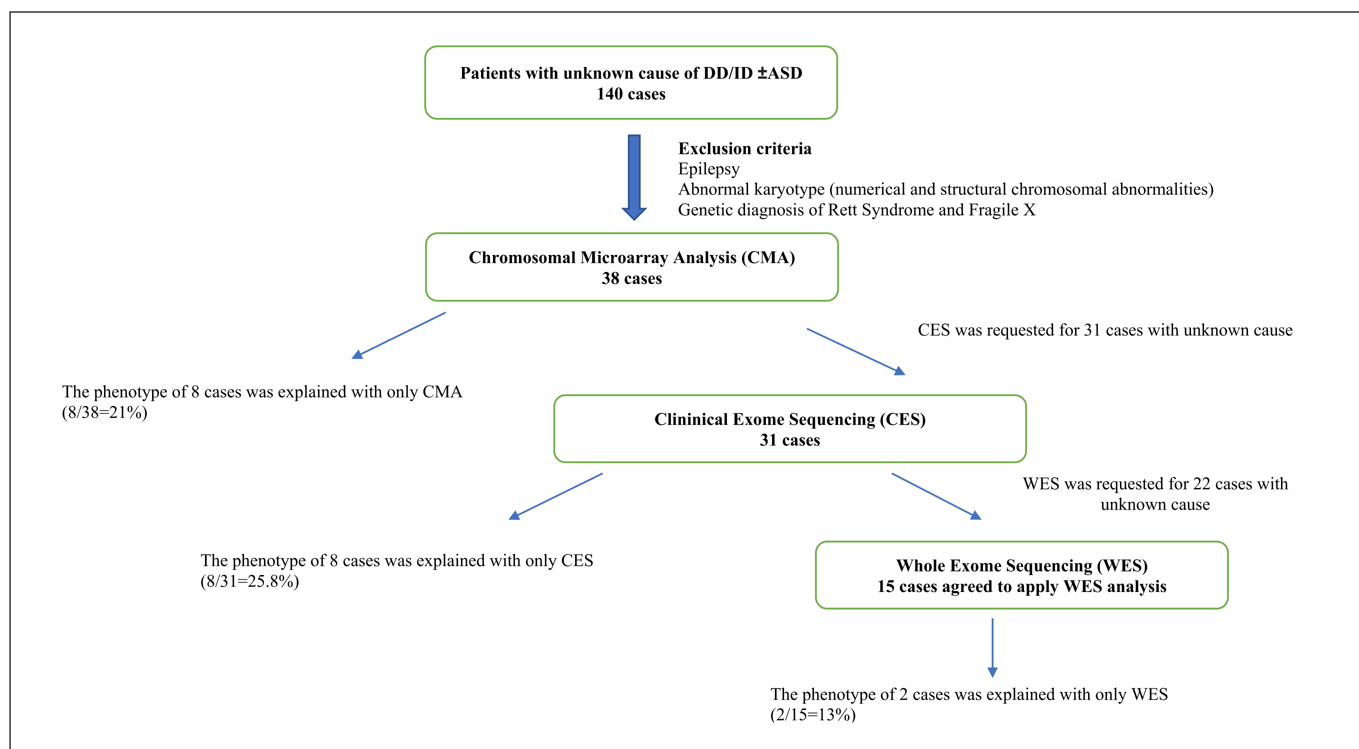

Fig. 1. Flow in the application of genetic testing, patient participation rates, and diagnostic rates.

Discussion and Conclusion

In this study, we investigated the genetic etiology of patients with unexplained ID/DD and/or ASD. We found a diagnostic rate of only CMA analysis as 21% presenting 8 pathogenic/likely pathogenic CNVs. In the previous studies including CMA analysis, CNVs were found in the range of 5–35% of the patients, depending on the DD/ID patient selection criteria and classification of detected variants [Miller et al., 2010; Gürkan et al., 2020; Liu et al., 2022]. In our study, we found that the diagnosis was mostly made by NGS methods. The rate of patients diagnosed with CES/WES methods was 32.2%. When all pathogenic and likely pathogenic variants were evaluated, the diagnosis rate was 44.7%. In studies investigating the genetic etiology of ID/DD patients, a genetic cause was found in approximately 40% of the patients (25–50%) [Nambot et al., 2018; Elmas et al., 2019; Kamath et al., 2022; Türkyılmaz et al., 2022].

Comorbidities of disorders such as dysmorphism, ASD, ADHD, and epilepsy are more common in DD/ID patients than in the general population [Misra et al., 2019; Wang et al., 2021; Türkyılmaz et al., 2022]. In our study, we excluded patients with epilepsy to obtain a more homogeneous group of patients specific to NDDs. Facial dysmorphism was the most common comorbidity, similar to the literature [Kamath et al., 2022].

In the presence of ASD comorbid to ID/DD, we detected 6 likely pathogenic and pathogenic variants (2 CNVs and 4 SNVs) in 14 patients (6/14; 42.9%). Wang et al. found that there are significantly more damaging de novo variants in the ASD with DD/ID [van Daalen et al., 2011; Nava et al., 2017; Wang et al., 2021]. We found disruptive SNVs, including 2 nonsense, 1 missense, and 1 start loss, supporting this association.

Among the defined pathogenic CNVs, we reported 6p21.31p21.1 microdeletion, 16p11.2 microduplication (OMIM #614671), chromosome 22q13 duplication (OMIM #615538) syndrome, and Williams-Beuren syndrome (OMIM #194050). The 6p21.3p21.2 deletion was first reported by Pillai et al. [2019]. To our knowledge, patient #4 is the second case with this deletion. The clinical findings of our patients were consistent with the first case reported by Pillai et al. [2019]. Differently, our patient had bilateral iris coloboma and abnormal brain MRI findings (thin corpus callosum, periventricular leukomalacia). Patient #7 with 16p11.2 microduplication had paternal inherited CNV. Father of patient #7

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also had diagnosis of ID. In addition, second-tier WES was performed in patient #7 because of abnormal brain magnetic resonance imaging findings. For patient #7, we detected a dual diagnosis of pathogenic 16p11.2 microduplication and de novo SNV. Dual diagnosis for resolution of disease phenotypes was implicated in the previous literature [Posey et al., 2017]. We detected a de novo heterozygous NM_001270399.1: c.787C>G variant in the *TUBA1A* gene. This change introduces a smaller residue at the same position. The wild-type residue is a proline (p.Pro263Ala). Prolines are known to be very rigid and therefore induce a special backbone conformation which might be required at this position. In addition, this mutation is located in a highly conserved position among other species [Yun et al., 2010]. This variant was previously unreported in the literature. For all of these reasons, it is classified as pathogenic (PM1, PM2, PM5, PP2, PP3) according to ACMG guidelines [Li et al., 2009]. Mutations in the alpha-tubulin 1A gene (*TUBA1A* [MIM #602529]) which encodes alpha-tubulin are associated with lissencephaly 3 (MIM #611603). As a result of the CMA analysis of patient #9, 8,480 kb (gene no: 65) duplication was identified in chromosome 22q13.2q13.33. To the best of our knowledge, patient #9 has the largest duplication in the region of chromosome 22q13.2q13.33. Unlike the other two reported cases, he had microcephaly and pectus carinatum.

All of the likely pathogenic CNVs were de novo except in patient #6 (adopted child). Patient #6 had a 3.1-Mb CNV in the 15q11.1q11.2 chromosomal region. Gürkan et al. [2020] reported a similar duplication of 4,170 kb in the 15q11.1q11.2 region in a patient with intrauterine growth retardation, DD, and motor retardation findings. De novo uncertain significance CNVs detected in patient #1 and patient #2 partially overlap with the clinical features of chromosome 2p16.3 deletion syndrome (OMIM #614332) and chromosome 2q37 deletion syndrome (OMIM #600430), respectively. It is expected that the findings of the patients will be milder due to the absence of genes in both regions. The uncertain significance-inherited CNV detected in patient #11 was not associated with any disease in the OMIM database. However, Prasad et al. [2012] reported a paternally inherited deletion containing the *SLC24A2* gene in the 9p22.1 region in a male patient with autism.

We identified eight novel rare variants in the *TUBA1A* (c.787C>G), *TMEM63A* (c.334-2A>G), *YY1AP1* (c.2051_2052del), *ABCA13* (c.12064C>T), *ABCA13* (c.13187G>A), *USP9X* (c.1189T>C), *ANKRD17* (c.328_330dup), and *GRIA4* (c.17G>A) genes using CES/WES analysis. Of these novel variants, 6 variants were associated with diseases having an autosomal-dominant inheritance pattern. Two cases, carrying variants of *ABCA13*:c.13187G>A and *GRIA4*:c.17G>A, presented maternal inheritance (patient #17 and patient #23, respectively). Mother of patient #17 suffered from learning disability. Variants of *GRIA4* gene were reported as causing a very rare disease with highly variable severity. The mother carrying the variant of *GRIA4*:c.17G>A variant presented microcephaly. For patient #13 carrying *TMEM63A*:c.334–2A>G variant and patient #16 carrying *ABCA13*:c.12064C>T variant, paternal inheritance was detected. Fathers of both cases suffered from ID. In addition, variant of *TUBA1A*:c.787C>G was found as de novo, while the inheritance pattern of *ANKRD17*:c.328_330dup variant was unclear.

We detected a novel *TMEM63A* (NM_014698.3):c.334-2A>G variation in patient #13. This variant, which has not been reported before in the literature, is classified as pathogenic (PVS1, PM2, PP3) according to ACMG [Richards et al., 2015]. Pathogenic variations of the *TMEM63A* [MIM 618685] gene cause HLD19 (MIM #618688). It was first reported in 2019 as a novel infantile onset transient leukodystrophy syndrome by Yan et al. [2019]. HLD19 is characterized by hypotonia, nystagmus, cognitive retardation, and hypomyelinating leukodystrophy findings on brain MR in the first months of life. In the few studies reported on HLD19, five different missense mutations were detected in eight different individuals [Yan et al., 2019; Tonduti et al., 2021; Yan et al., 2021; Fukumura et al., 2022]. To the best of our knowledge, our study presents the ninth patient.

Patient #15 was diagnosed as an extremely rare GRNG disease. GRNG (MIM #602531) is characterized by brachydactyly, syndactyly, learning disabilities, arterial occlusive disease, and cardiovascular anomalies. YY1-associated protein 1 (*YY1AP1*, MIM 607860]) gene mutations are associated with GRNG. It encodes YY1-associated protein 1, which is involved in cell cycle regulation and transcriptional regulation. The patient was not suffering from hypertension. She has a novel compound heterozygous variant combination c.1903_1906del/c.2051_2052del in the *YY1AP1* (NM_001198903.1) gene.

We detected two different novel variants in the *ABCA13* gene in two unrelated cases. We reported a case, patient #16, with a novel *ABCA13*:c.12064C>T variant. This variant was inherited from the patient's father, who also has an ID. Patient #17, an 8-year-old male, presented with ASD and DD. In this patient, we detected a maternally inherited heterozygous *ABCA13* gene: (NM_152701.5): c.13187G>A (p.Trp4396Ter) nonsense mutation. Both

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nonsense mutations are categorized as likely pathogenic and lead to a premature stop codon. The ATP-binding cassette, subfamily a, member 13 (*ABCA13*, MIM 607807) gene is located on chromosome 7p12.3. This gene encodes a protein involved in the ATP-mediated transport of cholesterol and gangliosides across the plasma membrane into the cell. *ABCA13* gene mutations were not associated with any disease in OMIM but were associated with increased susceptibility to bipolar disorder, schizophrenia, major depression, ADHD, and ASD [Chen et al., 2021; Liu et al., 2021]. Martin et al. [2014] investigated pathways of ADHD and ASD using CNV data analysis. In this study, they detected a deletion including the *ABCA13* gene in a case suffering from ASD. Also, *ABCA13* gene variants were associated with ASD in animal models [Iritani et al., 2018; Yamaguchi, 2018]. Liu et al. [2021] defined three different frame-shift mutations in the *ABCA13* gene in cases with ADHD. Our results support this association of *ABCA13* variants and NDDs.

The most important limitation of our study was the small sample size and missing cases in the WES analysis. The strengths of this study are the detailed clinical, radiological findings and complementary genetic analysis.

In conclusion, we presented our diagnostic rates and complementary approach to genetic analysis (CMA, CES, and WES) in patients with unexplained ID/DD and/or ASD. Our overall diagnosis rate was 44.7%. At the same time, the findings in this study demonstrated the importance of combined use of genetic testing in increasing the diagnostic yield for unexplained DD/ID. Genetic diagnosis is also very important in appropriate genetic counseling, prenatal testing, and routine clinical follow-up of the patient. In addition to diagnostic yield, we described novel rare variants in the *TUBA1A*, *TMEM63A*, *YY1AP1*, *ABCA13*, *USP9X*, *ANKRD17*, and *GRIA4* genes with detailed clinical characteristics to improve genotype-phenotype correlation in the literature.

Statement of Ethics

All procedures performed in this study were in accordance with the declaration of Helsinki. This study protocol was reviewed and approved by Balıkesir University Faculty of Medicine Clinical Research Ethics Committee, approval number E-94025189-050.04-59686 20 [dated August 25, 2021]. All the participants of the study have given their informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Hilal Aydin and Gul Unsel Bolat provided neurological and psychiatric evaluation. Hamide Betul Gerik Celebi and Hilmi Bolat provided genetic evaluation. Hamide Betul Gerik Celebi and Gul Unsel Bolat designed the study and then wrote the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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