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ORIGINAL ARTICLE

ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS IN CHILDREN WITH DEVELOPMENTAL DELAY/INTELLECTUAL DISABILITY

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ABSTRACT

Developmental delay (DD) is a condition wherein developmental milestones and learning skills do not occur at the expected age range for patients under 5 years of age. Intellectual disability (ID) is characterized by limited or insufficient development of mental abilities, including intellectual functioning impairments, such as learning and cause–effect relationships. Isolated and syndromic DD/ID cases show extreme genetic heterogeneity. Array-based comparative genomic hybridization aCGH) can detect copy number variations (CNVs) on the whole genome at higher resolution than conventional cytogenetic methods. The diagnostic yield of aCGH was 15.0-20.0% in DD/ID cases. The aim of this study was to discuss the clinical findings and aCGH analysis results of isolated and syndromic DD/ID cases in the context of genotype-phenotype correlation. The study included 139 cases (77 females, 62 males). Data analysis revealed 38 different CNVs in 35 cases. In this study, 19 cases with pathogenic CNVs (13.6%) and five cases with likely pathogenic CNVs (3.5%) were found in a total of 139 cases diagnosed with DD/ID. When all pathogenic and likely pathogenic cases were evaluated, the diagnosis rate was 17.1%. The use of aCGH analysis as a first-tier test in DD/ID cases contributes significantly to the diagnosis rates and enables the detection of rare microdeletion/microduplication syndromes. The clear determination of genetic etiology contributes to the literature in terms of genotype-phenotype correlation.

Keywords: Array-based comparative genomic hybridization (aCGH); Copy number variations (CNV); Developmental delay; Dysmorphic facial features; Genotype-phenotype correlation; Intellectual disability.

INTRODUCTION

Developmental delay (DD) is a condition wherein developmental milestones and learning skills do not occur at the expected age range for patients under 5 years of age. Areas used for evaluating developmental stages are gross and fine motor skills, speech and language skills, cognition, and personal-social development. Intellectual disability (ID) is characterized by limited or insufficient development of mental abilities, including intellectual functioning impairments, such as learning and cause-effect relationship [1]. Intellectual disability cases are often diagnosed in the early school-age period. The incidence of DD is 1.0-3.0% in the general population, whereas that of ID is approximately 2.7% among early school-age children [2]. Some cases have DD or ID as the only finding and are called isolated cases. Conversely, cases accompanied by facial dysmorphism, autism spectrum disorder (ASD), epilepsy and congenital anomalies, are called syndromic DD/ID [3]. Recent studies have shown that biological signaling pathways causing DD/ID, ASD, and epilepsy phenotypes are common. Additionally, the relationship between signaling pathways involved in early brain development, synaptic plasticity, and neuronal migration and the formation of these phenotypes has been demonstrated [4].

Isolated and syndromic DD/ID cases show extreme genetic heterogeneity. Genetic etiology can be detected in approximately 40.0% of the cases, whereas chromosomal abnormalities are observed in 25.0% [5,6]. Conventional cytogenetic testing can be used for detecting ≥5 Mb chro-

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mosome abnormalities. Moreover, specific chromosomal abnormalities can be investigated using fluorescence *in situ* hybridization (FISH) techniques. The diagnostic yield of both techniques for detecting DD/ID cases is approximately 5.0-6.0% [7]. Array-based comparative genomic hybridization (aCGH) can detect copy number variations (CNVs) in the whole genome at higher resolution than conventional cytogenetic methods. Copy number variations are defined as changes >1 kb resulting in an increase and/or decrease in the genomic DNA [8]. The CNVs are divided into two groups: recurrent and non recurrent. Recurrent CNVs often arise during meiosis from non-allelic homologous recombination (NAHR) between low copy repeat elements (LCRs). Non recurrent novel microdele-tion/microduplication syndromes have been identified in recent years owing to the widespread application of aCGH in diagnosis [9,10]. In a review published by Miller *et al.* [11] in 2010, the diagnostic yield of aCGH was 12.2% in 21,698 DD/ID cases retrieved from 33 different studies. The aCGH is currently recommended as the first-tier genetic test for DD/ID cases worldwide [11]. The aim of this study was to discuss the clinical findings and aCGH analysis results of isolated and syndromic DD/ID cases in the context of genotype-phenotype correlation.

MATERIALS AND METHODS

Patients. The study included 139 patients diagnosed with isolated or syndromic DD/ID (78 females, 62 males) at the Department of Pediatric Neurology, Giresun University, Giresun, Turkey; Department of Medical Genetics, Karadeniz Technical University, Trabzon, Turkey; Department of Medical Genetics, Erzurum City Hospital, Erzurum, Turkey and Department of Medical Genetics, Marmara University, Istanbul, Turkey. All patients were evaluated a by medical geneticist for dysmorphologic phenotyping. Patients with abnormal metabolic and thyroid function test results, brain tumor, brain infection, and signs of hypoxic ischemic encephalopathy, were excluded from the study. All cases were evaluated using prenatal history, family history, anthropometric measurements, detailed dysmorphological examination, hearing examination, eye examination and cardiac analysis (echocardiography). Electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) tests were performed in cases where it was deemed necessary. For genetic analysis, blood samples were obtained from all patients whose parents provided written informed consent.

Ethics Statement. All experimental procedures were conducted in accordance with the principles of the Declaration of Helsinki, and informed written consent was obtained from patients or their guardians. This was a retrospective clinical study approved by Erzurum Research and Training Hospital Ethics Committee, Erzurum, Turkey [Approval #2020/23-219].

Genetic Analysis. All patients first underwent standard karyotyping using the G-banding technique. At least 20 metaphases were analyzed at 450-500 band resolution for each patient. Chromosomal abnormalities were reported according to the recommendations of the International System for Human Cytogenetic Nomenclature 2016 [12].

For aCGH analysis, genomic DNA was isolated from peripheral blood leukocytes using Siam® DNA Mini Kit (Qiagen GmbH, Hilden, Germany). Affymetrix CytoScan Optima 315K arrays (Thermo Fisher Scientific, Waltham, MA, USA) were used according to the manufacturer's instructions for detecting CNVs. The aCGH results were evaluated using Chromosome Analysis Suite version 3.1.0 (Thermo Fisher Scientific). Technical specifications of the aCGH platform are available on the manufacturer's website (https://www.thermofisher.com/tr/en/home/life-sci ence/microarray-analysis/affymetrix.html). All CNVs were called and based on human assembly GRCh37 (hg19). Chromosomal abnormalities detected by aCGH analysis were confirmed using available FISH probes in available index cases and/or parents. The detected CNVs were evaluated according to the criteria of American College of Medical Genetics (ACMG) and were divided into three categories according to their size, gene content, inheritance pattern, presence in the literature, and population databases: pathogenic, variants of uncertain clinical significance (VUS) and benign [13]. Prevalent and known micro-deletion/microduplication syndromes and CNVs reported in several publications were considered pathogenic. Copy number variations that were reported in a single case report in the literature and explained the patient's phenotype including the genes, were considered VUS that were likely pathogenic. The CNVs that were identified in a small number of individuals in the general population and did not involve genes, were considered VUS that were likely benign. Copy number variations involving genes but having unclear dosage sensitivity status, with different opinions about its pathogenicity in the literature, were considered as VUS with no subclassification. Additionally, common polymorphisms in population databases and/or CNVs reported as benign in more than one publication, were considered benign. Pathogenicity of novel CNVs were analyzed by referring to current literature (PubMed), Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER, https://deciher. sanger.ac.uk/), Online Mendelian Inheritance in Man (OMIM, http://omim.org/), the Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home), and Clinical Genome Resource (ClinGen, https://dosage.clinicalge nome.org/index.html).

RESULTS

The study included 139 cases (77 females, 62 males) who met the patient selection criteria. The mean age was 6.3 ± 5.1 (range 1.0-25.0). Data analysis revealed 38 different CNVs in 35 cases. The average size of CNVs was 7.01 ± 11.38 Mb (range 0.215-50.379 Mb). Of the 38 CNVs, 19 were gains and 19 were losses. Additionally, 73.6% (28/38) of all CNVs were *de novo*, whereas 26.4% (10/38) were inherited. The CNVs were divided into three groups according to their pathogenicity: 21 were pathogenic, 10 were VUS (five VUS, likely pathogenic; four VUS, no subclassification; one VUS, likely benign), and seven were benign. Of the pathogenic CNVs, 10 (47.6%) were gains and 11 (52.4%) were losses. Pathogenic CNVs were further grouped by size: two (9.5%) were <1.0 Mb, five (23.7%) were 1.0-3.0 Mb, seven (33.4%) were 3.0-10.0 Mb, seven (33.4%) were >10.0 Mb. In addition, 66.6% (14/21) of the pathogenic CNVs were *de novo*, whereas 33.4% (7/21) were inherited. Two of the inherited pathogenic CNVs were inherited from a parent with a similar phenotype (one maternal and one paternal) and one from a healthy parent (maternal). Of the four inherited pathogenic CNVs, two resulted due to healthy carrier parents of balanced reciprocal translocation and two due to inversion. In two cases, more than one pathogenic CNVs was detected.

In this study, 19 cases with pathogenic CNVs (13.6%, $n = 19$) and five cases with likely pathogenic CNVs (3.5%, $n = 5$) were found in a total of 139 cases diagnosed with DD/ID. When all pathogenic and likely pathogenic cases were evaluated, the diagnosis rate was 17.1% ($n = 24/139$). The diagnosis rates for pathogenic/likely pathogenic CNVs in addition to DD/ID were as follows: ***i)*** 56.0% ($n = 13$) in cases with facial dysmorphism findings; ***ii)*** 50.0% ($n = 12$) in cases with congenital heart defects; ***iii)*** 45.8% ($n = 11$) in cases with epilepsy; ***iv)*** 41.6% ($n = 10$) in cases with microcephaly; ***v)*** 50.0% ($n = 12$) of cases with limb anomalies.

Of the 35 cases detected with CNVs, 16 had microcephaly, 15 had epilepsy, three had ASD, 14 had facial dysmorphism, 10 had short stature, two had congenital heart defect, and 10 had structural brain anomaly. The demographic and clinical findings and detailed neurological findings of the patients are summarized in Tables 1 and 2, respectively.

Table 1. Clinical and genetic features of the patients.

#	Sex-Age	Clinical Features	Karyotype	aCGH Results	Size (kb)
1	M-4	DD, hypotonia, short stature, microcephaly, micrognathia, small mouth, proximally placed thumb, fifth finger clinodactyly, broad forehead, strabismus, uplanting palpebral fissures, scoliosis	46,XY	1721.32q21.33 (47,346,528-48,900,875)x3	1554
2	M-6	DD, epilepsy, uncal dysplasia	46,XY	Xp11.23 (48,888,996-49,401,262)x2 Xq21.31q21.32 (91,579,532-92,176,985)x2	512 597
3	M-1	DD, IUGR, short stature failure to thrive, microcephaly, round face, low-set ears, epicanthus, hypotonia, cat-like cry	46,XY	5p15.33p15.2 (113,576-14,739,104)x1	14,625
4	M-6	DD, VSD, curly eyelashes, thin upper lip, prominent methopic suture, synophrys, triangular face, large ears, epilepsy	46,XY	8q24.21q24.3 (130,459,411-140,444,375)x1	9985
5	F-5	DD, epilepsy	46.XX.der(8) t(8,9)(p23.1; p23)	8p24.3p23.1 (158,048-10,161,482)x1 9p24.3p23 (203,861-13,947,653)x2	10,003 13,744
6	F-3	DD, short stature, failure to thrive, hypotonia, large ears, depressed nasal bridge, thin upper lip, epilepsy	46,XX	2q12.2q12.3 (106,925,594-188,257,773)x3	50,379
7	F-3	DD, bifid thumb, microcephaly, strabismus, broad nasal tip, depressed nasal bridge, telecanthus, short neck, low-set ears, epilepsy	46,XX,dup(4) (q28.2q35.1	4q28.2q35.1 (137,877,879-188,257,773)x3	50,379
8	F-1	DD, microcephaly, short stature, IUGR, prominent glabella, short philtrum, strasbismus, hypertelorism, epicanthus, epilepsy	46,XX	4p16.3 (68,345-1,881,435)x1	1800
9	F-4	DD, short stature, micrognathia, low-set ears, hyperterlorism, short philtrum, hypocalcemia	46,XX	23q11.21 (18,894,820-20,311,733)x1	1416
10	M-1	DD, microcephaly, hypertonicity, epilepsy	46,XY,der(3) (p25;q25)pat	3p26.3p26.1 (61,891-5,528,884)x1 3q25.32q29 (156,235,115-197,851,986)x3	5467 41,617
11	F-1	DD, hypotonia, iris coloboma	46,XX	15q13.1q13.3 (29,013,163-32,915,723)x1	3900
12	F-4	DD, epilepsy, ataxia, broad nasal tip	46,XX	6q21q23.31 (114,502,807-121,158,975)x1	6656
13	F-1	DD, hypotonia, brachycephaly, long eyelashes, small philtrum, telecanthus, pectus excavatum	46,XX	Xp22.2 (11,279,310-12,016,067)x4	737

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14	M-10	DD, epilepsy	46,XY	9q13q21.11 (68,240,211-70,984,588)x1	2744
15	M-3	DD, sensorineural hearing loss, ptosis, microcephaly	46,XY	9p24.3 (204,193-500,584)x3	296
16	F-10	DD, ASD, microcephaly, hypertonicity, self mutilation, optic atrophy, EEG abnormality	46,XX	16p12.2 (21,601,714-21,816,543)x1	215
17	F-8	DD, epilepsy, hypertonicity, hydrocephaly, obesity, short stature	46,XX	3p12 (44,626,845-45,983,652)x1	1357
18	F-10	DD, webbed neck, epilepsy, tall stature	46,X,der(X)	Xp22.2p21.3 (14,036,105026,666,672)x3	126
19	M-6	DD, ADHD, VSD, epilepsy, hypotonia, microcephaly	46,XY	Yp11.32q11.223 (118,546-25,415,912)x2	25,287
20	F-7	DD, pachygyria, lissencephaly, microcephaly, hypertonicity, epilepsy	46,XX	8q24.23 (137,278,410-138,539,014)x3	1261
21	M-4	DD, microcephaly, epilepsy, hypertonicity, macrodontia, optic atrophy, limb contractures	46,XY	16p13.11p12.3 (16,295,900-16,873,547)x1	578
22	F-3	DD, microcephaly	46,XX	14q32.33 (106,505,480-107,285,437)x1	780
23	F-2	DD, ASD, microcephaly, epilepsy, cone dystrophy	46,XX	8p1.21p11.1 (42,908,376-43,822,214)x3	914
24	F-1	DD, microcephaly, short stature, failure to thrive, prominent metopic suture, synophrys, asymetric head shape, triangular and asymetric face, telecanthus, epicanthal folds, down-slanting palpebral fissures, microphthalmia of the left eye, anteverted nares, smooth and tented philtrum, microretrognathia, low-set ears, auricular pits, high-arched palate, thin upper lip and hypotonia	46,XX,der(16)(q24)	16q121q23.5 (52,459,169-82,285,105)x3	29,800
25	F-2	DD, microcephaly, short stature, low-set ears, convex nasal ridge	46,XX	3p14.2 (60,681,991-61,207,077)x1	520
26	F-14	ID, obesity, behavioral problems	46,XX	8p21.3 (21,157,621-22,987,837)x3	1800
27	M-12	ID, impaired social interactions	46,XY	15q13.3 (31,999,631-32,914,239)x3	446
28	M-3	DD, epilepsy	46,XY	16p13.1 (14,866,283-16,391,910)x1	1500
29	F-7	ID, ASD, short stature, hand stereotypies	46,XX	14q32.2q32.33 (97,377,993-107,282,437)x3	9904
30	F-4	DD, epilepsy	46,XX	20p13 (2,911,855-4,931,592)x3	2020
31	M-14	ID, IUGR, hypotonia, microcephaly, short stature, low-set ears, small mouth, prominent forehead, hypertelorism	46,XY	19p13.3 (2,572,666-4,192,224)x3	1619
32	M-25	ID, diabetes mellitus, renal cysts, obesity, stereotyped movements	46,XY	15q11.2q13.1 (23,164,31-28,530,182)x3	5365
33	F-4	DD, epilepsy, microcephaly, micrognathia	46,XX	4q34.2q34.3 (177,322,096-180,306,130)x3	2984
34	M-14	DD, synophrys, thin upper lip, short fingernails	46,XY,der(10)t(10;13)(p15;p11)	10p15.3p15.1 (135,608-6,054,675)x1	5919
35	M-12	DD/ID, microcephaly, cerebral atrophy, synophrys, flat philtrum, 2-3-4-5 toe syndactyly	46,XY	2q31.1q31.3 (170,694,601-182,623,003)x1	11,900

Table 1. continued.

#	Sex-Age	OMIM Genes (*n*)	Critical Genes	Inheritance	Classification	Recurrent Microdeletion/ Duplication Syndrome
1	M-4	25	*PPP1R9B*, *COL1A1*, *CHAD*, *SGCA*	*de novo*	pathogenic	–
2	M-6	>30 1	*PCDH11X*	maternal	pathogenic	chromosome Xp11.23-11.22 duplication syndrome
3	M-1	10	*TPPP*, *TERT*, *NDUF6*, *SRD5A1*, *SEMA5A*, *MARCH6*, *CTNND2*, *DNAH5*	*de novo*	pathogenic	chromosome 5p deletion syndrome (Cri-du-Chat syndrome)
4	M-6	>30	*KCNQ3*	*de novo*	pathogenic	–
5	F-5	>30 >30	*FBX0025*, *TNKS*, *MSRA*, *MIR124-1*, *CLN8*, *DLGAP2* *SMARCA2*	paternal balanced reciprocal translocation	pathogenic	–
6	F-3	3	*PLGLA*, *RGPD3*, *ST6GAL2*	*de novo*	VUS, likely pathogenic	–

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7	F-3	>30	*NAA15*, *UCP1*, *MAB21L2*, *GRIA2*, *TLL1*, *VEGFC*	*de novo*	VUS, likely pathogenic	–
8	F-1	27	*ZNF141*, *PIGG*, *PDE6B*, *CPLX1*, *IDUA*, *RNF212*, *UVSSA*, *FGFR3*	*de novo*	pathogenic	chromosome 4p16.3 deletion syndrome (Wolf-Hirschhorn syndrome)
9	F-4	>30	*TUPLE1*, *TBX2*, *COMT*, *CRKL*	maternal	pathogenic	chromosome 11q11.2 deletion syndrome (DiGeorge syndrome)
10	M-1	13 >30	*CNTN6*, *CHL1* *LITRK3*, *SERPINI1*, *PDCD10*, *NAALA*, *DL2NGLN1*, *E1F2B5*, *ALG3*	paternal inv(3)(p25q25)	pathogenic	3p syndrome 3q26 microduplication syndrome
11	F-1	12	*OTUD7A*, *CHRNA7*	*de novo*	pathogenic	chromosome 15q13.3 deletion syndrome
12	F-4	18	*NUS1*, *SLC35F1*	*de novo*	pathogenic	–
13	F-1	3	*AMELX*, *MSL3*	*de novo*	VUS, no sub-classification	–
14	M-10	17	–	*de novo*	benign	–
15	M-3	1	*DOCK8*	*de novo*	benign	–
16	F-10	3	*METLLP*, *IGSF6*, *OTOA*	*de novo*	benign	–
17	F-8	4	*GUF1*, *GNPDA2*	*de novo*	VUS, no sub-classification	–
18	F-10	>30	*FANCB*, *PIGA*, *AP1S2*, *NHS*, *CDKL5*, *PDHA1*, *PHEX*, *SMPX*, *CNKSR2*, *RPS6KA3*, *ARX*	*de novo*	pathogenic	–
19	M-6	>30	*SHOX*, *CSF2RA*, *USP9Y*, *NLGN4Y*	*de novo*	VUS, likely benign	–
20	F-7	0	–	maternal	benign	–
21	M-4	2	*ABCC6*	*de novo*	VUS, no sub-classification	–
22	F-3	0	–	paternal	benign	–
23	F-2	4	–	maternal	benign	–
24	F-1	211	*CTCF*, *MAF*, *GNAO1*	*de novo*	pathogenic	–
25	F-2	1	*FHIT*	*de novo*	VUS, likely pathogenic	–
26	F-14	>30	*GFRA2*, *DOK2*, *XPO7*, *FGF17*	*de novo*	VUS, likely pathogenic	–
27	M-12	2	*OTUD7A*, *CHRNA7*	paternal	pathogenic	chromosome 15q13.3 duplication syndrome
28	M-3	12	*NDE1*, *NOMO1*, *NPIPA1*, *PDXDC1*, *NTAN1*, *RRN3*, *MARF1*, *MYH11*, *FOPNL*, *ABCC1*, *ABCC6*, *NOMO3*	*de novo*	pathogenic	–
29	F-7	>30	*DLK1*, *MIR134*, *MIR541*, *CCDC85C*, *EVL*, *YY1*, *BEGAIN*	*de novo*	pathogenic	–
30	F-4	29	*PTPRA*, *GNRH2*, *MRPS26*, *OXT*, *AVP*, *LZTS3*, *ITPA*, *SLC4111*, *ATRN*, *ADAM33*, *SIGLEC1*, *HSPA12B*	*de novo*	VUS, no sub-classification	–
31	M-14	>30	*PIAS4*, *ATCAY*, *EEF2*, *MAP2K2*	*de novo*	pathogenic	–
32	M-25	19	*MKRN3*, *MAGEL2*, *NDN*, *SNRPN*, *UBE3A*, *ATP10A*, *GABRB3*, *GABRA5*, *GABRG3*, *OCA2*, *HERC2*	*de novo*	pathogenic	chromosome 15q11-q13 duplication syndrome
33	F-4	4	*VEGFC*, *NEIL3*, *AGA*, *AGU*	*de novo*	VUS, likely pathogenic	–
34	M-14	19	*ZMYND11*	*de novo*	pathogenic	–
35	M-12	>30	*RAPGEF4*, *DLX1*, *DLX2*, *CHN1*, *SP3*, *HOXD* cluster	*de novo*	pathogenic	–

#: patient number; aCGH: array-based comparative genomic hybridization; M: male; F: female; DD: developmental delay; IUGR: intrauterine growth retardation; VSD: ventricular septal defect; VUS: uncertain clinical significance; DD/ID: developmental delay/intellectual disability; EEG: electroencephalogram; ASD: autism spectrum disorder; ADHD: attention deficit hyperactivity disorder.

Table 2. Detailed neurological findings of the patients.

#	Sex-Age	Epilepsy	Electroencephalogram	Brain Magnetic Resonance Imaging	Anti Epileptic Therapy Response
1	M-4	–	normal	normal	–
2	M-6	focal temporal lobe epilepsy started at the age of 5 months	left temporal discharges	left temporal uncal dysplasia	seizures controlled with the use of multi anti epileptic drugs
3	M-1	–	slowing of background activity	midbrain and pontine hypoplasia with enlargement of lateral ventricles	–
4	M-6	absence of seizures at the of 3 years	generalized SWDs maximally located at the post regions triggered with hyperventilation	normal	seizures controlled with the use of multi anti epileptic drugs
5	F-5	head drop seizures started at the age of 3 years	SWDs located on bilateral central regions	normal	seizures controlled with the use of multi anti epileptic drugs
6	F-3	focal motor seizures started at the age of 5 months	SWDs located on cetro-temporal regions	normal	seizures controlled with the use of multi anti epileptic drugs
7	F-3	focal motor seizures started at the age of 3 months	SWDs located on frontotemporal discharge	normal	seizures controlled with the use of multi anti epileptic drugs
8	F-1	focal motor seizures started at the age of 5 months	multifocal epileptic discharges with normal background activity	normal	seizures controlled with the use of multi anti epileptic drugs
9	F-4	–	normal	normal	–
10	M-1	focal motor seizures started at the age of 7 months	SWDs located on frontotemporal discharge	normal	seizures controlled with the use of multi anti epileptic drugs
11	F-1	–	normal	normal	–
12	F-4	myoclonic asthatic seizures started at the age of 3 years	3.0-3.5 hz generalized SWDs	normal	seizures controlled with the use of multi anti epileptic drugs
13	F-1	–	difuse slowing of the background activity without epileptic activity	cerebral and white matter atrophy	–
14	M-10	migratuar clonic seizures started as newborn	hypsarrhythmia	cerebral and white matter atrophy with enlargement of lateral ventricles	seizures controlled with the use of multi anti epileptic drugs
15	M-3	–	normal	normal	–
16	F-10	–	difuse slowing of the background activity without epileptic activity	cerebral and white matter atrophy with enlargement of lateral ventricles	–
17	F-8	focal hypomotor seizures started at the age of 5 months	SWDs located on temporoparietal and occipital regions	cerebral and white matter atrophy with enlargment of lateral ventricle and hydrocephalus	seizures controlled with the use of multi anti epileptic drugs
18	F-10	absence seizures started at the age of 4 years	3.0-3.5 hz generalized SWDs	normal	seizures controlled with the use of multi anti epileptic drugs
19	M-6	secondary generalized seizures and status epilepticus started at the age of 6 months	multifocal epileptic discharges with normal background activity	normal	seizures controlled with the use of multi anti epileptic drugs
20	F-7	multiple types of seizures started at the age of 18 months	multifocal epileptic discharges with slowing of background activity	Type 1 tip1 pachygyria, lis-sencephaly, nodular heterotropy	seizures were resistant to anti epileptic therapy
21	M-4	infantile spasm seizures started at the age of 4 months	hypsarrythmia	bilateral gliosis on the occipital regions	seizures were controlled with ACTH therapy
22	F-3	–	normal	normal	–
23	F-2	infantile spasm seizures started at the age of 4 months	multifocal epileptic discharges with slowing of background activity	bilateral gliosis on the occipital regions and enlargement of lateral ventricles	seizures were resistant to anti epileptic therapy
24	F-1	–	normal	normal	–
25	F-2	–	normal	normal	–
26	F-14	–	normal	normal	–

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27	M-12	–	normal	normal	–
28	M-3	febrile seizures started at the age of 12 months and restarted at the age of 2 years	normal	normal	seizures were controlled with the use of a single anti epileptic drug
29	F-7	–	normal	normal	–
30	F-4	myoclonic seizures started at the age of 18 months	generalized polyspike waves	normal	seizures were controlled with the use of multi anti epileptic drug
31	M-14	–	normal	normal	–
32	M-25	–	normal	normal	–
33	F-4		generalized polyspike waves	normal	seizures were controlled with ACTH therapy
34	M-14	–	normal	normal	–
35	M-12	–	normal	cerebral atrophy	–

#: patient number; M: male; F: female; EEG: electroencephalogram; MRI: magnetic resonance imaging; SWDs: sleep-wake disturbances, ACTH: adrenocorticotropic hormone.

The aCGH analysis revealed pathogenic CNVs showing clinical features in 19 (13.6%) of the total 139 cases. The findings of karyotype analysis were normal in 29 (n = 29/35, 82.8%) of the 35 cases with abnormalities detected by the aCGH analysis. Recurrent microdeletion/micro-duplication syndrome was detected in eight of the 35 cases, whereas 11 had rare microdeletion/microduplication syndrome. The karyotype analysis, aCGH analysis, inheritance pattern of CNVs, and pathogenicity classification of the patients are summarized in Table 1.

DISCUSSION

Array-based comparative genomic hybridization is recommended as the first-tier test in unexplained DD/ID cases as it can detect submicroscopic deletions and duplications below 5.0 Mb that cannot be detected by conventional karyotype analysis [11]. The widespread use of aCGH technology in recent years has resulted in increased diagnostic rates for DD/ID cases and identification of new microdeletion/microduplication syndromes.

The diagnosis rates vary between 5.1-35.0% in the literature [14,15]. The variability in diagnosis rates may be related to differences in criteria for patient selection, resolution of the aCGH platform used, and classification of detected CNVs. With the use of aCGH as a first-tier test in DD/ID cases, the frequency of VUS variants also increases in addition to the increase in diagnosis rates, making it difficult to demonstrate the genotype-phenotype correlation. CNVs associated with recurrent/well-defined syndromes, inherited CNVs from parents with a similar phenotype, and CNVs containing defined morbid genes in the OMIM database were identified as pathogenic, whereas polymorphic CNVs frequently seen in population databases were considered benign [16]. However, clinical interpretations of unique non recurrent CNVs are not always easy. The low number of these CNV cases in the literature, the unclear dosage sensitivity status of the genes, and the difference in penetrance, make interpretation difficult. In this study, pathogenic CNVs were detected in 19 cases according to the ACMG criteria, of which eight were cases of recurrent microdeletion/microduplication syndrome: 22q11.21 deletion (DiGeorge) syndrome in one, 5p deletion (Cri-Du-Chat) syndrome in one, 4p16.3 deletion (Wolf-Hirschhorn) syndrome in one, Xp11.23-p11.22 duplication syndrome in one, 3q26 microduplication syndrome and 3p deletion syndrome in one, 15q13.3 deletion syndrome in one, 15q13.3 duplication syndrome in one, and 15q11-q13 duplication syndrome in one. Additionally, rare pathogenic CNVs were detected in 11 cases: 2q31.1 q31.3 deletion in one, 10p15.3p15.1 deletion in one, 19p13.3 duplication in one, 14q32.2q32.33 duplication in one, 16p13.11 deletion in one, 16q12.1q23.3 duplication in one, Xp22.2p21.3 duplication in one, 6q21q22.31 deletion in one, 8p23.3p23.1 deletion and 9p24.3p23 duplication in one, 8q24.21q24.3 deletion in one, and 17q21.32q 21.33 duplication in one. All five CNVs that were considered likely pathogenic have been previously reported in at least one case with DD/ID in the literature and contain morbid genes. The CNVs detected in all four cases in the VUS, no subclassification group were previously reported as VUS in cases diagnosed with DD/ID in the DECIPHER database. Cases of frequent CNVs in the general population were grouped as benign. According to the two-hit model proposed by Girirajan *et al.* [17] for DD/ID cases, large CNVs that are observed more frequently in patients compared to the general population, were defined as "susceptibility loci." In these cases, it has been reported that there may be rare single nucleotide variations (SNVs) and small CNVs, which can be detected by whole-exome sequencing (WES) and whole-genome sequencing (WGS), that are responsible for the phenotype but cannot be detected due to the resolution

of microarray platform [17]. Therefore, it is believed that investigating cases of VUS CNVs with next-generation sequencing methods, such as WES and WGS, will increase the diagnostic rates.

Of the 26 pathogenic/likely pathogenic CNVs, 13 were gains and 13 were losses. Additionally, 88.4% (23/26) of these CNVs were alterations larger than 1.0 Mb. It has been reported in the literature that microdeletion syndromes are more frequently observed, and microduplication syndromes are overlooked owing to their mild phenotype [18,19]. The more frequent detection of microduplication syndromes in this study can be attributed to the inclusion of cases with a mild phenotype. The interpretation of pathogenicity of microduplications is more difficult due to incomplete penetrance and unclear triplosensitivity status of the genes it involves. Microduplications that are not found in the general population, larger than 1.0 Mb, *de novo*, and contain morbid genes, are more likely pathogenic [20].

Some cases of pathogenic CNVs with novel clinical and radiological findings are rarely described in the literature. Patient 1, a 4-year-old male with DD, short stature, microcephaly and scoliosis findings, was diagnosed with a *de novo* 1554 kb duplication in the 17q21.32q21.33 region. Two cases with duplication detected by aCGH analysis in a similar region were reported in the literature and two cases were reported in the DECIPHER database (DECIPHER ID 997 and 356717) [21,22]. Developmental delay, short stature, microcephaly, scoliosis, micrognathia, upslanting palpebral fissures were common in all the reported cases. In our case, proximally placed thumbs are a novel finding. It has been reported that *COL1A1*, *CHAD* and *SGCA* genes located in the duplication region may be responsible for skeletal abnormalities, whereas the *PPP1R9B* gene may be responsible for the DD phenotype [22]. In Patient 2, a 6-year-old male with ID and epilepsy findings, a 597 kb maternal duplication in the Xq21.31q 21.32 region, was detected. Left temporal uncal dysplasia, which was not previously reported in this syndrome, was detected in the brain MRI of the patient, who had clinical findings similar to the cases reported in the literature. It has been reported that the *PCDH11X* gene located in the duplication region, may be responsible for the ID phenotype [23,24]. In Patient 4, a 6-year-old male with ID in addition to ventricular septal defect (VSD) and dysmorphic features, epilepsy 9985 kb *de novo* deletion, was detected in the 8q24.21q24.3 region. Two cases with deletion in the same region have been reported in the literature; additionally, it has been reported that the *KCNQ3* gene may be responsible for the epilepsy phenotype [25]. Duplications were detected in patients 7 and 33 at 50.3 Mb in the 4q28.2q35.1 (137, 877, 879-188, 257, 773) region and 2.9 Mb in the 4q34.2q34.3 (177, 322, 096-180, 306, 130) region, respectively. Developmental delay, microcephaly, and epilepsy phenotype of both cases are in common with the cases reported in the literature [26]. The bifid thumb in patient 7 was a novel finding for this syndrome. Although the epilepsy types of both cases were different, seizures could be controlled by anti-epileptic treatment. More dysmorphic findings were observed in patient 7 who had a larger duplication. Chromosome 15q13.3 deletion and 15q13.3 duplication syndromes were detected in patients 11 and 27, respectively. In both cases, no significant facial dysmorphic findings were found except for ID. It has been reported that the *OTUD7A* and *CHRNA7* genes may be responsible for the phenotype in both cases [27]. Patient 24, who was previously presented in a case report, had a *de novo* pure partial trisomy 16q and contributed to the literature with its novel dysmorphic findings [28].

The widespread use of aCGH analysis in DD/ID cases increases the diagnostic rate. However, karyotype analysis must also be considered in each case for evaluating the cases for balanced translocations, inversions, and low-level mosaicisms that cannot be detected by the aCGH method. Determination of the location, size, and involved genes of the chromosomal abnormality using aCGH is important in terms of genotype-phenotype correlations. Additionally, a clear presentation of the chromosomal abnormality is critical for prognosis, clinical follow-up, and rehabilitation program planning. In terms of the family of the index case, it becomes possible to present prenatal diagnosis and pre-implantation genetic diagnosis options by screening other family members for chromosomal anomalies and explaining the risk of recurrence in subsequent pregnancies to the family [29].

The first limitation of this study is the small number of patients. The small sample size may have resulted in the low detection rate of frequently observed microdeletion/ microduplication cases. The second limitation of the study was that *FMR1* and/or *MECP2* gene analyses were not done before the aCGH analysis in these cases. Some guidelines recommend the analysis of these two genes in cases of DD/ID [30]. The strengths of this study are the detailed clinical, neurological, radiological and EEG findings of the cases.

In conclusion, the use of aCGH analysis as a first-tier test in DD/ID cases contributes significantly to the diagnosis rates and enables the detection of rare microdeletion/ microduplication syndromes. The clear determination of genetic etiology contributes to the literature in terms of genotype-phenotype correlation.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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