İSTANBUL UNIVERSITY PRESS

Çocuk Dergisi - Journal of Child 2022;22(3):151-158
DOI: 10.26650/jchild.2022.1112958

RESEARCH ARTICLE / *ARAŞTIRMA MAKALESİ*

# Clinical Characteristics of Children with Neurodevelopmental Delay and Pathogenic Copy Number Variations in Chromosomal Microarray Analysis

## *Nörogelişimsel Geriliği Olan ve Mikrodizin Analizinda Patojenik Kopya Sayısı Değişikliği Saptanan Çocukların Klinik Özellikleri*

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**Citation/Atıf:** Maras Genc H, Kendir Demirkol Y, Beklen H, Akgun Dogan O, Kutlubay B, Sozen HG. Clinical characteristics of children with neurodevelopmental delay and pathogenic copy number variations in chromosomal microarray analysis. Çocuk Dergisi - Journal of Child 2022;22(3):151-158. https://doi.org/10.26650/jchild.2022.1112958

**ABSTRACT**

**Objective:** In this study, we report the clinical characteristics of a small cohort of children with neurodevelopmental delay and pathogenic copy number variations (CNV) in chromosomal microarray.
**Materials and Methods:** We retrospectively analyzed children aged 0-18 years with neurodevelopmental delay and a pathogenic CNV in the chromosomal microarray analysis, who had been evaluated in the pediatric genetics and pediatric neurology outpatient clinics of a tertiary hospital between August 2017 and March 2021.
**Results:** Twenty-four patients were included, 15 (62.5%) of them were girls. The mean age at diagnosis was 47.0±42.0 months (age range: 4-133 months). Most of the children (n=17, 70.8%) were diagnosed with well-defined microdeletion/microduplication syndromes. Of 28 CNVs in 24 patients; 21 (75%) were deletions, 7 (25%) were duplications. Fifteen (62.5%) of them had GDD, seven (29.2%) had ID, and three (12.5%) had ASD. A history of preterm birth and small birth weight for gestational age were present in four and five children, respectively. Neuroimaging was compatible with hypoxic-ischemic injury in two children and hypoglycemic sequel in one child. Facial dysmorphism was present in 19 (79.2%), hypotonicity in 14 (58.3%), epilepsy in eight (33.3%), microcephaly in seven (29.2%), macrocephaly in two (8.3%), hearing impairment in two (8.3%), and visual impairment in three (12.5%) children.
**Conclusion:** Chromosomal microarray analysis is a valuable tool in patients with unexplained neurodevelopmental delay. Even in children with brain injury secondary to perinatal asphyxia and neonatal hypoglycemia, microarray analysis should be performed in cases with concomitant dysmorphism and/or multisystem involvement.

**Keywords:** Nerodevelopmental delay, cognitive impairment, global developmental delay, autism spectrum disorder, chromosomal microarray, copy number variations

**ÖZ**

**Amaç:** Bu çalışmada, nörogelişimsel geriliği olan ve kromozomal mikrodizin analizinde patojenik kopya sayısı değişikliği saptanan çocukların klinik özelliklerini tanımlamayı amaçladık.
**Gereç ve Yöntem:** Üçüncü basamak bir hastanenin pediatrik genetik ve pediatrik nöroloji polikliniğinde Ağustos 2017-Mart 2021 tarihleri arasında nörogelişimsel gecikme açısından değerlendirilen ve patojenik kopya sayısı değişikliği saptanan 0-18 yaş arası çocuklar retrospektif olarak analiz edildi.
**Bulgular:** Çalışmaya 24 hasta dahil edildi, 15'i (%62,5) kızdı. Ortalama tanı yaşı 47.0±42.0 ay (yaş aralığı: 4-133 ay). Çocukların çoğunda (n=17, %70,8) iyi tanımlanmış OMIM mikrodelesyon/mikroduplikasyon sendromları saptandı. Yirmi dört hastada saptanan 28 kopya sayısı değişikliklerinin 21'i (%75) delesyon, 7'si (%25) duplikasyondu. On beş hastada (%62,5) global gelişme geriliği, 7 hastada (%29.2) zihinsel yetersizlik ve 3 hastada (%12.5) otizm spektrum bozukluğu vardı. Sırasıyla 4 ve 5 çocukta erken doğum öyküsü ve gestasyonel yaşa göre düşük doğum ağırlığı mevcuttu. Nörogörüntüleme 2 çocukta hipoksik-iskemik hasar ve 1 çocukta hipoglisemik sekel ile uyumluydu. Fasiyal dismorfizm 19 (%79.2), hipotoni 14 (%58.3), epilepsi 8 (%33,3), mikrosefali 7 (%29.2), makrosefali 2 (%8.3), görme bozukluğu 3 (%12,5) ve işitme kaybı 2 (%8,3) hastada saptandı.
**Sonuç:** Kromozomal mikrodizin analizi, açıklanamayan nörogelişimsel gecikmesi olan hastalarda değerli bir tanısal araçtır. Perinatal asfiksi ve neonatal hipoglisemiye sekonder beyin hasarı olan çocuklarda bile, eşlik eden dismorfizm ve/veya multisistem tutulumu olan olgularda mikroarray analizi yapılmalıdır.

**Anahtar Kelimeler:** Nörogelişimsel gerilik, zihinsel yetersizlik, global gelişme geriliği, otizm spektrum bozukuğu, kromozomal mikroarray, kopya sayısı değişikliği

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**Submitted/Başvuru:** 08.05.2022 • **Revision Requested/Revizyon Talebi:** 07.09.2022 • **Last Revision Received/Son Revizyon:** 04.10.2022 • **Accepted/Kabul:** 04.10.2022 • **Published Online/Online Yayın:** 31.10.2022

## INTRODUCTION

Global developmental delay (GDD), intellectual disability (ID), and autism spectrum disorders (ASD) are neuro-developmental disorders that are frequently encountered in pediatric neurology practice. The term GDD is used to describe children under five years of age who have delays in two or more areas of development (gross/fine motor, speech/language, cognitive, personal/social) by more than two standard deviations compared to their peers. Intellectual disability is a condition characterized by deficits in mental functions (judging, learning, problem-solving) and adaptive skills, in which at least one of the conceptual, social or practical areas of life are affected. Although it is a childhood-onset disorder, this term is used after the age of 5 years. Two criteria are used to define ID: intelligence coefficient and adaptive skills. An intelligence quotient (IQ) of 70 or less is classified as intellectual disability. ASD is defined as a spectrum ranging from mild to severe, comprising a group of disorders characterized by deficits in social interaction and communication, and limiting and repetitive interests and behaviors (1-3). GDD and ID are seen in approximately 1-3% of the population (1,4,5). The prevalence of ASD was reported as 2-25 per 1000 people in studies conducted in Europe, Asia, and the United States (6).

Genetic or chromosomal disorders are the most common etiology in patients presenting with neuro-developmental disorders. If a specific diagnosis cannot be made after systemic clinical evaluation, chromosomal microarray (CMA) is the first-line test with the highest diagnostic value. CMA can detect copy number variations (CNV) smaller than 1Mb. A pathogenic CNV can be detected in 7.8% of patients with developmental delays and 10.6% in children with syndromic features (7).

The aim of this study is to evaluate the clinical features of a small cohort of patients with neuro-developmental delay pathogenic CNVs in CMA analysis.

## MATERIAL AND METHODS

This study enrolled 33 children aged 0-18 years with neuro-developmental delay who had been evaluated in the pediatric genetics and pediatric neurology clinics of our hospital between August 2017 and January 2021. Among 33 children who underwent CMA analysis and were found to have either variant of unknown significance (VUS), likely pathogenic or pathogenic CNVs; 24 patients with pathogenic CNVs are included in the study. Pathogenicity of CNVs was identified according to Miller et al. (8), and American College of Medical Genetics guidelines (9). To determine the clinically significant CNVs, we used databases including DECIPHER (https://decipher.sanger.ac.uk/application/), ClinGen (https://clinicalgenome.org), International Standard Cytogenomic Array Consortium (https:// isca.genetics.emory.edu), OMIM (http://www.ncbi.nlm.nih.gov/Omim), dbVar (http://www.ncbi.nlm. nih.gov/dbvar/) and peer-reviewed literature. Parental analyses could not be performed in all families due to loss of follow-up, lack of consent or financial constraint.

Platforms used for aCGH included Cytoscan HD-750K (Affymetrix, Santa Clara, CA, USA) and CytoSNP-12 (Illumina, San Diego, CA, USA) instruments the Infinium CytoSNP-850K v1.1 Beadchip (Illumina, San Diego, California, USA), and were used according to the manufacturer's instructions. Data analysis was undertaken using Chromosome Analysis Suite (ChAS) software, Genome Studio (v.2.0.4) and BlueFuse (v.4.5).

Clinical findings were retrospectively analyzed. Prenatal, natal, postnatal and family history was recorded. All patients underwent physical examination and dysmorphological evaluation and metabolic screening (detailed biochemistry, tandem MS/MS, plasma amino acids, urinary organic acids, ammonia, lactate). In case of clinical necessity, additional metabolic tests, brain magnetic resonance imaging (MRI), and electroencephalography (EEG) were requested.

The Statistical Package for Social Sciences (IBM SPSS version 20.0) was used for the statistical analysis. Descriptive statistical methods were used. The study was approved by the ethical committee of our hospital (15.03.2021-5417).

## RESULTS

The study included 24 children; fifteen (62.5 %) girls and 9 (37.5 %) boys. The mean age of the children at the time of diagnosis was 47 months (min 4, maximum 133 months, SD: 42.0). Parental consanguinity was present in 8 (33.3%) cases. A history of a preterm birth and small birth weight for gestational age were present in 4 (16.7%) and 5 (20.8 %) children respectively. One child was born large for gestational age (4.2%). GDD was present in 15 (62.5%), ID in 7 (29.2%) and ASD in 3 (12.5%) cases.

We detected a total of 28 CNVs in 24 patients; 21 (75%) were deletions, and 7 (25%) were duplications. Seventeen patients had single deletions, four had single duplications, and the remaining three had multiple CNVs comprising both deletions and duplications. One patient had mosaicism for tetrasomy of chromosome 12p (patient-14). Six (21.4%) of the CNVs were <1Mb, 12 (42.9%) were 1-5 Mb, 4 (14.3%) were 5-10 Mb and 6 (21.4%) were >10 Mb.

Seventeen children (70.8%) were diagnosed with common microdeletions/duplications or well-defined syndromes with OMIM (Online Mendelian Inheritance in Man) entries. These included three patients with the recurrent chromosome 16p11.2 deletion (MIM# 611913); three with 1p36 deletion syndrome (MIM# 607802); two with 15q13.3 deletion syndrome; and one patient each with the following: Angelman syndrome (MIM# 105830) due to a 15q11.2 deletion; Coffin-Siris syndrome (MIM# 135900) due to a 6q25.3 deletion encompassing the *ARID1B* gene; 22q13.3 deletion syndrome (MIM# 606232), 22q11.2 duplication syndrome (MIM# 608363), Tetrasomy 18p (MIM# 614290), 18q deletion syndrome (MIM# 601808), 17p13.1 deletion syndrome (MIM# 613776), Mosaic tetrasomy 12p syndrome (Pallister-Killian syndrome, MIM# 601803), and 9p deletion syndrome (MIM# 158170). The remaining patients had pathogenic CNVs that were not previously associated with a well-described entity.

Although patient 20, had a large deletion (11 Mb), prenatal genetic tests on chorionic villus samples, including karyotype and CMA analysis (with a lower resolution) and whole exome sequence analysis were found to be normal.

Three patients had an additional diagnoses of cerebral palsy: Patient-7, with tetrasomy 18p syndrome, was born at 33 gestational weeks, with a birth weight of 1300 g [small for gestational age (SGA)]. Mild periventricular leukomalacia was present in the cranial MRI. Patient-12, who had a diagnosis of 1p36 deletion syndrome, was born at term with a birth weight SGA. She had a history of respiratory arrest at 40-days-old, and was resuscitated. Follow-up cranial MRI revealed periventricular leukomalacia. Patient-16, who was diagnosed with 1p36 deletion syndrome, was born at term, and birth weight was appropriate for gestational age. He had a history of neonatal hypoglycemia. Follow-up cranial MRI revealed bilateral parieto-occipital encephalomalacia. All three patients underwent CMA analysis because of dysmorphic features.

A summary of the clinical features of the patients is shown in Table 1, and detailed phenotype and molecular cytogenetic data of patients are listed in Table 2.

**Table 1: Clinical findings of children with pathogenic copy number variations**

| Clinical findings | Frequency/ total number of patients evaluated (%) |
|---|---|
| History of preterm birth | 4/24 (16.7) |
| Birth weight | |
| AGA | 18/24 (75.0) |
| SGA | 5/24 (20.8) |
| LGA | 1/24 (4.2) |
| Parental consanguinity | 8/24 (33.3) |
| Positive first degree family history | 3/24 (12.5) |
| Global developmental delay | 15/24 (62.5) |
| Mild | 8 |
| Moderate to severe | 7 |
| Intellectual disability | 7 (29.2) |
| Mild | 5 |
| Moderate to severe | 2 |
| Autism spectrum disorder | 3 (12.5) |
| Head circumference | |
| Microcephaly | 7/24 (29.2) |
| Macrocephaly | 2/24 (8.3) |
| Tonus | |
| Hypotonicity | 14/24 (58.3) |
| Hypertonicity | 2/24 (8.3) |
| Facial dysmorphism | 19/24 (79.2) |
| Hearing impairment | 2/24 (8.3) |
| Visual impairment | 3/24 (12.5) |
| Epilepsy (seizure control is defined in 7 patients) | 8/24 (33.3) |
| Seizure control with monotherapy | 5/7 |
| Seizure control with two anti-seizure drugs | 1/7 |
| Drug-resistant epilepsy | 1/7 |
| EEG abnormality | 5/13 (38.5) |
| Focal anomaly | 3 |
| Generalized anomaly | 3 |
| Abnormal cranial MRI | 6/18 (33.3) |
| Accompanying major anomaly | 6/22 (27.3) |

AGA: Appropriate for gestational age, SGA: small for gestational age, LGA: large for gestational age, EEG: Electroencephalography, MRI: Magnetic resonance imaging

## DISCUSSION

Our cohort consists of children with neuro-developmental delay and pathogenic CNVs. The diagnostic yield of CMA is reported to be 7.8-30% in children with developmental delay and CMA has the highest diagnostic yield than any clinical test available in this patient group (7,10-13). Routine screening for inborn errors of metabolism has a lower diagnostic yield (up to 5%) (7). Metabolic screening tests and cranial MRI are usually included in the protocol of studies investigating the diagnostic yield of CMA in neurodevelopmental disorders (11,13). In our study, metabolic screening tests were performed before CMA analysis. Commonly used metabolic screening tests usually generate results faster than genetic tests, and they are primarily performed to exclude treatable causes of neuro-developmental delay. Diagnosis of a treatable cause of neurodevelopmental disorders has a great impact on patient treatment and outcome.

In our cohort, a significant part of the reported CNVs are over 5 Mb in size, which could be diagnosed by conventional chromosome analysis. In 2010, the International Standard Cytogenetic Array (ISCA) Consortium recommended CMA as the first-line diagnostic test for individuals with GDD/ID, ASDs, or multiple congenital anomalies (8). Standard karyotype analysis is recommended if the patient has a recognizable chromosomal syndrome (eg, Down syndrome, Turner syndrome), history of multiple abortions, chromosomal rearrangements or infertility. For patients with clinically recognizable microdeletion/microduplication syndromes (e.g., DiGeorge syndrome), FISH analysis for that specific syndrome can be used. In our cohort, CMA was selected as an initial genetic test, even for patients with clinically recognizable syndromes (eg. 1p36 deletion syndrome), because there may be significant phenotypic variation between affected patients, and clinical diagnosis may not be as certain as Down syndrome in very young pediatric patients, infants especially. Moreover, CMA has a superior resolution to FISH analysis and can detect all currently known microdeletion/microduplication syndromes (7,8).

Recently, in parallel with the rapid development in genomic technologies, it has been possible to perform microarray

**Table 2: Summary of phenotype and cytogenetic data of patients**

| Patient no/ gender/age | Clinical features | Copy number variations / karyotype | Size | Inheritance | Associated gene or chromosome locus, references |
|---|---|---|---|---|---|
| **1/F/8y5m** | Mild ID, epilepsy, preterm SGA | arr[GRCh37]3q27.1q29(184,326,528-192,863,237)x1 | 8.5 Mb | unknown | DECIPHER: 292123, 275722, 276534, 281790 |
| **2/F/10y3m** | ASD, mild ID | arr[GRCh37]7p22.3(1,132,971-2,343,853)x1 | 1.2 Mb | unknown | ISCA: nssv14082213 |
| **3/F/2y8m** | ASD, macrocephaly | arr[GRCh37]7p22.2p22.1(4,186,277-6,601,751)x3 | 2.4 Mb | de novo | 7p22.1 duplication syndrome (PMID: 27866048) (14) |
| **4/M/10y3**m | Epilepsy, mild ID, macrocephaly | arr[GRCh37]22q11.21(20,716,876-21,927,646)x1 | 1.2 MB | unknown | Central 22q11.2 deletion (PMID 25123976) (24) |
| **5/M/7y4**m | Severe ID, hypotonia, dysmorphism, corpus callosum dysgenesis, hirsutism, feeding problems | arr[GRCh37]6q25.3(155,751,813-157,398,175)x1/ karyotype: 46, XY | 1.6 Mb | unknown | ARID1B gene, Coffin Siris syndrome (OMIM# 135900) |
| **6/F/9m** | GDD, hypotonia, dysmorphism, hypomyelination, corpus callosum dysgenesis | arr[GRCh37]4q34.1(173,854,451-175,026,757)x1 / karyotype: 46, XX | 1.2 Mb | de novo | DECIPHER: 267783 ISCA: nssv578000 |
| **7/F/6**m | GDD, dysmorphism, preterm SGA, hearing loss, microcephaly, hypertonia, periventricular leukomalacia | arr[GRCh37]18p11.32p11.21(136,226-14,978,127) x4 karyotip: 47,XX+mar | 14.8 Mb | de novo | Tetrasomy 18 p, isochromosome 18p syndrome (OMIM# 614290) |
| **8/F/13**m | GDD, hypotonia, dysmorphism, | arr[GRCh37]18q12.2q21.1(34,504,506-46,532,088)X1 / karyotype: 46,XX,del(18)(q12.2q21.1) | 12 Mb | de novo | Chromosome 18q deletion syndrome (OMIM# 601808) |
| **9/F/3y11**m | GDD, hypotonia, term SGA, bilateral anophthalmia, dysmorphism, extremity anomalies | 1-arr[GRCh37]3p26.3(61,891-2,070,846) x1<br>2-arr[GRCh37]4q35.1q35.2(185,721,369-190,957,473) x1<br>3-arr[GRCh37]13q31.3q34(92,732,335-115,107,733) x3* | 1-2 Mb<br>2-5.2 Mb<br>3-22.4Mb | 1-paternal (2Mb)<br>2-de novo<br>3- de novo<br>mother karyotype: 46,XX,t(4:13) (q35.1;q31.3) | 3* PMID: 25337073 (15) |
| **10/M/4m** | GDD, preterm SGA, hypertonicity, microcephaly, duplicated collecting system in kidneys | 1- arr[GRCh37]21q22(45,973,877-48,097,372)x3<br>2- arr[GRCh37]22q13.2q13.33 (42,592,238-51,197,838) x1* / karyotype: 46,XY,der(22)t(21;22)(q22.3;q13.31) | 1-2 Mb<br>2- 8.6 Mb | unknown | 2*Chromosome 22q13.3 deletion syndrome (Phelan McDermid syndrome) (OMIM# 606232) |
| **11/F/ 11y1m** | mild ID, dysmorphism, sensorineural hearing loss | arr[GRCh37]22q11.21(20,742,449-21,804,886)x3 | 1.1 Mb | unknown | Chromosome 22q11.2 microduplication syndrome (OMIM# 608363) |
| **12/F/2y1**m | GDD, hypotonia, microcephaly, epilepsy, dysmorphism, tracheostomised, cardiomyopathy, strabismus, periventricular leukomalacia | arr[GRCh37]1p36.33p.36.32(849,466-5,021,200) 46,XX,del(1)(p36.3) / karyotype: 46,XX,del(1)(p36.3) | 4.2Mb | de novo | 1p36 del syndrome (OMIM# 607872) |
| **13/M/20m** | GDD, hypotonia, microcephaly, dysmorphism | arr[GRCh37]15q11.2q13.1(23,291,158-28,828,168)X1 confirmed with methylation specific MLPA analysis | 5.5Mb | unknown | Angelman syndrome (OMIM# 105830) |

**Table 2: Summary of phenotype and cytogenetic data of patients *(Continued)***

| Patient no/ gender/age | Clinical features | Copy number variations / karyotype | Size | Inheritance | Associated gene or chromosome locus, references |
|---|---|---|---|---|---|
| **14/F/9 m** | GDD, hypotonia, dysmorphism, hyperpigmentation along Blaschko's lines, optic atrophy | arr[GRCh37]12p13.33p11.22(191619-28756107)x2-4 | 28.5Mb | unknown | Mozaic tetrasomy 12p syndrome (Pallister-Killian syndrome) (OMIM# 601803) |
| **15/M/2y4m** | GDD, hypotonia, term LGA | arr[GRCh37]15q13.2q13.3(31,073,668-32,914,239)x1 / karyotype: 46,XY | 1.8 Mb | de novo | 15q13.3 deletion syndrome (OMIM# 612001) |
| **16/M/6y10**m | Severe ID, refractory epilepsy, hypotonia, microcephaly, visual impairment, dysmorphism. Clinical findings and MRI are compatible with neonatal hypoglycemic sequel | arr[GRCh37]1p36.33(849,466-1,992,748)x1 / karyotype: 46, XY | 1.1 Mb | de novo | 1p36 deletion syndrome (OMIM# 607872) |
| **17/M/7m** | GDD, hypotonia, dysmorphism | arr[GRCh37]16p11.2(29,567,295-30,226,930)x1 / karyotype: 46, XY | 660Kb | unknown | Chromosome 16p11.2 deletion syndrome-593 Kb (OMIM# 611913) |
| **18/M/4y** | GDD, epilepsy | arr[GRCh37]16p11.2(29634212_30199805)x1 | 566Kb | de novo | Chromosome 16p11.2 deletion syndrome-593 Kb (OMIM# 611913) |
| **19/F/14**m | GDD, epilepsy, hypotonia | arr[GRCh37]16p11.2(29634212-30199805)x1 | 565Kb | unknown | Chromosome 16p11.2 deletion syndrome-593 Kb (OMIM# 611913) |
| **20/F/13**m | GDD, microcephaly, hypotonia, epilepsy, dysmorphism, term SGA, Ebstein anomaly | arr[GRCh37]1p36.33p36.22(82154_1160306)1x1 | 11.5Mb | unknown | 1p36 deletion syndrome (OMIM# 607872) |
| **21/F/5y9m** | GDD, hypotonia, dysmorphism | arr[GRCh37]9p24.3p22.3(203,861-14,831,003)x1 / karyotype from chorionic villus sampling: 46,XX | 14.6Mb | unknown | Chromosome 9p deletion syndrome (OMIM# 158170) |
| **22/F/19m** | ASD, dysmorphism | arr[GRCh37]17q24.2(65,738,331-66,344,703)x1 / karyotype: 46,XX | 606Kb | maternal (294Kb) | BPTF gene (this gene is not deleted in mother) PMID: 22166941 (16) |
| **23/M/6y8**m | Mild ID, dysmorphism, epilepsy, macrocephaly, prematurity | arr[GRCh37]15q13.2q13.3(30,940,398-32,922,947)x1 karyotype: 46,XX | 2Mb | unknown | 15q13.3 deletion syndrome (OMIM# 612001) |
| **24/F/3**y | GDD, hypotonia, microcephaly, epilepsy, dysmorphism, | 1-arr[GRCh37]1q21.2(147391614- 147826658)x3 2-arr[GRCh37]17p13.1(7082687_7277907)x1* / karyotype: 46, XX | 1-435 Kb 2- 195Kb | 1- maternal (102 Kb) 2- de novo | 2*Chromosome 17p13.1 deletion syndrome (OMIM# 613776) |

GDD: Global developmental delay, ID: Intellectual delay, ASD: Autism spectrum disorder. SGA: small for gestational age, LGA: large for gestational age *copy number variation that is evaluated as pathogenic

analyses with higher resolutions, increasing the diagnostic rate in patients. However, this has also led to an increase in the reporting of VUS, which causes difficulties for clinicians, patients, and families. The ACMG 2019 guidelines propose many evidence categories for the classification of a CNV, including genomic content, dosage sensitivity, clinical overlap and segregation in similarly affected family members. While *de novo* occurrence of a CNV is not always enough to classify it as pathogenic, it can be accepted as individual evidence for pathogenicity in most cases. Likewise, while detection of a VUS variant in healthy parents suggests that the CNV is more likely to be a benign variation, it does not rule out pathogenicity due to certain CNVs representing low-penetrance risk alleles for disease (8). We did not include VUS CNVs in the present study because we could not perform parental analyses in most cases. This may be the reason why well-defined micro-deletion/micro-duplication syndromes were reported in most of the patients included in our study.

Most of the CNVs were deletions similar to other reports in the literature (10,11,13). In our study, three patients had an additional diagnosis of cerebral palsy, which would explain the developmental delay. Therefore, even in the presence of clinical and radiological findings consistent with cerebral palsy, further genetic testing should be performed if there are accompanying dysmorphic or syndromic findings.

Facial dysmorphism (79.2%), hypotonicity (53.8%), epilepsy (33.3%) and microcephaly (29.2%) were the most frequent additional findings of developmental delay in our study. Misra et al. (12) studied the yield of CMA in pediatric neurology practice; they found that phenotypes that predicted the presence of a pathogenic CNV were developmental delay (odds ratio [OR] 3.69 [1.30–10.51]), dysmorphism (OR 2.75 [1.38–5.50]), cortical visual impairment (OR 2.73 [1.18–6.28]), and microcephaly (OR 2.16 [1.01–4.61]). Each additional clinical feature increased the likelihood of detecting a pathogenic CNV even more.

In another study by D'arrigo et al. (11), the diagnostic yield of CMA in children with intellectual or developmental delay was high, regardless of the severity. Likewise, mild developmental delay/intellectual disability comprised the majority of our study cohort, indicating the importance of the diagnostic role of CMA in mild GDD or mild ID.

Three children with GDD were diagnosed with 16p11.2 deletion syndrome. Two of them had epilepsy and two of them were hypotonic. With recurrent CNV of about 600 Kb at chromosome 16p11.2, deletions are the most frequent etiologies of neurodevelopmental delay. This is also known as 'Autism susceptibility 14A'. Common neurologic problems in children with 16p11.2 deletion syndrome are language and speech impairment (>70%), hypotonia (50%), ASD (20-25%) and unprovoked seizures (24%) (17).

Three children were diagnosed with 1p36 deletion syndrome (patients 13, 17 and 21). All had microcephaly and epilepsy. Patient-13 had a history of cardiopulmonary arrest at 40 days old, and periventricular leukomalacia was present. Patient-17 had a history of neonatal hypoglycemia, and parieto-occipital encephalomalacia was present. Array analysis and whole exome sequencing on chorionic villus samples were found to be normal in patient-20. Carter et al. (18), reviewed the records of children with 1p36 deletion syndrome for perinatal distress and/or hypoxic injury; 59% of term patients and 75% of preterm patients needed resuscitation, and 18% had cardiac arrest. Periventricular leukomalacia or suggestion of hypoxic insult was present in 18% of term and 45% of preterm children (18). Prenatally, one of them was investigated because of cardiac defects and microcephaly. In case of doubts, postnatal genetic tests should be repeated. CMA with a lower resolution or inappropriate sampling may lead to false negative results. Congenital heart disease and cardiomyopathy are well described in 1p36 deletion syndrome (19). In our cohort, significant cardiac involvement was present in two cases, both with a diagnosis of 1p36 deletion syndrome (patient-12 and 20).

Patient-5 with Coffin-Siris syndrome due to a 6q25.3 deletion encompassing the *ARID1B* gene, had severe ID, hypotonia, hirsutism, thick eyebrows, long eyelashes, feeding problems and corpus callosum dysgenesis. He wasn't able to produce meaningful words and needed support with walking. He was investigated for mucopolysaccharidosis because of his coarse face. He had no marked fifth digit involvement. Intellectual disability, severe speech impairment, corpus callosum abnormalities, dysmorphic findings including thick eyebrows, long eyelashes, coarse face and hirsutism are common findings in patients with haploinsufficiency of *ARID1B.* Most affected patients have finger/toe abnormalities, however it may not be seen in all cases. Coffin-Siris syndrome is a clinical diagnosis, and phenotypic data are obtained from patients with a clinical diagnosis of Coffin-Siris syndrome. Patients with non-syndromic ID and *ARID1B* mutations will broaden the phenotypic spectrum (20).

Patient-10, who had a history of in utero growth retardation (IUGR), preterm SGA birth, microcephaly, hypertonicity, and renal anomaly, was diagnosed with 22q13.3 syndrome (Phelan-Mc Dermid syndrome) at 4 months old. ASD is seen in most patients with this syndrome and the patient was referred for physical therapy and special education early in infancy. Although the normal head size and normal growth are reported in the majority of patients, our patient had IUGR and microcephaly. These findings may be related to perinatal hypoxia. A cranial MRI at four months old was normal and was planned to be repeated after 1-2 years because early neuroimaging may overlook white matter lesions due to unmyelinated areas.

The *BPTF* gene on 17q24.2 was affected in a patient with ASD and dysmorphism (patient-22). Although the CNV was inherited from a healthy mother, it was expanded, encompassing the *BPTF* gene which was not involved in the mother. *BPTF* is the largest subunit of nucleosome remodeling factor (NURF) complex that belongs to the chromatin-remodeling-complex family. BPTF variants are rare and were found to be pathogenic in patients with neurodevelopmental delay, postnatal microcephaly and dysmorphism (21).

Pallister-Killian syndrome is also a clinically recognizable syndrome. Patient-14, who had a diagnosis of Pallister Killian syndrome, had GDD, hypotonia, hyperpigmentation along Blaschko's lines, optic atrophy and dysmorphic findings including hypertelorism, temporal sparse hair, long philtrum, and flat nasal bridge. Epilepsy is commonly seen in these patients, yet it was not present in our 6-month-old patient. Chromosomal mosaicism was suspected at the initial presentation. Diagnosis of Pallister-Killian syndrome is made by the presence of tetrasomy of 12p. Karyotype from peripheral blood is usually found to be normal, and diagnosis requires analysis of cultured fibroblasts. CMA is another alternative for the diagnosis of Pallister-Killian syndrome; it does not require cell culture and can detect cells in all cell cycles. However, it cannot detect mosaic abnormal cells with a prevalence lower than 10-20 % (22). In our case, CMA was preferred since it was non-invasive. If CMA was normal, then we would continue with cytogenetic analysis of fibroblast sampling.

The limitations of our study are as follows: Segregation analysis of CNVs and parental karyotype analysis were not available for all children. If it had been possible to access the data of all the children who had undergone CMA for the neuro-developmental delay, we could have defined the diagnostic yield of CMA in our cohort. In children with neuro-developmental delay for whom CMA was not diagnostic, additional follow-up data including whole exome sequencing would contribute to the diagnostic yield of genetic tests in this patient group.

## CONCLUSION

We presented a small cohort of children with neurodevelopmental delay and pathogenic CNVs in CMA. Most of them were diagnosed with well-defined microdeletion or microduplication syndrome. GDD and ID were mild in more than half of our patients. Dysmorphism, head circumference abnormalities, and hypotonia were the most frequent accompanying findings. CMA is an important tool for the diagnosis of patients with neurodevelopmental delay. Enabling an accurate etiologic diagnosis in patients with neurodevelopmental delay is important for better clinical management, follow-up for possible complications, and genetic counselling after segregation analysis for subsequent pregnancies and avoiding unnecessary tests. Even if the patient has a diagnosis of cerebral palsy, due to a hypoxic insult, CMA analysis should be performed in the presence of dysmorphic findings and/or multisystem involvement.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ümraniye Training and Research Hospital Ethics Committee (15.03.2021-5417).

**Informed Consent:** Informed consent was not obtained since the study is retrospective.

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- H.M.G., Y.K.D Data Acquisition- H.M.G., Y.K.D., B.K., Ö.A.D., H.G.S. Data Analysis/Interpretation- H.M.G., Y.K.D., H.B., Ö.A.D..; Drafting Manuscript- H.M.G., Y.K.D., H.B., B.K., Ö.A.D., H.G.S.; Critical Revision of Manuscript- H.M.G., Y.K.D.,H.B., Ö.A.D.; Final Approval and Accountability- H.M.G., Y.K.D., H.B., B.K., Ö.A.D., H.G.S.

**Conflict of Interest:** Authors declared no conflict of interest.

**Financial Disclosure:** Authors declared no financial support.

**Etik Komite Onayı:** Bu çalışma için etik kurul onayı Ümraniye Eğitim ve Araştırma Hastanesi Etik Kurulu'ndan alınmıştır. (15.03.2021-5417).

**Bilgilendirilmiş Onam:** Katılımcılardan bilgilendirilmiş onam alınmıştır.

**Hakem Değerlendirmesi:** Dış bağımsız.

**Yazar Katkıları:** Çalışma Konsepti/Tasarım- H.M.G., Y.K.D.; Veri Toplama- H.M.G., Y.K.D., B.K., Ö.A.D., H.G.S.; Veri Analizi/Yorumlama- H.B., Y.K.D., Ö.A.D.; Yazı Taslağı- H.M.G., Y.K.D., H.B., B.K., Ö.A.D., H.G.S.; İçeriğin Eleştirel İncelemesi- H.M.G., Y.K.D., Ö.A.D.; Son Onay ve Sorumluluk- H.M.G., Y.K.D., H.B., B.K., Ö.A.D., H.G.S.

**Çıkar Çatışması:** Yazarlar çıkar çatışması beyan etmemişlerdir.

**Finansal Destek:** Yazarlar finansal destek beyan etmemişlerdir.

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