DOI: 10.24938/kutfd.1495687 Kırıkkale Üniversitesi Tıp Fakültesi Dergisi 2024;26(3):300-307
Original Article *Özgün Araştırma*

DIAGNOSTIC VALUE OF CHROMOSOMAL MICROARRAY ANALYSIS IN PATIENTS WITH CONGENITAL ANOMALIES AND DYSMORPHIC FEATURES; DETAILS OF TWO NEW PATIENTS WITH 2q33 DELETIONS

Konjenital Anomalisi ve Dismorfik Özellikleri Olan Hastalarda Kromozomal Mikrodizin Analizinin Tanısal Değeri; 2q33 Delesyonu Olan İki Yeni Hastanın Ayrıntıları

Pelin ÖZYAVUZ ÇUBUK[1] Ece KESKİN[1] Lamiya MARDAN HACIZADE[1] Tuğba AKIN DUMAN[1] Fatma Nihal ÖZTÜRK[1] Ömer Faruk KARAÇORLU[1] Hasan TAŞLIDERE[1] Mehmet Buğrahan DÜZ[2]

[1] *Department of Medical Genetics, Haseki Training and Research Hospital, University of Health Sciences, ISTANBUL, TÜRKİYE*
[2] *Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA*

ABSTRACT

Objective: Detection of copy number variations (CNVs) through molecular karyotyping is a useful method to assess the genetic anomalies with dysmorphic features and multiple congenital anomalies. This study demonstrated the diagnostic rate of chromosomal microarray analysis in patients with specific phenotype, and also contributed the literature with presenting new patients with very rare CNVs.

Material and Methods: Chromosomal microarray analysis was performed in 419 patients with dysmorphic features and multiple congenital anomalies.

Results: A total of 61 CNVs were detected in 50 patients (12%). Two of these patients exhibited a 2q33.1 deletion. While patient 13 presented with speech delay, seizures, behavioral abnormalities, and a 469 kbp deletion disrupting *SATB2*, patient 14 displayed immune deficiency, failure to thrive, hypothyroidism, diarrhea, cryptorchidism, and ectodermal features such as alopecia, nail dysplasia, and oligodontia. This patient had a 7.5 Mb deletion on 2q33.1q34, which included *CTLA4* and was responsible for the immune deficiency symptoms of the patient. The *CASP10*, was not included in the deletion region.

Conclusions: According to our study, co-deletion of *CTLA4* and *CASP10* did not lead to phenotypic effects like immune deficiency. Rather, only the deletion of the *CTLA4* gene may result in hypogammaglobulinemia and immune deficiency. These findings suggest that chromosomal microarray analysis can be a valuable tool in diagnosing rare CNVs and guiding clinical management, particularly in patients with immune deficiency and other congenital anomalies. Identifying specific gene deletions, such as *CTLA4*, may inform personalized treatment approaches, including immune-modulating therapies, and provide insights for genetic counseling in affected families.

Keywords: *Congenital anomaly, dysmorphic features, molecular karyotyping, 2q33.1 deletion, chromosomal microarray*

ÖZ

Amaç: Moleküler karyotipleme yoluyla kopya sayısı varyasyonlarının (CNV'ler) tespiti, dismorfik özellikler ve birden fazla konjenital anomali (MCA) ile ilişkili genetik anomalileri ortaya çıkarmak için kullanışlıdır. Bu çalışma, belirli fenotipe sahip hastalarda kromozomal mikrodizin analizinin tanısal oranını göstermekte ve çok nadir CNV'lere sahip yeni hastaları sunarak literatüre katkıda bulunmaktadır.

Gereç ve Yöntemler: Dismorfik özellikler ve birden fazla konjenital anomaliye sahip 419 hastada kromozomal mikrodizin analizi gerçekleştirildi.

Bulgular: Toplamda 50 hastada (%12) 61 CNV tespit edildi. Bu hastalardan ikisinde 2q33.1 delesyonu saptandı. Hasta 13 konuşma gecikmesi, nöbetler, davranış bozuklukları ve *SATB2* genini bozan 469 kbp delesyon ile kendini gösterirken; hasta 14, immün yetmezlik, büyüme geriliği, hipotiroidizm, ishal, inmemiş testisler ve alopesi, tırnak displazisi ve oligodonti gibi ektodermal özellikler sergiledi. Bu hastada, 2q33.1q34 bölgesinde *CTLA4* genini içeren ve immün yetmezlik belirtilerine neden olan 7.5 Mb delesyon mevcuttu. *CASP10* geni ise delesyon bölgesinde yer almamaktadır.

Sonuç: Çalışmamıza göre, *CTLA4 ve CASP10* genlerinin birlikte delesyonu bağışıklık yetmezliği gibi fenotipik etkilere yol açmaz iken, yalnızca *CTLA4* geninin delesyonu hipogamaglobulinemi ve bağışıklık yetmezliği ile sonuçlanabilir. Bu bulgular, moleküler karyotiplemenin nadir CNV'lerin tanısında ve doğumsal anomalileri olan hastaların klinik yönetiminde değerli bir araç olabileceğini göstermektedir. *CTLA4* gibi spesifik gen delesyonlarının belirlenmesi, bağışıklık düzenleyici tedaviler de dahil olmak üzere kişiselleştirilmiş tedavi yaklaşımlarına kapı açabilir ve etkilenen ailelerde genetik danışmanlık için önemli bilgiler sağlayabilir.

Anahtar Kelimeler: *Konjenital anomali, dismorfik bulgular, moleküler karyotipleme, 2q33.1 delesyonu, kromozomal mikrodizin*

Correspondence / Yazışma Adresi: **Dr. Pelin ÖZYAVUZ ÇUBUK**
Department of Medical Genetics, Haseki Training and Research Hospital, University o f Health Sciences, ISTANBUL, TÜRKİYE
Phone / Tel: +905558050387 **E-mail** / E-posta: ozyavuzpelin@gmail.com
Received / Geliş Tarihi: 05.06.2024 **Accepted** / Kabul Tarihi: 17.10.2024

KÜ Tıp Fak Derg 2024;26(3):300-307
Doi: 10.24938/kutfd.1495687

INTRODUCTION

Chromosomal microarray analysis (CMA) was a pivotal diagnostic tool to identify microdeletion/micro-duplication syndromes, and serve as a first-tier test for a wide range of childhood diseases which included developmental delay, congenital anomalies, and intellectual disability (1,2). This routine molecular genetic diagnostic method is not only useful to define well-known syndromes but also provides insights into the functioning of genes and their effect on phenotype. The diagnostic success rate of CMA in individuals with global developmental delay is estimated to range between 10-25%, varying based on the platform used and the criteria for patient selection (1-3). In this study, we aimed to emphasize the diagnostic importance of CMA in patients presenting with multiple congenital anomalies and dysmorphic features, as well as to contribute to the existing literature with new cases. Additionally, we intended to delineate the clinical features of two novel patients with 2q33.1 deletion, both exhibiting distinct clinical manifestations despite sharing the 2q33.1 microdeletion syndrome region. This study stands out as one of the largest series reported from Türkiye in terms of patient number and diversity.

MATERIALS AND METHODS

Patient Enrollment and Study Design

A total of 419 patients with developmental delay, multiple congenital anomalies, and dysmorphic features, who applied to Haseki Training and Research Hospital, Istanbul, Türkiye, between January 2017 and May 2020, and were assessed with chromosomal microarray (CMA), were enrolled in this study. All patients included in this retrospective study were evaluated in the outpatient clinic of the medical genetics department and provided written informed consent. Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Haseki Training and Research Hospital (decision no: 2020/86, date: 24.06.2020). The study design adhered to the principles outlined in the Declaration of Helsinki.

DNA Isolation and Microarray Analysis

Blood samples were collected from the enrolled patients and, where available, from their family members. Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany), following the manufacturer's guidelines. The procedure involves cell lysis, protein precipitation, and selective DNA binding to a silica-based membrane.

The DNA was eluted in a low-salt buffer for storage. The quality and quantity of the extracted DNA were assessed using spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific, MA, USA) and gel electrophoresis to ensure high-purity DNA for subsequent microarray analysis. All microarray procedures were performed using the CytoScan Optima Array Kit (ThermoFisher Scientific, MA, United States), which contains over 315,000 non-polymorphic markers, DNA fragmentation, labeling, and hybridization were carried out according to the manufacturer's protocols, with each sample hybridized onto a CytoScan Optima Array chip.

Data Interpretation

The resulting microarray data were analyzed using Chromosome Analysis Suite (ChAS) 4.3 software from Affymetrix. ChAS supports the analysis of large genomic datasets by utilizing GRCh37/hg19 reference libraries for CNV detection. The software integrates robust statistical algorithms for automated data processing, including quality control metrics, normalization, and background correction, ensuring accurate detection of genomic imbalances. Additional features include visualization tools for identifying chromosomal aberrations and reporting functions for variant classification.

Variant Classification and Databases

Interpretation of CNVs was conducted following the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) guidelines. Detected variants were compared against public and in-house databases, including the Database of Genomic Variants (DGV), DECIPHER, ClinVar, ISCA, HGMD, and PubMed. Additionally, our department's in-house database was consulted to further characterize the variants. Based on ACMG 2015 criteria, CNVs were classified into five categories: benign, likely benign, variant of unknown clinical significance (VUS), likely pathogenic, and pathogenic. Only variants classified as VUS, likely pathogenic, or pathogenic were discussed in this study, while patients with benign or likely benign findings were excluded from further analysis.

RESULTS

A total of 419 patients (184 (44%) female, 235 (56%) male) were included in this study. While no CNVs were detected in 369 patients (88%), CNVs that potentially explained clinical findings were identified in 50 (12%) patients. A total of 61 CNVs were detected in 50 patients, with multiple CNVs found in 10 of these patients. Of the identified CNVs, 45 were deletions and 16 were duplications. Two CNVs (patient 32 and 35) were classified as VUS, while the others were classified as pathogenic/likely pathogenic. Table 1 displays the phenotypic characteristics of the patients and their CMA results.

CNVs that were identified in 33 patients, overlapped with the known microdeletion/microduplication syndrome regions. The identified microdeletions included 7q11.23 microdeletion (5 patients), 1p36 microdeletion (4 patients), 15q11.2q13 microdeletion (3

KÜ Tıp Fak Derg 2024;26(3):300-307
Doi: 10.24938/kutfd.1495687

patients), WAGR syndrome (2 patients), and 22q11 microdeletion (2 patients). Homozygous deletions were detected in patient 28, and homozygous duplications were found in patient 3.

In 11 patients with CNVs of 10 Mb and above, changes were demonstrated using chromosome analysis. CNVs between 5 and 10 Mb could not be detected by chromosome analysis due to the inability to perform high-resolution banding (HRB)

DISCUSSION

Chromosomal microarray (CMA) is a versatile tool that serves as a well-established primary diagnostic method in the assessment of individuals with congenital anomalies, intellectual disabilities, and developmental delays. It is also useful for detecting genetic variations across diverse patient groups. CMA enables the detection of submicroscopic copy number variations (CNVs) in pediatric patients with autism spectrum disorders, guides prognosis and treatment in oncology, and allows for the identification of microdeletions and aneuploidies in prenatal diagnosis. It not only facilitates the identification of known microdeletion and duplication syndromes but also helps unveil novel syndromes. Furthermore, CMA contributes to the understanding of the genetic underpinnings of various syndromes by elucidating genetic heterogeneity and identifying new loci harboring potential candidate genes (1,2).

This study shows the diagnostic yield of CMA in our patients with congenital anomalies and dysmorphic features, with a rate of 12%, which was consistent with other studies from Türkiye (5-35%) (4-12). This variability was probably due to the differences in the number and diversity of patients.

Patient 28 had a homozygous 239 kbp deletion including exon 1-17 of *TOP3B*, deletions of which were associated with epilepsy, autism, and intellectual disability (13-15). Similarly, with our patient, a homozygous deletion of *TOP3B* was identified in an adult male patient with bilateral renal cancer (16). Loss of *TOP3B* disrupted genomic stability and led to cancer, so we continued to monitor our patient, who was diagnosed at the age of six, from this perspective.

Patient 13 presented with a 467 kb deletion that included *SATB2* exon 4-11, leading to a diagnosis of *SATB2*-associated syndrome. Born from a first-degree cousin marriage, the female infant exhibited normal percentiles at birth with no history of hypotonia, feeding problems, or cleft palate. Head control was achieved at 2 years old, walking commenced at 4 years old, and the eruption of primary dentition began at 3 years old. Since the age of two, the patient has been under medication for seizures and currently exhibited limited verbal expression. Intragenic deletions of *SATB2* were very rare and have been reported in only four cases to date (17). Patients with intragenic deletions, such as patient 13, commonly presented with intellectual disability and speech delay. Additionally, dysmorphic features and cleft palate were seen in some affected individuals. Seizures have usually been documented in patients with *SATB2* point mutations or large deletions; however, our case represented the first instance of seizures among patients with intragenic deletions (18). This was likely due to the deletion that disrupted the *SATB2* gene and resulted in effects similar to point mutations.

Patient 14, a 7-year-old male, presented with a large deletion on chromosome 2q33.1q34 without *SATB2*. The patient was born to nonconsanguineous parents following uneventful prenatal follow-ups, with a birth weight of 2230 gr and a length of 45 cm at 38 weeks gestation. Due to respiratory issues, the patient required incubation for one week after birth and was readmitted for an additional week due to signs of pneumonia. Milestones included head control at 2 months, walking at 18 months, and speech at 12 months. Surgical interventions were performed for cryptorchidism and hydrocele, while ongoing treatment for hypothyroidism was administered. The first hospitalization occurred at age one due to chronic diarrhea and poor weight gain. At three years old, the patient had oligodontia and developed a 3x3 cm alopecia areata in the temporoparietal region, followed by the loss of lower eyelashes. While hair regrowth occurred spontaneously, eyelash regrowth did not, and subsequent hair loss was permanent. Nail anomalies manifested following a hand-foot-mouth disease episode at age 5. Laboratory investigations revealed low levels of IGF1 and IGFBP3, with immunoglobulin subgroup analysis indicating low levels of IgG2 and IgG4, and also IgG, IgA, and IgE which were detected below -2SDS. Following consultation at the immunology clinic, the patient was diagnosed with immunodeficiency and initiated IV Immunoglobulin replacement therapy every three weeks.

Deletion of patient 14, encompassed the 2q33.1 microdeletion syndrome (Glass syndrome) region and extended distally. Genes previously associated with disease within the deletion region included *ALS2, BMPR2, CRYGB, CRYGC, CRYGD, FASTKD2, NDUFS1, ICOS, CTLA4, PIKFYVE, and TMEM237*. However, evidence of phenotypic association was shown only for *CTLA4* among these genes. Symptoms observed in our patient, such as recurrent respiratory tract infections, autoimmune enteropathy, growth retardation, and hypogammaglobulinemia, may be attributed to *CTLA4* gene involvement. According to the four reported cases of severe immune dysregulation associated with heterozygous germline mutations in the *CTLA4*, *CTLA4* plays a critical role in maintaining T and B lymphocyte homeostasis (19-21).

KÜ Tıp Fak Derg 2024;26(3):300-307
Doi: 10.24938/kutfd.1495687

Table 1: Clinical and molecular findings of patients

P	Year / Sex	Phenotype	Karyotype	CNV	Size	OMIM
1	4/F	Short stature, DD	46,XX	3q29(195734932_197356334)x1	1,621Mb	3q29 microdeletion syndrome #609425
2	0/M	Pulmoner stenosis,DD	46,XY	7q11.23(72765457_74154634)x1	1,389Mb	Williams-Beuren syndrome #194050
3	1/M	DD, epilepsy, scoliosis, constipation, round face, metopic synostosis,	47,XY,+i(18)(p10) dn	18p11.32p11.21(136226_15198990)x4	15 Mb	tetrasomy 18p #614290
4	0/F	Anal atresia, low set ears, renal pelviektasia, dysmorphisim	46.XX	22q11.21(18917030_21465662)x1	2,549Mb	22q11.2 deletion syndrome #611867
5	2/F	Postnatal overgrowth, severe DD, prominent forehead, epichantal folds, depressed nasal bridge, anteverte nares, agenesis of corpus callosum	46,XX	3q13.2q13.31(112170371_115523299)x1	3,353 Mb	3q13.31 deletion syndrome #615433
6	0/M	Dysmorphism,laringomalasia, diaphragmatic hernia, narrow forhead, prominent metopic ridge, upslanting palpebral fissures	46,XY	7q11.23(72765457_74197135)x1	1,432 Mb	Williams-Beuren syndrome #194050
7	0/F	Hypotonia, dysmorphism	46,XX	15q11.2q13.1(22770421_28660038)x1	5,890 Mb	Prader willi
8	9/F	DD, prominent ears, long face	46,XX	7q11.23(72765457_74175640)x1	1,410 Mb	Williams-Beuren syndrome #194050
9	3/M	DD, dysmorphism	46,XY	17p11.2(16581800_20271898)x3	3,690 Mb	Potocki-Lupski 17p11.2 dup send 610883
10	0/M	Hydrocephaly, dysmorphism	46,XY	1q21.1q21.2(146117290_147814694)x1	1,697 Mb	1q21.1 deletion syndrome #612474
11	0/F	Hypotonia, Low set ears, pes echinovarus, displaced nipples, short neck, labial adhesion, sacral hypertrichosis	46,XX	15q11.2q13.1(22770421_28660038)x1	5,8 Mb	Prader-Willi syndrome #176270
12	0/M	Renal agenesis, aniridia	46,XY	11p14.1p13(30561122_34633745)x1 dn	4 Mb	WAGR syndrome #194072
13	13/F	DD, speech delay, seizures, behavioral abnormalities	46,XX	2q33.1(199826786_200293924)x1 dn	467kb	Glass syndrome
14	7/M	Failure to thrive,hypothyroidism, frequent infection history, diarrhea, dysmorphism, ectodermal signs alopesi areata, nail dysplasia, oligodontia, cryptorchidism	46,XY	2q33.1q34(202450560-209933795)x1 dn	7.5 Mb	Pathogenic CNV
15	1/F	Failure to thrive hypothyroidism, tiz sesli ağlama, triangular face, prominent forehead, sparse hair, olygodactyly, preauricular apendix, bilateral hallux nail hypoplasia, clinodactyly	46,XX,del(3)(p11p13) de novo	3p13p11.1(74115297_88803526)x1 dn	14.7 Mb	Pathogenic CNV
16	11/M	Bilateral hearing loss, inguinal hernia, cleft palate, DD, decrease in subcutaneous fat tissue, hypertrichosis, messy eyebrows, joint contractures	NA segregation: NA	22q11.1q11.21(16888899_20295420)x3, 11q23.3q25(116589651_134938470)x3	3,407Mb/18,349Mb	Emanuel syndrome #609029
17	3/M	DD, pulmoner stenosis, long palpebral fissures, hypertelorism, large ears	N/A de novo	3p26.3p25.3(61891_9635471)x1, 9p24.3p21.3(203861_20688117)x3 dn	9,5 mb 20,4 Mb	Likely pathogenic CNV

18	20/F	DD, language delay, short stature, microcephaly, scoliosis, syndactyly, irreguler periods hyperglisemia, narrow torax, prominent forehead, triangular face	46, XX segregation: NA	7q36.1(148931521_152009647)x1	3 Mb	Likely pathogenic CNV
19	0/M	DD, hypotonia, VSD, hirschprung with ileostomia, dysmorphism	46,XY segregation: NA	11q13.2q13.5(67,979,509-75,279,846)x3	7.3 Mb	pathogenic CNV
20	0/F	Narrow forehead, epicantus, upslant palpebral fissures, thick eyebrows, depressed nasal bridge, intrauterin growth retardation, microcephaly, hemangioma	46,XX, del(7)(q33-35) pat	7q33q36.3(136971100_159119707)x1, 15q26.3(99595514_102429112)x3	22 Mb/2.8 Mb	pathogenic CNV
21	9/M	Multiple congenital anomalies, attention deficit, malar hypoplasia, short philtrum, asymmetric cup shaped ear, talipes equinovarus, camptodactyly	46,XY	22q11.21(18917030-21465662)x1 dn	2.5 Mb	22q11.2 deletion syndrome #611867
22	8/F	Aortic regurgitation and aortic stenosis, prominent eyes, thick lower lip, delayed speech, tongue tie, oligodontia	46,XX	7q11.23(72654597-74175640)x1	1.3 Mb	Williams-Beuren syndrome #194050
23	4/M	Growth retardation, aortic coarctation, strabismus, thin eyebrows, hypertelorism, cup-shaped ears, thin upper lip, fetal finger pad, undescended testicle, scrotum hypoplasia, 2-3 toe syndactyly	46,XY	Xq28(150633501_155233731)x2 dn	4.6 Mb	Xq28 duplication syndrome #300815
24	14/M	Wilms tumor, aniridia, undescended testicle	46,XY N/A	11p13(31781828_35494887)x1	3.7 Mb	WAGR syndrome #194072
25	4/F	Cleft palate, micrognathia, retrognathia, sandal gap	46,XY	17q24.2q24.3(66925909_69578693)x1	2.7 Mb	Pierre Robin sequence due to upstream of SOX9
26	1/M	Congenital hypotonia, dysmorphism	46,XY,der(8)t(8;17)(p23;q25)dn	8p23.3p23.1(158048_6982980)x1, 8p23.1p22(12527948_13509999)x3, 17q25.3(76092552_81041938)x3 dn	6,825kbp/4,949kbp	Likely pathogenic CNV
27	2/M	Developmental delay, language delay, dysmorphism, unable to speak or walk	46,XY	1p36.33p36.32(849466-2936815)x1	2.1 Mb	1p36 microdeletion syndrome #607872
28	6/M	MMR, epilepsy	46,XY	22q11.22(22312374_22551837)x0	239kbp	TOP3B deletion #603582
29	1/M	MMR, corpus callosum hypoplasia, hypertonia, joint contractures	46,XY	10p12.31p12.1(21616814_27323287)x1	5.7 Mb	Pathogenic CNV
30	15/F	MODY, dysmorphism	46,XX,der(13)t(9;13)(p23;q33) segregation: NA	9p24.3p22.3(203861_14292138)x3, 13q33.1q34(102778024_115107733)x1	14Mb /12Mb	13q34 deletion syndrome including CHAMP1
31	1/F	DD, dysmorphism	46,XX,del(18)(q22)	18q22.1q23(65202259_78014123)x1 dn	12 Mb	18q del syndrome #601808
32	25/F	Short stature, recurrent pregnancy loss	46,XX,r(15)(p11.2q26)	15q26.3(102,052,633-102,429,112)x1 dn	376 kb	VUS, 15q26.3 del
33	6/F	MMR, dysmorphism	46,XX	1p36.32p36.22(5141292_12559766)x1	7.4 Mb	1p36 microdeletion syndrome #607872
34	4/M	Short stature, language delay, coarse face, prominent ears, undescended testicle, micropenis	46,XY,add(7)(q36).ish der(7)t(4;7)(p16.3;q36.3)	4p16.3p15.31(68345_18464078)x3, 7q36.3(157473221_159119707)x1	18 Mb/1.6 Mb	4p16.3 microduplication syndrome
35	1/F	Small mouth, prominent lips, epicanthus, developmental delay	46,XX	2q11.2q12.1(102281806_105132332)x1	2.8 Mb	VUS CNV
36	34/M	Recurrent first trimester abortion in partner	46,XY	11q24.3q25(129054558_134938470)x1	5.8 Mb	Jacobsen syndrome # 147791

KÜ Tıp Fak Derg 2024;26(3):300-307
Doi: 10.24938/kutfd.1495687

37	1/F	SGA,DD,coarse face, hypertelorism,hypertrichosis, cerebral atrophy, ASD	46,XX,der(10)t(4;10)(q28;p15)mat	4q28.3q35.2(134093149_190957473)x3, 10p15.3(100026_2144362)x1	56 Mb/2 Mb	10p15.3 microdeletion syndrome # 616083, including ZMYND11
38	6/F	MMR, obesity, dysmorphism	46,XY	1p36.33p36.32(1908648_5195317)x1	3,2 Mb	1p36 microdeletion syndrome #607872
39	7/M	MMR, strabismus, hypothyroidism, factor 7 and 10 deficiency	46,XY der(13)t(7;13)(p22;q34) mat	7p22.3p22.2(43360_4071034)x3, 13q33.3q34(109387132_115107733)x1	4 Mb/ 5,7 Mb	13q33-34 deletion syndrome #619148
40	0/M	Hypotonia, undescended testicle	46,XY	15q11.2q13.1(22770421_28828168)x1	6 Mb	Prader-Willi syndrome #176270
41	M	Multiple fetal abnormalities, dandy walker, corpus callosum agenesis, membranous VSD, bilateral renal pelviectasis, oligodactyly, terminated	46,--,der(6)t(3;6)(q25;p25)pat	3q25.1q29(151956588_197851986)x3, 6p25.3p25.2(156974_4101567)x1	45 Mb/ 3,9 Mb	Pathogenic CNV
42	2/M	MMR, dysmorphism	46,XY segregation N/A	7q22.3q31.1(105182994_108794174)x1	3,6 MB	pathogenic CNV
43	1/M	Developmental delay, torticollis pulmonary stenosis	46,XY	7q11.23(72765457_74217755)x1	1,3 Mb	Williams-Beuren syndrome #194050
44	1/F	Lissencephaly, band heterotopia, epilepsy	46,XX	17p13.3(2335809_2583616)x1	247kbp	Miller-Dieker lissencephaly syndrome #247200
45	0/F	Down synrome like dysmorhic features	N/A	8p23.3(244625_2223283)x1 8p23.2p11.23(2520625_38026262)x3	1,9 Mb/35 Mb	Inverted duplication deletion 8p (invdupdel 8p) syndrome
46	1/M	Hypertelorism, epicantus, large nose, atrioventricular septal defect, supravalvular pulmoner stenosis	46,XY	9q34.3(136808975_138198846)x1 dn	1,3 Mb	Ehlers-Danlos syndrome, including COL5A1
47	9/F	Neurofibromatosis, axillary-inguinal freckling, cafe-a-lait macules	46,XX	17q11.2(30742545_32015647)x1 dn	1,2 Mb	17q11.2 microdeletion syndrome #613675
48	1/F	Prenatal growth retardation, seizures, ebstein anomaly, ASD, thick eyebrows, synophris, depressed nasal bridge, nail distrophy, gastroesophageal reflux	46,XX	1p36.33p36.23(811228_9064551)x1 dn	8,2 Mb	1p36 microdeletion syndrome #607872
49	8/F	DD, dysmorphism, strabismus, ASD, accessory spleen, prominent metopic suture, enlargement of both lateral ventricles on brain MRI, atrophy of the cerebral sulci, large anterior fontanel	46,XX	17q21.32q21.33(48645540_50218581)x1 dn	1,5 Mb	Likely pathogenic CNV
50	4/M	Language delay, febril convulsion, autism	46,XY	16p11.2(29662635_30187279)x1 dn	524kbp	16p11.2 microdeletion syndrome #611913

P: Patient, MMR: Mental motor retardation, ASD: Atrial septal defect, VSD: Ventricular septal defect, DD: Developmental delay, ID: Intellectual disability, SGA: Small for gestational age

Similarly, two cases (P1 and P2) with 2q33.2q33.3 deletions including *CTLA4*, have reported immunodeficiency and hypogammaglobulinemia (22). Interestingly, although *CTLA4* is located within the critical region of the 2q33 microdeletion syndrome, no cases of immunodeficiency have been reported in these patients. The reason behind this is that might be the balancing effect of another gene in this region.

Mutations in the *CASP10* gene, which is also located in the 2q33 microdeletion syndrome region, lead to autoimmune lymphoproliferative syndrome type IIA (23). We hypothesized that this discrepancy may be due to the inclusion of the *CASP10* gene in the deletion region with *CTLA4*. Further functional studies are needed to evaluate which genes are responsible for the immunophenotypic findings in patients.

To enhance diagnostic accuracy, additional analyses such as whole exome sequencing (WES), next-generation sequencing (NGS), and non-invasive prenatal testing (NIPT) may complement CMA findings. Incorporating functional studies like RNA sequencing and exploring epigenetic factors can further refine diagnoses, providing a comprehensive understanding of genetic abnormalities and enabling personalized medical care.

Microarray-based genome-wide analysis not only aids in the diagnosis of microdeletion/microduplication syndromes but also provides crucial insights into gene function. In this study, we found a diagnostic yield of 12% for CMA testing in the 419 cases included. One of our cases exhibited a 239 kbp homozygous deletion affecting only the *TOP3B* gene and was monitored for renal cancer risk. Furthermore, by discussing in detail two patients with 2q33 deletion, we demonstrated that while immune deficiency was not observed in patients with deletions encompassing both *CTLA4*, which can cause hypogammaglobulinemia, and *CASP10*, which can cause hypergammaglobulinemia, the deletion of the *CTLA4* gene alone can lead to immune deficiency with hypogammaglobulinemia. We hope that further complementary studies will elucidate the functional changes that deletions involving either one or both of these genes may cause.

This study emphasizes that the application of CMA in patients with various clinical findings can help in determining the etiology of developmental delay and dysmorphism. Identifying the specific genetic cause of congenital anomalies and dysmorphic features in each patient is essential for better personalized medical care, leading to improved clinical management and access to relevant support services.

*Conflict of Interest***:** The authors have no conflict of interest to declare.

*Researchers' Contribution Rate Statement***:** Concept/Design: PÖÇ, MBD, EK, TAD, AA; Analysis/Interpretation: PÖÇ, TAD, HT, MBD, EK, LMH, FNÖ, ÖFK; Data Collection: PÖÇ, TAD, HT, MBD, EK, LMH, FNÖ, ÖFK; Writer: PÖÇ; Critical Review: PÖÇ, TAD, HT, MBD, EK, LMH, FNÖ, ÖFK; Approver: PÖÇ, TAD, HT, MBD, EK, LMH, FNÖ, ÖFK.

Support and Acknowledgment: No financial support was received from any institution or person.

*Ethics Committee Approval***:** The study protocol was approved by the Clinical Research Ethics Committee of the Haseki Training and Research Hospital (decision no: 2020/86, date: 24.06.2020). All participants gave written informed consent.

REFERENCES

1. Sagoo GS, Butterworth AS, Sanderson S, Shaw-Smith C, Higgins JP, Burton H. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: Updated systematic review and meta-analysis of 19 studies and 13,926 subjects. *Genet Med*. 2009;11(3):139-146.
2. Miller DT, Adam MP, Aradhya S, 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.
3. Hochstenbach R, van Binsbergen E, Engelen J, et al. Array analysis and karyotyping: workflow consequences based on a retrospective study of 36,325 patients with idiopathic developmental delay in the Netherlands. *Eur J Med Genet*. 2009;52(4):161-169.
4. Gerik-Celebi HB, Aydin H, Bolat H, Unsel-Bolat G. Clinical and genetic characteristics of patients with unexplained intellectual disability/developmental delay without epilepsy. *Mol Syndromol.* 2023;14(3):208-218.
5. Ceylan AC, Citli S, Erdem HB, Sahin I, Acar Arslan E, Erdogan M. Importance and usage of chromosomal microarray analysis in diagnosing intellectual disability, global developmental delay, and autism; and discovering new loci for these disorders. *Mol Cytogenet.* 2018;11:54.
6. Unal AT, Durmaz CD. Chromosomal microarray experience in Turkish pediatric patients with congenital anomalies and/or neurodevelopmental disorders: Review of ASXL2 gene duplication and Xq13 deletion. *Dicle Med J*. 2024;50(1):135-148.
7. Çebi AH, Altıner Ş. Application of chromosome microarray analysis in the investigation of developmental disabilities and congenital anomalies: Single center experience and review of NRXN3 and NEDD4L deletions. *Mol Syndromol*. 2020;11(4):197-206.
8. Utine GE, Haliloglu G, Volkan-Salanci B, et al. Etiological yield of SNP microarrays in idiopathic intellectual disability. *Eur J Paediatr Neurol*. 2014;18:327-337.
9. Arican P, Olgac Dundar N, Ozyilmaz B, et al. Chromosomal microarray analysis in children with unexplained developmental delay/intellectual disability. *J Pediatr Genet*. 2019;8(1):1-9.

10. Ayaz A, Gezdirici A, Yilmaz Gulec E, et al. Diagnostic value of microarray method in autism spectrum disorder, intellectual disability, and multiple congenital anomalies and some candidate genes for autism: Experience of two centers. *Medeni Med J*. 2022;23;37(2):180-193.
11. Ozyilmaz B, Kirbiyik O, Koc A, et al. Experiences in microarray-based evaluation of developmental disabilities and congenital anomalies. *Clin Genet*. 2017;92(4):372-379.
12. Sagoo GS, Butterworth AS, Sanderson S, Shaw-Smith C, Higgins JP, Burton H. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: Updated systematic review and meta-analysis of 19 studies and 13,926 subjects. *Genet Med.* 2009;11:139-146.
13. Daghsni M, Lahbib S, Fradj M, et al. TOP3B: A novel candidate gene in juvenile myoclonic epilepsy? *Cytogenet Genome Res*. 2018;154(1):1-5.
14. Riley JD, Delahunty C, Alsadah A, Mazzola S, Astbury C. Further evidence of GABRA4 and TOP3B as autism susceptibility genes. *Eur J Med Genet.* 2020;63(5):103876.
15. Kaufman CS, Genovese A, Butler MG. Deletion of TOP3B is associated with cognitive impairment and facial dysmorphism. *Cytogenet Genome Res*. 2016;150(2):106-111.
16. Zhang T, Wallis M, Petrovic V, Challis J, Kalitsis P, Hudson DF. Loss of TOP3B leads to increased R-loop formation and genome instability. *Open Biol.* 2019;9(12):190222.
17. Balasubramanian M, Smith K, Basel-Vanagaite L, et al. 2011. Case series: 2q33.1 microdeletion syndrome-further delineation of the phenotype. *J Med Genet* 48:290-298.
18. Zarate YA, Fish JL. SATB2-associated syndrome: Mechanisms, phenotype, and practical recommendations. *Am J Med Genet A*. 2017;173(2):327-337.
19. Schubert D, Bode C, Kenefeck R, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat Med*. 2014;20(12):14106.
20. Zeissig S, Petersen BS, Tomczak M, et al. Early-onset Crohn's disease and autoimmunity associated with a variant in CTLA-4. *Gut*. 2015;64(12):188997.
21. Kuehn HS, Ouyang W, Lo B, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science.* 2014;345(6204):1623-1627.
22. Schwab C, Gabrysch A, Olbrich P, et al. Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. *J Allergy Clin Immunol.* 2018;142(6):1932-1946.
23. Wang J, Zheng L, Lobito A, et al. Inherited human Caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. *Cell.* 1999;98(1):47-58.