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Copy Number Variations in a Turkish Cohort of Children with Intellectual Disability*

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ABSTRACT

Objective: Intellectual disability (ID) is a complex, variable, and clinically heterogeneous neurodevelopmental disorder that affects 1% – 3% of the global population. Copy number variations (CNVs) contribute to approximately 15%–20% of ID cases. Array comparative genomic hybridization (aCGH) is the first-line test for diagnosing patients with ID with/without multiple congenital anomalies (MCAs). This study aimed to present CNVs identified in a retrospective aCGH cohort of Turkish patients with ID with/without other medical conditions.

Materials and Methods: The study population consisted of 210 patients (139 male, 71 female) aged 2–18 years. aCGH analysis was performed using oligo and bacterial artificial chromosome (BAC)-based microarray platforms. CNVs were interpreted using public databases and literature mining and categorized according to international guidelines.

Results: Forty-five CNVs were detected in 38 (18%) patients. Among these CNVs, 21 (46.6%) were pathogenic, 4 (8.8%) were likely pathogenic, and 8 (17.7%) were variants of uncertain clinical significance (VUS). Nineteen CNVs corresponded to rare microdeletion/microduplication syndromes.

Conclusions: This study reports rare CNVs or syndromes among Turkish patients with ID with/without other medical conditions. Data revealed an overall diagnostic rate of 11.43%, which confirms aCGH as the first-line technology allowing geneticists to diagnose complex phenotypes, identify candidate genes involved in ID, and explore novel CNV effects.

Keywords: Intellectual disability, copy number variation, Turkish cohort, array CGH, deletion, duplication

INTRODUCTION

The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), describes intellectual disability (ID) as a neurodevelopmental disorder that is complex, variable, and clinically heterogeneous (1). Approximately 1%–3% of the global population has ID (2) with or without multiple congenital anomalies (MCAs) (3). Environmental factors and genetics play a role in ID etiology (4). Genetic causes include copy number variations (CNVs), chromosomal aberrations, and single gene mutations (5).

Current analyses have indicated that submicroscopic CNVs contribute to approximately 15%–20% of ID cases (3). Conventional karyotyping has a resolution of 5–10 Mb and detects chromosomal aberrations in 5% of individuals with ID (except clinically recognizable chromosomal syndromes such as Down syndrome). Array comparative genomic hybridization (aCGH) enables identifying CNVs responsible for ID, with a commonly reported average diagnosis rate of 15%–20% (6). aCGH is still accepted as a first-line test for diagnosing patients with ID, global developmental delay,

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Table 1. Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical Features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM morbid genes/associated syndromes	Classification
1	F	14	Specific learning disability, ID, nasal speech, tubular nose, thin and long fingers, and cardiac anomaly			arr[GRCh37] 21q22.3(47,316,377_ 48,075,565)x1	0.759	13	*PCNT, COL6A2, COL6A1, LSS, FTCD*	Pathogenic
2	F	11	ID, epilepsy, and dysmorphic face			arr[GRCh37] Xq26.2(130,675,805 _130,963,121)x3	0.287	2	*IGSF1*	VUS
3	M	8	ID, epilepsy, and dysmorphic face			arr[GRCh37] Xp22.33 (113,071_ 2,709,818)x2	2.596	16	*CSF2RA, SHOX*	VUS
						arr[GRCh37] Xq28 (154,560,225_ 155,223,860)x2	0.663	8	*TMLHE, CLIC2*	VUS
4	M	11	ID, microcephaly, and dysmorphic face			arr[GRCh37] Xp22.31 (6,554,861_ 7,932,908)x2	1.328	4	*STS*	VUS
5	F	13	ID, behavioral problems, Simian crease on the left hand, partial Simian crease on the right hand, operated strabismus, epicanthus, long face, talipes equinovarus, and heart valve defects			arr[GRCh37] Xp22.33p22.2 (466,805_ 11,623,233)x3	11.1	41	*ARSL, STS, HCCS, SHOX, GPR143, MID1, CLCN4, NLGN4X*	Pathogenic
						arr[GRCh37] 6q27 (168,145,844_ 170,926,453)x1	2.780	16	*THBS2, TBP, SMOC2, ERMARD, DLL1, PSMB1* 6q27 Terminal Deletion Syndrome	Pathogenic
6	M	7	ID, anteverted ears, blue eyes, tubular nose, narrow nares, thin upper lip, systolic heart murmurs, and prognathia			arr[GRCh37] 10q26.2q26.3 (128,993,707_ 133,811,505)x3	4.8	14	*EBF3* Distal trisomy 10q syndrome	Likely pathogenic

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM Morbid genes/ associated syndromes	Classification
7	M	15	ID, autism, big hands and feet, downslanted palpebral fissures, retrognathia, hypoplasia of the maxilla, prominent forehead, hypertelorism, rotting teeth, and genu valgus			arr[GRCh37] 15q11.2q13.1 (23,639,473_ 28,356,321)x3	4.839	14	*UBE3A, OCA2, HERC2, GABRB3, MKRN3, GABRA5* 15q11q13 microduplication syndrome	Pathogenic
8	F	11	ID, epilepsy, and dysmorphic face			a arr[GRCh37] 15q11.2q13.2 (22,777,056_ 30,370,684)x3	7.593	27	*UBE3A, OCA2, HERC2, GABRB3, MKRN3, GABRA5, NSMCE3, MAGEL2* 15q11q13 microduplication syndrome	Pathogenic
9	M	10	ID, deep-set eyes, downslanted palpebral fissures, prognathia, bulbous nose, dysplastic ears, and large forehead			arr[GRCh37] 16p11.2 (32,066,962_ 33,933,923)x1	1.866	13		Benign
10	M	7	ID, tubular nose, behavioral problems			arr[GRCh37] 16p11.2 (32,066,962_33,862,112) x1	1.795	13		Benign
11	M	4	ID, behavioral problems, pes planus, narrow forehead, deep-set eyes, tubular nose, long philtrum, thin upper and lower lip, epicanthus, hyperextansibility, and upslanted palpebral fissures			arr[GRCh37] 1q23.2q23.3 (159,785,281 _164,270,792)x3	4.5	76	*MPZ, ATP1A2, SDHC, APOA2, FCGR2A, KCNJ10, FCGR2B, DDR2, CFAP45, PIGM, CASQ1, DCAF8, PEX19, COPA, NCSTN, VANGL2, CD244, USF1, NECTIN4, UFC1, PPOX, NDUFS2, FCGR3A, ATF6, NOS1AP, RGS5*	Pathogenic

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical Features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM morbid genes/ associated syndromes	Classification
12	F	3	ID, epilepsy, deafness			arr[GRCh37] 2p11.2p11.1 (89,323,730_91,413,804)x1	2.09			Benign
13	F	4	ID, delayed walking, speech delay, corpus callosum hypoplasia, delayed myelination, deep-set eyes, high palate, short hand fingers, down-slated palpebral fissures, micrognathia, and microcephaly			arr[GRCh37] 2q22.3 (145,149,256_145,269,121) x3	0.12	1	*ZEB2* Mowat–Wilson syndrome	Likely pathogenic
14	M	7	Mild ID, depressed nasal bridge, hypertelorism, irregular teeth, and Fragile X syndrome phenotype			arr[GRCh37] 16p11.2 (32,066,962_33,862,112)x1	1.795	13		Benign
						arr[GRCh37] Xq27.3 (146,410,692_147,029,643) x2	0.618	1	*FMR1* Fragile X syndrome	Likely pathogenic
15	F	5	ID, retrognathia, and temporal bossing			arr[GRCh37] 17p11.2 (17,393,323_19,977,668)x1	2.584	41	*RAI1, SREBF1, TOP3A, B9D1, ATPAF2, MYO15A, MIEF2, GRAP, ALDH3A2* Smith–Magenis syndrome	Pathogenic
16	M	7	ID, autism, dolichocephaly, slanted eyes, long philtrum, clinodactyly, and prognathia	Patient: 46,XY,del(4) (q12q13)[20] Mother:46,XX[20] Father:46,XY[20]		arr[GRCh37] 4q12q13.3 (58,520,002_71,755,523)x1	13.235	46	*UGT2B17, MUC7, GNRHR, ENAM, TCRL, AMTN, AMBN*	Pathogenic

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM morbid genes/associated syndromes	Classification
17	F	8	ID, agenesis of corpus callosum, upslanted palpebral fissures, tubular nose, and thin lips			arr[GRCh37] 16p11.2 (32,070,356_34,197,186)x1	2.076	13		Benign
18	M	17	ID, pulmonary valve stenosis, cubitus valgus, micropenis, cafe au lait spots, Simian crease, hypospadias, pes cavus, and short metacarpals	46,XY[20]		arr[GRCh37] 8p23.1 (9,165,634_10,952,305)x1	1.8	9	*RP1L1* 8p23.1 microdeletion syndrome	Pathogenic
19	M	4	ID			arr[GRCh37] 8p23.1 (7,011,414_7,689,941)x1	0.678	32		Benign
20	F	15	ID, behavioral problems, epilepsy, prognathia, long face, tubular nose, cubitus valgus, high palate, deep-set eyes, prominent glabella, short philtrum, and hirsutism			arr[GRCh37] 8p23.1 (6,919,229_7,689,941)x1	0.77	32		Benign
21	M	10	Moderate ID, nasal speech, seizures, scoliosis, strabismus, high palate, anteverted nares, dysplastic ears, and thoracal mass			arr[GRCh37] 6q26q27 (163,503,546_170,921,603) x1	7.3	32	*PDE10A, TBXT, RNASET2, CEP43, SMOC2, ERMARD, DLL1, THBS2, TBP, MPC1* 6q terminal deletion syndrome	Pathogenic
22	M	6	ID, downslanted palpebral fissures, thin upper lip, high palate, and dysplastic ears			arr[GRCh37] 13q21.33q22.2 (71,772,677_75,318,330)x1	3.546	7	*PIBF1*	Pathogenic

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM Morbid genes/ associated syndromes	Classification
23	F	5	ID, epilepsy, behavioral problems			arr[GRCh37] 14q22.3q23.1 (57,622,904_58,397,426)x1	0.774	5		VUS
24	M	3	ID, Angelman syndrome phenotype			arr[GRCh37] 15q11.2q13.1 (23,813,089_28,525,601)x1	4.835	13	*UBE3A, GABRB3, HERC2, OCA2, GABRA5, MAGEL2, MKRN3* Angelman syndrome	Pathogenic
25	M	6	Mild ID, autism, hyperextensibility			arr[GRCh37] 16p13.11 (15,078,280_15,366,031)x1	0.28	3	16p13.11 Microdeletion Syndrome	Pathogenic
						arr[GRCh37] 16p11.2(32,066,962_33,961,234) x1	1.89	13		Benign
26	F	5	ID, speech delay, Smith–Magenis syndrome phenotype		Nuc ish(SMCRx1) [100]	arr[GRCh37] 17p11.2(16,723,001_20,291,167) x1	3.56	47	*MYO15A, TNFRSF13B, ATPAF2, RAI1, ALDH3A2, FLCN, SREBF1, TOP3A, B9D1, MIEF2, GRAP* Smith–Magenis syndrome	Pathogenic
27	M	8	Mild ID, depressed nasal bridge, upslanted palpebral fissures, short philtrum, thin upper lip, hyperextensibility, and light-colored eyes and hair		Nuc ish(N25x1) [100]	arr[GRCh37] 22q11.21(18,641,420_21,457,610) x1	2.766	51	*GP1BB, SNAP29, RTN4R, COMT, TBX1, PRODH, PI4KA, LZTR1, SLC25A1, USP18, CDC45, TXNRD2, TANGO2, SCARF2, SERPIND1* 22q11.2 deletion syndrome	Pathogenic

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM morbid genes/associated syndromes	Classification
28	M	14	ID, hyperactivity, upslanted palpebral fissures, long philtrum, retrognathia, tubular nose, high palate, hypotelorism, anteverted alae nasi, irregular teeth, and thick lower and upper lips			arr[GRCh37] 5p14.3 (20,693,806_21,723,899) x1	1.03			Likely benign
						arr[GRCh37] 7q11.23 (75,985,508_76,810,806) x1	0.825	10	*YWHAG, ZP3* Distal chromosome 7q11.23 deletion syndrome	Likely pathogenic
29	M	17	Moderate ID, triangular face, prominent nasal bridge, thick ala nasi, broad nasal tip, deep-set eyes, low-set posteriorly located ears, downslanted palpebral fissures, chin dimple, prognathism, short distal phalanx of the hands and broad thumb, hyperextensibility, and large testis	Patient: 46,XY[20]	Nuc ish(8q11.1–q11.23x1)[100]	arr[GRCh37] 8q11.1q11.23 (47,355,673_54,495,215) x1	7.183	16	PRKDC, RB1CC1, *MCM4, SPIDR* Silver–Russel-like syndrome	Pathogenic
30	M	9	Severe ID, autistic behaviors, epilepsy, speech delay, attention deficit, strabismus, cryptorchidism, tubular nose, short stature, microcephaly, prognathia, flat forehead, long palpebral fissures, long philtrum, dysplastic ears, bilateral epicanthus, and thin upper lip			arr[GRCh37] 8p23.1 (6,925,491_7,689,941)x1	0.764	32		Benign
						arr[GRCh37] 16p13.3 (446,285_1,421,168)x1	0.97	42	*CACNA1H, STUB1, PIGQ, LMF1, CAPN15, CCDC78, GNPTG* ATR-16 syndrome	Pathogenic
31	F	11	ID, epilepsy, high palate, narrow forehead, low-set frontal hairline, cubitus valgus, thin upper lip, retrognathia, low-set dysplastic ears, short philtrum, malocclusion, and hypotonia	47,XX+mar[20]		arr[GRCh37] 15q11.2q13.3 (20,406,312_32,757,361) x3	12.351	64	*NIPA1, UBE3A, GABRB3, OCA2, HERC2, MAGEL2, TRPM1, FAN1, NSMCE3, GABRA5, MKRN3* 15q11q13 microduplication syndrome	Pathogenic

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM Morbid genes/associated syndromes	Classification
32	F	8	ID, epilepsy, narrow forehead, high palate, bulbous nose, tapering fingers, deep-set eyes, upslanted palpebral fissures, obesity, supernumerary nipple, and cardiac murmur		Nuc ish(1p34.3–p34.2x3) [100]	arr[GRCh37] 1p34.3p34.2 (34,785,012_41,468,186)x3	6.68	86	*GJB3, COL8A2, CSF3R, ZMPSTE24, COL9A2, GJB4, NCDN, AGO1, ADPRS, SNIP1, DNALI1, RSPO1, EPHA10, YRDC, MACF1, TRIT1, MFSD2A, PPT1, KCNQ4, CTPS1*	Pathogenic
33	M	15	ID, high palate, narrow forehead, prognathia, anteverted ears, thick lower lip, and pes cavus			arr[GRCh37] 10q26.3 (135,110,555_135,405,799)x3	0.295	13	*SYC1, ECHS1, TUBGCP2*	VUS
34	M	13	ID, epilepsy, micropenis, scoliosis, narrow forehead, tubular nose, cubitus valgus, downslanted palpebral fissures, short philtrum, thick upper and lower lips, hypoplasia of the maxilla, and high palate			arr[GRCh37] 10q26.3 (135,095,033_135,405,799)x3	0.31	13	*SYC1, ECHS1, TUBGCP2*	VUS
						arr[GRCh37] 15q11.2 (22,763,424_23,221,732)x1	0.45	4	*NIPA1* 15q11.2 BP1–BP2 microdeletion syndrome	Pathogenic
35	M	7	Moderate ID, epilepsy, behavioral problems, speech impairment, hypotonia, high palate, and low-set frontal hairline	46,XY[20]	Nuc ish(3p12.1–p11.1x1) [100]	arr[GRCh37] 3p12.1p11.1 (85,301,035_88,258,694)x1	2.95	8	*CHMP2B, POU1F1* 3p11.2–p12.1 deletion syndrome	Pathogenic
36	M	3	ID, hyperactivity, narrow forehead, downslanted palpebral fissures, epicanthus, tubular nose, facial hemiparesis, short philtrum, micrognathia, and hirsutism on the back			arr[GRCh37] Xp22.2 (11,291,711_11,913,836)x2	0.62	4	*AMELX, MSL3, FRMPD4*	VUS

Table 1 (continuous). Summary of clinical and molecular features of patients with CNVs.

Case	Sex	Age (year)	Clinical features	Karyotyping	FISH	aCGH result	Size (Mb)	Number of affected coding genes	OMIM norbid genes/associated syndromes	Classification
37	M	11	ID, epilepsy, narrow forehead, tubular nose			arr[GRCh37] Xp22.33 (2,167,170_2,270,373)x2	0.1	1		Benign
38	M	11	ID, epilepsy, short philtrum, high palate, synophrys, and thick lower lip	46,XY[20]		arr[GRCh37] Xp22.33 (1,804,303_2,131,027)x2	0.327			Benign

v* according to the International System for Human Cytogenetic Nomenclature (ISCN) 2020
F, female; M, male; ID, intellectual disability; VUS, variant of uncertain clinical significance.
Patients in gray were detected using BAC array platform.

MCAs, and autism spectrum disorders (ASDs) (2).

Thus, this study aimed to present CNVs identified in a retrospective cohort of 210 patients having ID with/without other medical conditions (such as ASDs, psychomotor retardation, epilepsy, attention deficit disorder, dysmorphic facial features, and/or MCAs) referred to our laboratory between 2009 and 2012. The CNVs identified in 38 patients with ID were summarized.

MATERIALS AND METHODS

Patients

This single-center retrospective cross-sectional study included 210 patients (139 male, 71 female, male/female ratio of 1.96) with unexplained ID with/without other medical conditions (such as ASD, psychomotor retardation, epilepsy, attention deficit disorder, dysmorphic facial features, and/or MCAs) and referred to our genetic laboratory from Pediatric Neurology, Pediatric Psychiatry, Pediatric Cardiology and Pediatric Endocrinology Departments of Kocaeli University, between 2009 and 2012. The median age was 8 (range, 2–18) years. The medical history (anamnesis, personal and family histories, and physical and dysmorphological examination) of the patients was provided by medical geneticists. Patients who refused to provide informed consent and whose genetic alterations explaining their clinical features were detected by one of the other techniques (e.g., karyotyping, fluorescent in situ hybridization (FISH), multiplex ligation-dependent probe amplification, and sequencing) before the aCGH were excluded from the study.

All procedures were conducted following the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent revisions. Owing to the patient's age, guardians or parents signed the informed consent forms approved by the Human Subjects Research Ethical Committee of Kocaeli University, under Project number 2009/102.

aCGH

In this study, 3 mL of peripheral blood was collected from each patient, and genomic DNA was extracted using DNeasy Blood & Tissue kit (Qiagen, Germany), following the manufacturer's instructions. aCGH analysis was performed using the oligo-based CytoSure Syndrome Plus ISCA Design (v2) Microarray 4 × 44K (Oxford Gene Technology, Oxford, UK) in 142 patients and bacterial artificial chromosome (BAC) CytoChip Focus Constitutional (v1.1) arrays (BlueGnome Ltd., Cambridge, UK) in 68 patients according to the manufacturer's recommendations. Data analysis was performed using CytoSure visualization software (Oxford Gene Technology) for oligo arrays and BlueFuse Software v2.2 (BlueGnome Ltd.) for BAC arrays. Karyotyping was performed in only six patients according to standardized procedures (7) and was used for segregation analysis in one patient (case 16). Chromosome observations were performed using an Olympus microscope and CytoVision analysis software. FISH was performed for validation using BlueFish tile BAC probes (BlueGnome Ltd.) RP11-101E19 (Chr:8, Start:47728696, and Stop:47901824), RP11-327P22 (Chr:1, Start:34876928, and Stop:35068982), RP11-14B7 (Chr:3, Start:85446954, and Stop:85621157), and Cytocell FISH probes LPU 007-S (Smith–Magenis (FLII)/Miller–Dieker Probe Combination) and LPU 010 (VCFS N25) according to the manufacturer's instructions.

CNV interpretation

To determine the pathogenicities of CNVs, they were evaluated using public databases and literature mining. The UCSC Genome Browser was used to display gene distributions. Coordinates of aberrations were based on the UCSC NCBI36/hg18. For the translation of the coordinates to hg19, UCSC LiftOver was used (https://genome.ucsc.edu/cgi-bin/hgLiftOver). Benign CNVs were identified using the Database of Genomic Variants, which contains CNVs of the normal population. CNVs were compared with the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER), dbVar, ClinVar, and ClinGen that provide CNVs with clinical features. The ClinGen Dosage Sensitivity Map was used to determine the dosage sensitivity of the genes in the aberrant region. The NCBI Gene Database, GeneCards, PubMed, Genetic Home Reference, and OMIM provided information on the functions of genes located in genomic aberrations. Genes related to neurodevelopmental disorders were searched using Gene2Phenotype.

According to ACMG/ClinGen guidelines, CNVs were classified as pathogenic, likely pathogenic, VUS, likely benign, and benign (8, 9). In addition, Franklin by genoox (https://franklin.genoox.com/clinical-db/home) and XCNV (http://119.3.41.228/XCNV/index.php) were used in CNV interpretation.

RESULTS

In this study, 45 CNVs were found in 38 (18%) patients. Among the CNVs, 18 (40%) were duplications, and 27 (60%) were deletions. Thirteen CNVs (28.8%) were large genomic aberrations encompassing a region of ≥3 Mb. Forty-five CNVs were classified into five categories: 21 (46.7%) were pathogenic, 4 (8.9%) were likely pathogenic, 8 (17.8%) were VUS, 1 (2.2%) was likely benign, and 11 (24.4%) were benign.

Multiple CNVs were found in 7 (18.4%) patients. Segregation analysis could be performed in only one patient (case 16) using karyotyping, which resulted in a *de novo* deletion. Detected CNVs were validated by FISH in five patients (cases 26, 27, 29, 32, and 35) and karyotyping in one patient (case 16). The clinical features of patients with CNVs are shown in Table 1.

Among 45 CNVs, 19 corresponded to rare microdeletion/microduplication syndromes. Microdeletions associated with syndromes were Smith–Magenis syndrome (n = 2), 8p23.1 microdeletion syndrome, 6q terminal deletion syndrome, Angelman syndrome, distal chromosome 7q11.23 deletion

syndrome, Silver–Russel-like syndrome,15q11.2 BP1-BP2 microdeletion syndrome, 3p11.2–p12.1 deletion syndrome, 16p13.11 microdeletion syndrome, ATR-16 syndrome, and 22q11.2 deletion syndrome. Microduplications associated with syndromes were 15q11.q13 microduplication syndrome (n = 3), Mowat–Wilson syndrome, distal trisomy 10q syndrome, and fragile X syndrome.

In this study, pathogenic CNVs, including deletions of 21q22.3 and 4q12q13.3 and duplications of Xp22.33p22.2, 1q23.2q23.3, and 1p34.3-p34.2, were not found to be associated with a syndrome.

Breakpoints of the marker chromosome detected by karyotyping were identified using aCGH in one patient. Case 31 showed a gain for the region 15q11.2q13.3 with a size of approximately 12 Mb. In 172 patients (82%), no aberrations were observed.

The pathogenic/likely pathogenic chromosomal changes that could explain the phenotype or be related to the patient's findings were detected in 24 of 210 (11.43%) patients. The diagnostic rate in this study was 11.43%.

DISCUSSION

In the present study, 45 CNVs were found in 38 (18%) patients. Among the CNVs, 18 (40%) were duplications, and 27 (60%) were deletions. A study reported that random duplications may occur less frequently than random deletions in the genome (6).

Among the CNVs, 21 (46.7%) were pathogenic, and 4 (8.9%) were likely pathogenic. Pathogenic CNVs were more prevalent in our study by detecting large CNVs, and the detected CNVs were predominantly deletions.

In this study, deletions were found to be associated with Smith–Magenis syndrome (n = 2), 8p23.1 microdeletion syndrome, 6q terminal deletion syndrome, Angelman syndrome, distal chromosome 7q11.23 deletion syndrome, Silver–Russel-like syndrome,15q11.2 BP1-BP2 microdeletion syndrome, 3p11.2–p12.1 deletion syndrome, 16p13.11 microdeletion syndrome, ATR-16 syndrome, and 22q11.2 deletion syndrome. Duplications were associated with 15q11.q13 microduplication syndrome (n = 3), Mowat–Wilson syndrome, distal trisomy 10q syndrome, and fragile X syndrome. Microduplication syndromes are frequently unnoticed because of their mild phenotype, although microdeletion syndromes have been more frequent owing to their recognizable features (10).

In this study, pathogenic CNVs, which could not be found to be associated with a syndrome, have been also detected. A pathogenic deletion of 21q22.3 encompassing *PCNT, COL6A2, COL6A1, LSS,* and *FTCD* was detected in case 1 with a specific learning disability, ID, nasal speech, tubular nose, thin and long fingers, and a cardiac anomaly. *S100B, DIP2A, PCNT,* and *PRMT2,* which are located in the breakpoints of our CNV, are candidate genes for dyslexia. A study suggested that *COL18A1, COL6A1*, and *COL6A2* are causal for cardiac abnormalities such as ascending aorta dilatation (11).

A complex CNV (pathogenic 6q27 deletion and pathogenic Xp22.33p22.2 duplication) was found in case 5 with ID, behavioral problems, Simian crease on the left hand, partial Simian crease on the right hand, operated strabismus, epicanthus, long face, talipes equinovarus, and heart valve defects. Interpreting the phenotypic consequences of patients with complex CNVs is challenging. Strabismus, ID, epicanthus, and behavioral problems have been reported in 6q27 terminal deletion syndrome (12). A large pathogenic Xp22.33p22.2 duplication including *ARSL, STS, HCCS, SHOX, GPR143, MID1, CLCN4,* and *NLGN4X* was detected in the same patient. Among these genes, *CLCN4* is a morbid OMIM gene associated with Raynaud–Claes syndrome. In Genereviews (https://www.ncbi.nlm.nih.gov/books/NBK575836/), *CLCN4*-related neurodevelopmental disorder (*CLCN4*-NDD) has been reported with phenotypic features such as developmental delay or ID, behavioral problems (e.g., ASD, hyperactivity, anxiety, and bipolar disorder), epilepsy, and gastrointestinal dysfunction. In Genereviews, chromosomal microarray analysis (CMA) has been suggested as the first genetic test for diagnosis in children with developmental delay or older patients with ID. We think that the patient findings were caused by the combined effects of the detected deletion and duplication. Complex CNVs should be verified whether they arise from a parental balanced rearrangement.

Marker chromosomes cannot be identified by conventional cytogenetic methods (13). In this study, the CNV size, chromosomal breakpoints, and gene content of the marker chromosome detected in case 31 were identified using aCGH. A large pathogenic duplication was found in case 11. Pure and partial trisomy 1q very rarely occur (14). The reported duplications are predominantly distal trisomy 1q and are caused by unbalanced translocations with partial deletions at other chromosomes. The size, location, and genes implicated in the duplication determine the severity of its symptoms. Individuals with chromosome 1q duplications may have various features including developmental delay, learning disabilities, slow growth, short stature, birth defects (e.g., cleft palate and heart defect), and facial dysmorphic features (e.g., retrognathia). To our knowledge, no 1q23.2q23.3 duplication was reported in the literature. In addition, in DECIPHER, no duplications overlap exactly with our region, and there are either smaller (95–953 kb) or larger (7–103 Mb) ones. Patient 342100 with ID and autism, reported in DECIPHER, has a duplication with a size of 953.57 Kb. This duplication contains *MPZ, SDHC, APOA2, CD244, NECTIN4, UFC1, PPOX, USF1,* and *NDUFS2* overlapping with our region. In Franklin genoox, *SDHC, UFC1, PPOX,* and *NDUFS2* were found to be associated with ID and behavioral problems phenotypes. These genes may be responsible for the ID and behavioral problems in our patient.

A large 4q12q13.3 deletion was detected in case 16 with ID, autism, dolichocephaly, slanted eyes, long philtrum, clinodactyly, and prognathia. Proximal 4q aberrations (deletions/duplications) have been reported in different sizes and regions so far. ID and autism findings in our patient were associated with *UBA6* located in the deleted region (15). Thus, this gene may be responsible for the cognitive and behavioral features.

A pathogenic 13q21.33q22.2 deletion was found in a male patient (case 22) with ID, downslanted palpebral fissures, thin upper lip, high palate, and dysplastic ears. Partial 13q deletions are uncommon. No pure 13q21.33q22.2 deletion was reported in the literature. The reported 13q deletions are larger CNVs including our breakpoints. Kirchhoff et al. have evaluated molecular and clinical data, belonging to 14 European patients who had *de novo* 13q deletions, for the genotype–phenotype mapping of 13q. Their data and earlier study have indicated that 13q21.1–q21.33 and 13q31 are associated with mild ID or even normal mental development (16). In addition, our CNV region contains one of the ID-related gene, i.e., *PIBF1*, which has been associated with Joubert syndrome 33.

A rare 1p34.3–p34.2 duplication of 6.68 Mb in size was detected in a female patient (case 32) with ID, epilepsy, narrow forehead, high palate, bulbous nose, tapering fingers, deep-set eyes, upslanted palpebral fissures, obesity, supernumerary nipple, and cardiac murmur. Few interstitial 1p duplications were described. Reported duplications have been larger than our duplicated region and have been associated with phenotypic features such as severe intrauterine growth retardation, ambiguous genitalia, Kabuki syndrome-like symptoms, sex reversal, and MCAs including a heart defect. A girl presenting with heart defects, developmental delay, midface hypoplasia, speech delay, broad nasal bridge, frontal bossing, fifth finger clinodactyly, low-set posteriorly rotated ears, tapering fingers, microdontia, pes planus, and varus positioning of feet was reported previously. She had interstitial 1p34.1–p34.3 duplication detected by FISH. In that report, *COL8A2,* which is located in our duplicated region, was suggested to be responsible for congenital heart defects (17). Jacher et al. described a female patient who had *de novo* 1p34.3p34.2 deletion with a size of 2.3 Mb and presented delayed development, mild ID, bone age delay, vocal cord paralysis, bilateral metatarsus adductus, bilateral vesicoureteral reflux, aberrant right subclavian artery, kyphoscoliosis, and genu valgum. They suggested that the haploinsufficiencies of *AGO1, SLC2A1, AGO3*, *RIMS3,* and *GRIK3* may cause neurocognitive impairments and other symptoms presented in their patient, and *SNIP1* may have an important role in central nervous system disorders, particularly delayed development, cognitive impairment, epilepsy, structural brain deformities, and ID (18). To understand the triplosensitivity effect of *SNIP* and other genes in the 1p34.3–p34.2 region, more cases of patients with similar duplications must be reported.

This study presents CNV data from a cohort of 210 Turkish patients with ID. Pathogenic/likely pathogenic CNVs were found in 24 of 210 (11.43%) patients. The diagnostic rate of aCGH is variable and is determined by various factors such as the phenotype complexity of the patients being tested and the array design being used (19). Our diagnostic rate (11.43%) was lower than the average diagnostic rate of 15%–20% reported recently (6) but in concordance with the 10%–20% reported in previous aCGH studies (20, 21).

In this study, any CNV could not be observed in 172 (82%) patients. One of the next-generation sequencing methods such as whole-exome sequencing is recommended for patients in whom no CNVs were detected or whom VUS/likely benign/benign CNV is detected that does not clarify their phenotypes. In addition, VUS CNVs should be followed because their pathogenicity may change over time.

This study has some limitations. First, parental inheritance could not be identified. Parental inheritance information would have been useful to interpret CNV data, particularly VUS. Second, high-resolution aCGH could not be used. If a high-resolution aCGH could be used, a higher diagnostic yield could be achieved. Third, the study analyzed a small sample. Fourth, few patients (n = 6) with CNVs detected by aCGH underwent FISH or karyotyping to validate the results of aCGH.

In summary, this study presents rare CNVs or syndromes among Turkish patients having ID with/without other medical conditions. In addition, our results identified VUS CNVs that may be reclassified after further functional studies. CGH remains the first-tier technology allowing geneticists to diagnose complex phenotypes, identify candidate genes involved in ID, and explore novel CNV effects.

Ethics Committee Approval: All procedures were conducted following the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent revisions. Approved by the Human Subjects Research Ethical Committee of Kocaeli University, under Project number 2009/102.

Informed Consent: Owing to the patient's age, guardians or parents signed the informed consent forms.

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