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. A 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-
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 microdeletion/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 by a 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-sciences/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://decipher.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.clinicalgenome.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: seven (33.4%) were 3.0-10.0 Mb, seven (33.4%) were >10.0 Mb, two (9.5%) were <1.0 Mb, five (23.7%) were 1.0-3.0 Mb, and five (23.7%) 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 CNV 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 | 4 | DD, hypotonia, short stature, microcephaly, micrognathia, small mouth, proximally placed thumb, fifth finger clinodactyly, broad forehead, strabismus, upplanting palpebral fissures, scoliosis | 46,XY | 1721.32q21.33 (47,346,528-48,900,875)x3 | 1554 |
| 2 | M-6 | 4 | DD, epilepsy, uncal dysplasia | 46,XY | Xp11.23 (48,888,996-49,401,262)x2 | 512 |
| 3 | M-1 | 4 | 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)x2 | 14,625 |
| 4 | M-6 | 4 | DD, VSD, curly eyelashes, thin upper lip, prominent methopic suture, synphrys, triangular face, large ears, epilepsy | 46,XY | 8q24.21q24.3 (130,459,411-140,444,375)x1 | 9985 |
| 5 | F-5 | 4 | DD, epilepsy | 46,XX,del(8) (8,9)(p23.1; p23) | 8p24.3p23.1 (158,048-10,161,482)x1 | 10,003 |
| 6 | F-3 | 4 | DD, short stature, failure to thrive, hypotonia, large ears, depressed nasal bridge, thin upper lip, epilepsy | 46,XX | 2q12.2q12.3 (106,925,594-188,257,775)x1 | 50,379 |
| 7 | F-3 | 4 | DD, bifid thumb, microepiphyseal, strabismus, broad nasal tip, depressed nasal bridge, telecanthus, short neck, low-set ears, epilepsy | 46,XX,del(4) (q28.2q35.1) | 4q28.2q35.1 (137,877,879-188,257,775)x3 | 50,379 |
| 8 | F-1 | 4 | DD, microcephaly, short stature, IUGR, prominent glabella, short philtrum, strabismus, hypertelorism, epicanthus, epilepsy | 46,XX | 4p16.3 (68,345-1,881,435)x1 | 1800 |
| 9 | F-4 | 4 | DD, short stature, micrognathia, low-set ears, hypertelorism, short philtrum, hypocalcemia | 46,XX | 23q11.21 (18,894,820-30,117,733)x1 | 1416 |
| 10 | M-1 | 4 | DD, microcephaly, hypertonicity, epilepsy | 46,XY,del(3) (p25q25)patt | 3p26.3p26.1 (61,891-5,528,884)x1 | 5467 |
| 11 | F-1 | 4 | DD, hypotonia, irid coloboma | 46,XX | 15q13.1q33 (29,013,163-32,915,723)x1 | 3900 |
| 12 | F-4 | 4 | DD, epilepsy, ataxia, broad nasal tip | 46,XX | 6q21q23.31 (114,502,807-121,158,975)x1 | 6656 |
| 13 | F-1 | 4 | DD, hypotonia, brachycephaly, long eyelashes, small philtrum, telecanthus, pectus excavatum | 46,XX | Xp22.2 (11,279,310-12,016,066)x4 | 737 |

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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     | >301            | PCDH11X         | maternal | pathogenic | chromosome Xp11.23-11.22 duplication syndrome |
| 3 | M-1     | 10              | TPP5, TERT, NDUF6, SRD5A1, SEMA5A, MARCH6, CTNN2, DNAH5 | de novo | pathogenic | chromosome 5p deletion syndrome (Cri-du-Chat syndrome) |
| 4 | M-6     | >30             | KCNQ3           | de novo | pathogenic | – |
| 5 | F-5     | >301            | FBXO025, TNKS, MSRA, MIR124-1, CLN8, DLGAP2, SMARCA2 | paternal balanced reciprocal translocation | pathogenic | – |
| 6 | F-3     | 3               | PLGLA, RGPD3, ST6GAL2 | de novo | VUS, likely 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. |
|---|---|---|---|---|
| 7 | F-3 | >30 | NAA13, UCP1, MB21L2, GRIA2, TLL1, VEGFC | de novo | VUS, likely pathogenic | – |
| 8 | F-1 | 27 | ZNF141, PIGG, PDE6B, CPLX1, IDUA, RNF212, UVS4, 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 | CNTN6, CHL1, LITRK3, SERPIN11, 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 | NUSI, 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 | METTLP, IGF6, OTOA | de novo | benign | – |
| 17 | F-8 | 4 | GUF1, GNPDA2 | de novo | VUS, no sub-classification | – |
| 18 | F-10 | >30 | FANCZ, PIGA, AP1S2, NHS, CDKL5, PDHA1, PHEX, SMPX, CNKSR2, RPS6KA3, ARX | de novo | pathogenic | – |
| 19 | M-6 | >30 | SHOX, CSF2R4, USP9Y, NGLN4Y | 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 | FHT | de novo | VUS, likely pathogenic | – |
| 26 | F-14 | >30 | GFRA2, DOK2, XP07, FGFI7 | de novo | VUS, likely pathogenic | – |
| 27 | M-12 | 2 | OTUD7A, CHRNA7 | paternal | pathogenic | chromosome 15q13.3 duplication syndrome |
| 28 | M-3 | 12 | NDE1, NOMO1, NPIP1A, PDADC1, NTAN1, RN3, MARF1, MTH11, FOPNL, ABC2C1, ABC2C, NOMO3 | de novo | pathogenic | – |
| 29 | F-7 | >30 | DLK1, MIR314, MIR541, CCDC83C, EVL, YY1, BEGAIN | de novo | pathogenic | – |
| 30 | F-4 | 29 | PTAPA, GNRH2, MRPS26, OXT, AVP, LZTS3, ITPA, SLCA111, ATRN, ADAM33, SIGLEC1, HSPA1B2 | de novo | VUS, no sub-classification | – |
| 31 | M-14 | >30 | PIAS4, ATCAY, EFED, MAP2K2 | de novo | pathogenic | – |
| 32 | M-25 | 19 | MKRN3, MAGEL2, NDN, SRPN, UBE3A, ATP10A, GABBR3, GABRG3, 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, HOMD cluster | de novo | pathogenic | – |
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     | –                                       | diffuse 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    | –                                       | diffuse 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 enlargement of lateral ventricles 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 heterotopy | seizures were resistant to anti epileptic therapy |
| 21 | M-4     | infantile spasm seizures started at the age of 4 months | hypsarrhythmia       | 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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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, Xp22.2p23.1 deletion in one, 6q21q22.31 deletion in one, 8p23.3p23.1 deletion and 9p24.3p23 duplication in one, 17q21.32q21.33 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.

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

| #: patient number; M: male; F: female; EEG: electroencephalogram; MRI: magnetic resonance imaging; SWDs: sleep-wake disturbances; ACTH: adrenocorticotropic hormone. | | |
|---|---|---|
| 27 | M-12 | normal normal | 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 | normal normal |
| 30 | F-4 | myoclonic seizures started at the age of 18 months | generalized polyspike waves normal normal | seizures were controlled with the use of multi anti epileptic drug |
| 31 | M-14 | normal normal | normal normal |
| 32 | M-25 | normal normal | normal normal |
| 33 | F-4 | generalized polyspike waves | normal normal | seizures were controlled with ACTH therapy |
| 34 | M-14 | normal normal | normal normal |
| 35 | M-12 | normal cerebral atrophy | normal normal |
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 COLIA1, 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 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 mosaicism 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.
REFERENCES

1. Choo YY, Agarwal P, How CH, Yeleswarapu SP. Developmental delay: Identification and management at primary care level. Singapore Med J. 2019; 60(3): 119-123.

2. Michelson DJ, Shevell MI, Sherr EH, Moeschler JB, Gropman AL, Ashwal S. Evidence report: Genetic and metabolic testing on children with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology. 2011; 77(17): 1629-635.

3. Kaufman L, Ayub M, Vincent JB. The genetic basis of non-syndromic intellectual disability: A review. J Neurodev Disord. 2010; 2(4): 182-193.

4. Lo-Castro A, Curatolo P. Epilepsy associated with autism and attention deficit hyperactivity disorder: Is there a genetic link? Brain Dev. 2014; 36(3): 185-193.

5. Moeschler JB, Shevell M; Committee on Genetics. Comprehensive evaluation of the child with intellectual disability or global developmental delays. Pediatrics. 2014; 134(3): e903-e918.

6. Miclea D, Peca L, Cuzmici Z, Pop IV. Genetic testing in patients with global developmental delay/intellectual disabilities. A review. Clujul Med. 2015; 88(3): 288-292.

7. Wang R, Lei T, Fu F, Li R, Jing X, Yang X, et al. Application of chromosome microarray analysis in patients with unexplained developmental delay/intellectual disability in South China. Pediatr Neonatol. 2019; 60(1): 35-42.

8. Lee C, Iafrate AJ, Brothman AR. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. Nat Genet. 2007; 39(7 Suppl): S48-S54.

9. Weise A, Mrasek K, Klein E, Mutalinho M, Llerena JC Jr, Hardekopf D, et al. Microdeletion and microduplication syndromes. J Histochem Cytochem. 2012; 60(5): 346-358.

10. Watson CT, Marques-Bonet T, Sharp AJ, Mefford HC. The genetics of microdeletion and microduplication syndromes: an update. Annu Rev Genomics Hum Genet. 2014; 15: 215-244.

11. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, et al. Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet. 2010; 86(5): 749-764.

12. McGowan-Jordan J, Simons A, Schmid M. An International System for Human Cytogenetic Nomenclature. Basel, Switzerland: Karger, 2016.

13. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. Genet Med. 2011; 13(7): 680-685.

14. Ballif BC, Rorem EA, Sundin K, Lincicum M, Gaskin S, Copping J, et al. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. Am J Med Genet A. 2006; 140(24): 2757-2767.

15. Aston E, Whitby H, Maxwell T, Glaus N, Cowley B, Lowry D, et al. Comparison of targeted and whole genome analysis of postnatal specimens using a commercially available array based comparative genomic hybridisation (aCGH) microarray platform. J Med Genet. 2008; 45(5): 268-274.

16. Lee KY, Shin E. Application of array comparative genomic hybridization in Korean children under 6 years old with global developmental delay. Korean J Pediatr. 2010; 42(3): 203-209.

17. Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat Genet. 2010; 42(3): 203-209.

18. Conrad B, Antonarakis SE. Gene duplication: A drive for phenotypic diversity and cause of human disease. Annu Rev Genomics Hum Genet. 2007; 8: 17-35.

19. Rice AM, McLysaght A. Dosage sensitivity is a major determinant of human copy number variant pathogenicity. Nat Commun. 2017; 8: 14366.

20. Stankiewicz P, Pursley AN, Cheung SW. Challenges in clinical interpretation of microduplications detected by array CGH analysis. Am J Med Genet A. 2010; 152A(5): 1089-1100.

21. Zahir FR, Langlois S, Gall K, Eydoux P, Marra MA, Friedman JM. A novel de novo 1.1 Mb duplication of 17q21.33 associated with cognitive impairment and other anomalies. Am J Med Genet A. 2009; 149A(6): 1257-1262.

22. Kemeny S, Pebrel-Richard C, Eymard-Pierre E, Gay-Bellile M, Gouas L, Goumy C, et al. Clinical and molecular description of a 17q21.33 microduplication in a girl with severe kyphoscoliosis and developmental delay. Eur J Med Genet. 2014; 57(10): 552-557.

23. Speevak MD, Farrell SA. Non-syndromic language delay in a child with disruption in the
Protocadherin11X/Y gene pair. Am J Med Genet B Neuropsychiatr Genet. 2011; 156B(4): 484-489.

24. Cianci P, Agosti M, Modena P, Selicorni A. De novo Xq21.31-q21.32 duplication in intellectual disability: A new report. Clin Dysmorphol. 2019; 28(2): 98-100.

25. Verheij JB, de Munnik SA, Dijkhuizen T, de Leeuw N, Olde Weghuis D, van den Hoek GJ, et al. An 8.35 Mb overlapping interstitial deletion of 8q24 in two patients with coloboma, congenital heart defect, limb abnormalities, psychomotor retardation and convulsions. Eur J Med Genet. 2009; 52(5): 353-357.

26. Rinaldi R, De Bernardo C, Assumma M, Grammatico B, Buffone E, Poscente M, et al. Cytogenetic and molecular characterization of a de novo 4q24qter duplication and correlation to the associated phenotype. Am J Med Genet A. 2003; 118A(2): 122-126.

27. van Bon BW, Mefford HC, Menten B, Koolen DA, Sharp AJ, Nillesen WM, et al. Further delineation of the 15q13 microdeletion and duplication syndromes: A clinical spectrum varying from non-pathogenic to a severe outcome. J Med Genet. 2009; 46(8): 511-523.

28. Türkyılmaz A, Yaralı O. A very rare partial trisomy syndrome: De novo duplication of 16q12.1q23.3 in a Turkish girl with developmental delay and facial dysmorphic features. Balkan J Med Genet. 2020; 23(1): 103-108.

29. D’Arrigo S, Gavazzi F, Alfèi E, Zuffardi O, Montomoli C, Corso B, et al. The diagnostic yield of array comparative genomic hybridization is high regardless of severity of intellectual disability/developmental delay in children. J Child Neurol. 2016; 31(6): 691-699.

30. Shevell M, Ashwal S, Donley D, Flint J, Gingold M, Hirtz D, et al. Practice parameter: Evaluation of the child with global developmental delay: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology. 2003; 60(3): 367-380.