Application of Chromosome Microarray Analysis in the Investigation of Developmental Disabilities and Congenital Anomalies: Single Center Experience and Review of *NRXN3* and *NEDD4L* Deletions

Alper Han Çebi^a Şule Altın^{b, c}

^aDepartment of Medical Genetics, Karadeniz Technical University School of Medicine, Trabzon, Turkey; ^bDepartment of Medical Genetics, University of Health Sciences, Trabzon Kanuni Training and Research Hospital, Trabzon, Turkey;

^cDepartment of Medical Genetics, Ankara University School of Medicine, Ankara, Turkey

Keywords

Chromosomal microarray · Copy number variation · Developmental disabilities · *NEDD4L* · *NRXN3*

Abstract

Chromosomal microarray analysis (CMA) is a first step test used for the diagnosis of patients with developmental delay, intellectual disability, autistic spectrum disorder, and multiple congenital anomalies. Its widespread usage has allowed genome-wide identification of copy number variations (CNVs). In our study, we performed a retrospective study on clinical and microarray data of 237 patients with developmental disabilities and/or multiple congenital anomalies and investigated the clinical utility of CMA. Phenotype-associated CNVs were detected in 15.18% of patients. Besides, we detected submicroscopic losses on 14q24.3q31.1 in a patient with speech delay and on 18q21.31q21.32 in twin patients with seizures. Deletions of *NRXN3* and *NEDD4L* were responsible for the phenotypes, respectively. This study showed that CMA is a powerful diagnostic tool in this patient group and expands the genotype-phenotype correlations on developmental disabilities.

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Introduction

Developmental delay/intellectual disability (DD/ID) is a common condition with a prevalence of 1–3% [Roeleveld et al., 1997]. The International Standard for the Consortium of Cytogenomic Array Consortium recommended chromosomal microarray (CMA) analysis as a first diagnostic step in patients with developmental disabilities [unexplained DD/ID or autism spectrum disorders (ASD)] or multiple congenital anomalies (MCA) [Miller et al., 2010].

CMA detects genomic gains and losses at a much higher resolution than G-banded karyotyping. Besides, it allows the identification of new copy number variations by its genome-wide design. Microarray platform, patient selection, and interpretation criteria affect the diagnostic yield of CMA, which ranges from 5–20% according to the current literature [Sagoo et al., 2009; Miller et al., 2010].

A total of 237 patients with unexplained developmental disabilities and/or MCA were tested with CMA in this study. Genomic changes that could be associated with the phenotypes of the patients were identified in 15.18%.

DD/ID is an umbrella term that contains many conditions with variable severity. DD can affect motor, language, social, or intelligent skills. Besides, DD/ID can be accompanied by other neurological features or congenital anomalies. The genetic causes of DD/ID have been described as accelerating in the recent years. Despite these improvements, the interpretation of some cases could be challenging due to the rarity of genetic aberrations. We present 2 detailed reports with relatively rare CNVs in order to contribute to the literature. We detected a deletion in *NRXN3* (neurexin 3) in a patient with speech delay. *NRXN3* is a member of the human neurexin gene family that expresses presynaptic transmembrane proteins [Vaags et al., 2012]. Also, deletion of *NEDD4L* in twin patients with seizures has been accounted responsible for their phenotype. *NEDD4L* encodes an E3 ubiquitin ligase that regulates channel turnover and has a role in neuronal functions [Yanpallewar et al., 2016].

Briefly, the aim of this study was to investigate the diagnostic yield of CMA in a group of Turkish patients with unexplained developmental disabilities and/or MCA and to contribute to the genetic causes in DD/ID.

Methods

A total of 237 patients with unexplained developmental disabilities and/or MCA who were referred to the Department of Medical Genetics, Karadeniz Technical University School of Medicine, Trabzon, Turkey were enrolled from 2014–2019 for CMA analysis. CMA was performed as the first step test. Every patient underwent a detailed evaluation by a medical geneticist, which included prenatal and birth history, pedigree, family history, and detailed clinical and dysmorphology examinations.

Peripheral blood specimens were used for all genetic tests mentioned below. Genomic DNA was extracted using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostic). From January 2014 to December 2017, Affymetrix, CytoScan 315K (Affymetrix CytoScan Optima®), CytoScan 750K SNP microarray platforms, and Chromosome Analysis Suite (ChAS) software were used. Each finding was analyzed with the criteria for detection by at least 20 oligonucleotide probes on the array matrix. From January 2018 to December 2019, human CytoSNP-12 Beadchip 300K array, Infinium Global Screening Array-24 V2.0 665K platforms (Illumina), and Karyostudio software was used. A copy number change was called when more than 10 consecutive probes were involved in a segment. Genomic positions were based on the UCSC February 2009 human reference sequence (hg19) (Genome Reference Consortium Human Build 37-GRCh37).

CNVs were analyzed and evaluated based on the phenotype. DECIPHER [Firth et al., 2009], Database of Genomic Variants [MacDonald et al., 2013], and Online Mendelian Inheritance in Man (OMIM) [Online Mendelian Inheritance in Man, 2020] databases were used for interpretation. CNVs were classified as benign, uncertain clinical significance, and pathogenic accord-

ing to gene content and function, inheritance pattern, and documentation of multiple peer-reviewed publications [Kearney et al., 2011; Nowakowska, 2017]. Molecular karyotype results were described according to the International System for Human Cytogenomic Nomenclature [McGowan-Jordan et al., 2016] and GRCh37.

Confirmation and inheritance studies were done by karyotyping/FISH for gross aberrations, terminal aberrations, and unbalanced translocations. Unbalanced translocations were supposed in subjects with concurrent terminal copy number loss of one chromosome and terminal copy number gain of another. CNVs <5 Mb could not be detected with conventional karyotyping, and therefore, 5 Mb was the line between small sized and gross aberrations. FISH and microarray studies were performed for small sized and interstitial CNVs.

Results

Our study consisted of 237 patients with MCA/mental retardation, 139 males and 98 females, between the age of 1 month and 16 years. Out of all patients studied, phenotype-associated CNVs were detected in 36 (15.18%).

This study was done in patients with unexplained developmental disabilities and/or MCA, and CMA was performed as the first step test. We detected 44 pathogenic or likely pathogenic CNVs in 36 patients: 27 patients had one pathogenic CNV, while 7 patients showed more than one and 2 patients, who were identical twins, had a likely pathogenic CNV with uncertain clinical significance. Table 1 provides detailed descriptions of the patients.

A defined microdeletion/microduplication syndrome in OMIM was detected in 16 of the patients (#607872, Chromosome 1p36 Deletion Syndrome (2 patients); #612474, Chromosome 1q21.1 Deletion Syndrome; #194050, Williams-Beuren Syndrome; #601362, DiGeorge Syndrome Velocardiofacial Syndrome Complex 2; # 194072, Wilms Tumor, Aniridia, Genitourinary Anomalies and Mental Retardation Syndrome; #130650, Beckwith-Wiedemann Syndrome; #608636, Chromosome 15q11-q13 Duplication Syndrome; #615656, Chromosome 15q11.2 Deletion Syndrome; #614526, Chromosome 17q12 Duplication Syndrome; #601808, Chromosome 18q Deletion Syndrome; # 611867, Chromosome 22q11.2 Distal Deletion Syndrome; # 606232, Phelan-McDermid Syndrome (2 patients); #300979, Xq25 Duplication Syndrome; #300815, Chromosome Xq28 Duplication Syndrome).

The size of these CNVs ranged between 108 kb and 79.7 Mb, and 18 of them were <5 Mb. The majority of aberrations were losses (25 losses vs. 19 gains).

Table 1. Clinical and molecular cytogenetic findings in patients with pathogenic/likely pathogenic CNVs

Case No.	Age/sex	Platform	arrayCGH (hg19)	Size, Mb	Classification of aberrations	Inheritance	MIM disease/likely pathogenic genes	DD/ID	Facial dysmorphism	Other findings
1	8 years/F	Illumina Infinium Global Screening Array-24 V2.0 665K	1p36.3p36.32 (565433_4418667)×1	3,853	Pathogenic	Not performed	#607872, Chromosome 1p36 deletion syndrome/ <i>PVL1, ATAD3A, CDK11A, GNB1, GABRD, SKI, PEX10, PRDM16</i>	+	Microcephaly, myopia, posteriorly rotated ears, cleft lip, cleft palate	Secundum ASD
2	5 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	1q21.1q21.2 (145126272_147419078)×1	2,293	Pathogenic	Maternal, mother had ID	#612474, Chromosome 1q21.1 deletion syndrome/ <i>RBMS1, NBPFI10, HYDIN2, NBPFI12, PRKAB2, FMO5, CHD1L, BCL9, ACP6, GJA5, GJA8, GPR89B</i>	+	Brachycephaly	Cryptorchidism, seizures, ADHD, aggressive behavior, metopic and sagittal synostosis
3	2 months/M	Affymetrix CytoScan Optima	4p16.1q11 (8355801_52685686)×3	44,330	Pathogenic	de novo	<i>HMAX1, DRD5, NKX3-2, CC2D2A, TAP11, SEPS2C, RPB1, STIM2, CCKAR, LG2, WDR19, RFC1, SLCS0A9, GUF1, GABRA2, GABRB1</i>	+	High palate, big ears	Pyloric stenosis, metopic synostosis
4	1 year/F	Illumina Infinium Global Screening Array-24 V2.0 665K	7q11.23 (72708505_74129824)×1	1,421	Pathogenic	de novo	#194050, Williams-Beuren syndrome/ <i>NRUN5, TRIM50, FKBP6, PZD9, BAZ1B, BCL7B, TBL2, MLX1PL, VPS37D, DNAJC30, WBSCR22, STX1A, ABHD11, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, LAT12, RFC2, CLIP2, GTF2IRD1, GTF2I</i>	+	Epicantal folds, thick lips	Pulmonary valve stenosis, gastroesophageal reflux, hypothyroidism, seizures
5	10 years/M	Affymetrix CytoScan Optima	8p11.21q21.2 (4095139_85038334)×3	44,043	Pathogenic	de novo	<i>KAT6A, POMK, SOX17, IMPAD1, CAA8, CHD7, CSPP1, CPA6, EYAL1, TMEM70, JPH1, GDAP1, PMP2, IMPA1</i>	+	Brachycephaly, proptosis, large ears, bulbous nasal tip	Myopia, sagittal and metopic synostosis, scoliosis, atrial septal defect, partial anomalous pulmonary venous return, cryptorchidism, hypospadias, vesicoureteral reflux, inguinal hernia, gastroesophageal reflux, aggressive behavior
6	8 years/M	Illumina human CytoSNP-12	10p15.3p15.1 (135708_3986323)×1	3,851	Pathogenic	Not performed	<i>ZMYND11, DIP2C, LARP4B, GTPB4, WDR37, KLF6</i>	+	Prominent glabella, large ears	Delayed speech
7	8 years/F	Illumina human CytoSNP-12	10p15.1p12.31 (6077845_21015343)×1	14,937	Pathogenic	Not performed	#601362, DiGeorge syndrome, Velocardiofacial syndrome complex, <i>21L2RA, RBM17, PRKCQ, SEMB27, TAF3, GATA3, CELF2, UPF2, SEC61A2, DUTKDI, PHTH, FRMD4A, DCLRE1C, ITGA8, CUBN, VIM, CACNB2</i>	+	High-bridged nose, low-set ears	Cataract, seizures, hypocalcemia
8	5 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	11p14.3p11.2 (21795746_43785206)×1	21,989	Pathogenic	de novo	#194072, Wilms Tumor, Aniridia, Genitourinary anomalies and mental retardation syndrome/ <i>BDNF, ELP4, PAX6, PRRG4, WT1, TRIM44</i>	+	Aniridia	Glaucoma, hypospadias, cryptorchidism, hydrocele, gastroesophageal reflux, seizures
9	10 years/M	Illumina human CytoSNP-12	11p15.5p15.3 (326718_10786175)×3	10,459	Pathogenic	de novo	#130650, Beckwith-Wiedemann syndrome/ <i>11P1T3, HRAS, H19, IGF2, INS, KCNQ1, KCNQ1OT1, CDKN1C</i>	+	Macroglossia	Cryptorchidism, mitral valve regurgitation, seizures, congenital hypothyroidism
10	9 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	12p13.2p11.22 (11482928_28337382)×1	16,854	Pathogenic	de novo	<i>ETV6, GRIN2B, EPS8, PDE3A, ABCC9, SOX5, KRAS, PTHLH</i>	+	Epicantal folds, strabismus, low nasal bridge	Gastroesophageal reflux, aortic valve regurgitation, ventriculomegaly, anal stenosis
11	6 months/F	Affymetrix CytoScan Optima	14q11.2q13.1 (20511672_35048345)×3	14,537	Pathogenic	de novo	<i>OSGEP, CHD8, SUBT16H, MYH6, MYH7, FOXG1, PRKD1</i>	+	High palate	Secundum ASD, orbital dermoid cyst, hydrocephaly, seizures
12	2 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	14q21.1q21.2 (38814819_43829964)×1	5,015	Pathogenic	Not performed	<i>SEC23A, TRAPP6B, LRFN5</i>	+	Macrocephaly	Secundum ASD, seizures
13	3 years/F	Affymetrix CytoScan Optima	14q24.3q31.1 (78531783_79729394)×1	1,198	Pathogenic	de novo	<i>NRXN3</i>	+	Deep set eyes, strabismus, low nasal bridge, full cheeks	Delayed speech, subependymal nodular heterotopia
14	3 years/M	Affymetrix CytoScan Optima	14q31.3q32.2 (89525993_100453010)×1	10,927	Pathogenic	de novo	<i>TDPI, CALM1, GPR68, CCDC88C, FBLN5, TRIP11, ATXN3, SLC24A4, SERPINA6, SERPINA1, GSC, DICER1, GLRX5, VRK1, BCL11B, CCNK, EML1</i>	+	Large forehead, thin upper lips, micrognathia	ASD, pulmonary valve stenosis, cryptorchidism, hydrocephaly, meningomyelocele, delayed speech, seizures
15	8 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	15q11.2 (22320346_23616115)×3	1,296	Pathogenic	Paternal, father normal	#608636, Chromosome 15q11-q13 duplication syndrome/ <i>TUBGCP5, CYFIP1, NIPA2, NIPA1</i>	+		ADHD
16	7 years/M	Affymetrix CytoScan 750K	15q11.2 (22770421_23679133)×1	0,909	Pathogenic	de novo	#615656, Chromosome 15q11.2 deletion syndrome/ <i>TUBGCP5, CYFIP1, NIPA2, NIPA1</i>	+	Macrocephaly	Obesity, hypospadias, focal gliosis in occipital lobe

Table 1 (continued)

Case No.	Age/sex	Platform	arrayCGH (hg19)	Size, Mb	Classification of aberrations	Inheritance	MIM disease/likely pathogenic genes	DD/ID	Facial dysmorphism	Other findings
17	2 months/F	Affymetrix CytoScan Optima	17p13.3p11.2 (525_22084950)×3	22,084	Pathogenic	Mother balanced translocation carrier	YWHAE, CRK, MYO1C, INP5K, PITPNA, SCARF1, RILP, PRPF8, PAFAH1B1, PM22, RAI1	+	Triangular face, low-set ears, high palate	Hypotonia, poor growth, single transverse palmar crease, secundum ASD, agenesis of corpus callosum
18	3 years/F	Illumina Infinium Global Screening Array-24 V2.0 665K	17q12 (34815551_36245768)×3	1,430	Pathogenic	Not performed	#614526, Chromosome 17q12 duplication syndrome/ ZNF173, MYO19, PIGW, MRMI, LHX1, ACACA, TADA2A, SYNRG, HNF1B	+	Microcephaly, strabismus, large ears, micrognathia	Overriding toes, inguinal hernia, seizures, hypotonia, cerebral atrophy
19	4 years/M	Illumina human CytoSNP-12	18q21.2q23 (48586344_78014582)×1	29,428	Pathogenic	de novo	#601808, Chromosome 18q deletion syndrome/ MBD2, TCF4, TXNL1, WDR7, NETO1, ZNF407, ZADH2, TSHZ1, MBP, GALNK, NEATC1	+	Microcephaly, midface hypoplasia, malformed ear lobes, large ears, wide nasal bridge, wide mouth, high palate, thin upper lip	Proximally placed thumbs, overriding toes, minimal mitral valve regurgitation, hypothyroidism, partial agenesis of corpus callosum, delayed myelination
20	10 years/F	Illumina Infinium Global Screening Array-24 V2.0 665K	18q21.31q21.32 (55090993_56556611)×1	1,466	Likely pathogenic	de novo	NEDD4L	+	High forehead	Myopia, seizures, asymmetric dilatation of lateral ventricle
21	10 years/F	Illumina Infinium Global Screening Array-24 V2.0 665K	18q21.31q21.32 (55090993_56556611)×1	1,466	Likely pathogenic	de novo	NEDD4L	+	High forehead	Myopia, seizures, periventricular focal gliosis
22	2 months/F	Affymetrix CytoScan Optima	20p13q11.21 (357569_29420339)×3	29,063	Pathogenic	Mother balanced translocation carrier	PLCB4, PAK7, JAG1, MACROD2, NKX2-2, PAX1, CST3, VXX1	+	Large ears, broad nasal tip, anteverted nares, micro-retrognathia	Hearing loss, unilateral renal hypoplasia and ectopia
23	7 years/F	Affymetrix CytoScan Optima	22q11.21q11.23 (20742449_23900510)×1	3,158	Pathogenic	de novo	# 611867, Chromosome 22q11.2 distal deletion syndrome/CRKL, UBE2L3, MAPK1, TOP3B	+	Metopic synostosis, narrow forehead, large ears, lacrimal duct stenosis, thin upper lip	Prematurity, IUGR, short stature, seizures
24	3 years/F	Illumina Infinium Global Screening Array-24 V2.0 665K	22q13.31q13.33 (45985018_51186276)×1	5,201	Pathogenic	de novo	# 606232, Phelan-McDermid syndrome/PIPM3, MAPK8IP2, SHANK3, ACR	+	High forehead, pointed chin	Neonatal hypotonia, delayed speech, autistic features
25	1 month/F	Affymetrix CytoScan Optima	Xp22.33p22.12 (665238_20629197)×1	19,964	Pathogenic	de novo	NIGM4X, MSL3, FRIMPD4, OFD1, AP1S2, CDKL5, RP66KA3	+	Posteriorly rotated ears	Seizures
26	6 years/M	Affymetrix CytoScan Optima	Xp22.33p22.11 (168546_23108187)×2	22,939	Pathogenic	de novo	SHOX, NIGM4X, KALL1, AP1S2, GRPR, NHS, CDKL5, RP66KA3, MBTPS2, SMS	+	Prominent glabella, high forehead, wide nasal bridge	Secundum ASD, radiolunar synostosis, cryptorchidism, absent speech, obsessive behavior
27	2 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	Xq24q26.2 (119145820_131644216)×3	12,498	Pathogenic	Maternal, mother had ID	#300979, Xq25 duplication syndrome/GRIA3, THOC2, XIAP, STAG2, OCRL, ZDHHC9, AIEF1, RAB33A, IGSF1	+	Microcephaly, nystagmus, overriding toes	IUGR, poor growth, micropenis, hypospadias, inguinal hernia, gastroesophageal reflux, seizures, gliosis in white matter, thin corpus callosum
28	5 months/F	Affymetrix CytoScan Optima	Xq25q27.2 (128494500_142000467)×3	13,506	Pathogenic	de novo	OCRL, ZDHHC9, AIEF1, RAB33A, IGSF1, GPC4, GPC3, ARHGEP6, PHF6, HPR17, SLIC9A6, SOX3	+	Microphthalmia, large ears, long philtrum	SCA, brachydactyly
29	6 years/M	Affymetrix CytoScan Optima	Xq28 (153605826_153714301)×2	0,108	Pathogenic	de novo	#300815, Chromosome Xq28 duplication syndrome/ RPL10, ATP6AP1, GDI1	+	Epicantal folds, high palate, broad neck	Hallux valgus, bicuspid aortic valve, aortic valve regurgitation, delayed speech
30	3 years/M	Illumina Infinium Global Screening Array-24 V2.0 665K	1p36.33 (801536_1859176)×1	1,057	Pathogenic	Not performed	#607872, Chromosome 1p36 deletion syndrome/ DVLL1, ATAD3A, CDKL1A, GNB1	+	Deep-set eyes, strabismus, low-set ears, flat nose	Pes planus, nephrolithiasis, seizures
31	2 months/M	Illumina Infinium Global Screening Array-24 V2.0 665K	18p11.32p11.22 (111358_9920784)×3	9,909	Pathogenic	Mother balanced translocation carrier	SMCHD1, LPIN2, TGIF1, LAMA1, NDUFV2	+	Prominent forehead, hypertelorism, malformed ears, high palate	IUGR, single transverse palmar crease, sacral dimple, hearing loss, secundum ASD, seizures
32	5 years/F	Illumina Infinium Global Screening Array-24 V2.0 665K	14q32.32q32.33 (103617637_107283150)×1	3,666	Pathogenic	Mother balanced translocation carrier	CKB, INF2, AKT1, BRF1, BTBD6, JAG2, PACS2, MTA1, IGHM	+	Hypertelorism, strabismus, high palate	Umbilical hernia, hypothyroidism

Table 1 (continued)

Case No.	Age/sex	Platform	arrayCGH (hg19)	Size, Mb	Classification of aberrations	Inheritance	MIM disease/likely pathogenic genes	DD/ID	Facial dysmorphism	Other findings
33	5 years/F	Affymetrix CytoScan Optima	7p22.3(43,360_21,49736)×1 9p24.3p13.3 (203861_34631162)×3	2,106 34,427	Pathogenic Pathogenic	Not performed	<i>FAM20C, PDGFA, HEATR2, COX19, MAD1L1</i> <i>KANK1, SMARCA2, VLDLR, MPDZ, NFIB, FREM1, PLAA, IFIT4, TEK, C9orf72, DDX58, APTX, UBAP1, KIAA1161, DNAIL</i>	+	Epicantal folds, deep-set eyes, high-bridged nose, wide nasal bridge, low-set ears	Focal gliosis in frontoparietal lobe, pulmonary stenosis, mitral and tricuspid valve regurgitation, Ebstein anomaly
34	1 year/F	Illumina human CytoScan-12	9p24.3p22.3 (46,587_155,74853)×1	15,528	Pathogenic	Father pericentric inversion carrier	<i>DOCK8, KANK1, KCNV2, GLIS3, SLIC1A1, GLDC, TYRP1, CER1, FREM1</i>	+	Microcephaly, trigonocephaly, midface hypoplasia, anteverted nares, low-set ears, malformed ears, thin upper lip, micrognathia	Tapering fingers, pes planus, seizures
35	5 years/F	Illumina human CytoScan-12	9q34.2q34.3 (137,155,945_141,044,489)×3 11q23.3q25 (11,668,1008_134,944,006)×3	3,889 18,263	Pathogenic Pathogenic	Mother balanced translocation carrier	<i>COL5A1, KCNT1, LHX3, PMPCA, INPP5E, NOTCH1, AGPAT2, MAN1B1, GRIN1, EHM1, CACNA1B, ARCN1, DDX6, VPS11, MFRP, PVRL1, HEPACAM, PUS3, NCAPD3</i>	+	Low-set ears, thick helices, micrognathia, broad nasal tip	Lacrimal duct stenosis, dilatation of third and lateral ventricle, ductal ectasia, seizures
36	8 months/F	Affymetrix CytoScan Optima	22q11.1.q11.21 (161,142,44_207,407,78)×3 22q13.31.q13.33 (47,632,018_51,197,838)×1 Xp22.33p11.22 (16,854,6_51,963,669)×1 Xq13.3Xq28 (75,532,540_155,233,713)×1	4,627 3,566 51,795 79,701	Pathogenic Pathogenic Pathogenic Pathogenic	de novo	<i>CECR7, ATP6V1E1, HIRA, TXNRD2, COMT, ARVCF, #606322, Phelan-McDermid syndrome/P1M3, MAPK8IP2, SHANK3, ACR, SHOX, NLGN4X, MSL3, FRMPD4, OFD1, APIS2, CDKL5, RP6K43, CNKSR2, SMS, ZFX, POLA1, ILIRAPL1, NR0B1, GK, DMD, TSPAN7, ATP6AP2, USP9X, DDX3X, SLC9A7, WDR45, USP27X, BMP15, ATRX, BRWD3, ZNF711, POF1B, DIAPH2, PCDH19, SRPX2, HNRNP2, MID2, COL4A6, COL4A5, ACSL4, PAK3, DCX, ALG13, C9orf56, UBE2A, UBE3A, CUL4B, GRIA3, THOC2, OCRL, ZDHHC9, SLC9A6, RBMX, CD40LG, ZIC3, SOX3, EMR1, AFF2, L1CAM, MECP2, RPL10, RAB39B, CLIC2, TM6HE</i>	+	Microcephaly, maxillary hypoplasia, thin upper lip, smooth philtrum	IUGR, reduced central white matter volume

Fig. 1. Microarray analysis shows B allele frequency (middle panel) and Log R ratio (lower panel). The result represents a hemizygous copy number loss on chromosome 18q21.31q21.32 from 55090993 to 56556611 according to GRCh37/hg19. The region of loss is shown in red on chromosome 18 (upper panel). This de novo aberration was detected in both siblings (Case#20 and #21).

Inheritance studies were completed in 80.6% (29/36) of patients, and 20 had de novo CNVs. Structural chromosomal imbalance was detected in parents of 6 cases (case 17, 22, 31, 32, 34, and 35). The remaining 3 CNVs were inherited from parents with ID in 2 cases (case 2 and 27) and from phenotypically normal parents in one (case 15). In order to contribute to the literature, 2 cases are explained in detail below.

Case 13

The patient was born at term by spontaneous vaginal birth with a birth weight of 3,150 g. She was the third child of nonconsanguineous parents and had 2 healthy siblings. The mother had no history of miscarriages or intra-uterine fetal deaths. A 6 × 8 mm interhemispheric cyst

was detected prenatally. The family did not give consent for a prenatal genetic study.

Her measurements at 3 years of age were as follows: head circumference: 48 cm (25–50th percentile), height: 94 cm (25–50th percentile), and weight: 16 kg (75–90th percentile). She had dysmorphic features such as deep set eyes, strabismus, low nasal bridge, and full cheeks. She could control her head at 3, sit unassisted at 7, and walk at 13 months. She could say a few words at 24 months and 10 words at 36 months of age. On the other hand, she did not have stereotypical movements and developmental problems in social interaction. Subependymal nodular heterotopia was shown on a brain MRI. CMA revealed a de novo 1.198 Mb loss at 14q24.3q31.1 (chr14:78531783_79729394) in the proband. The dele-

Fig. 2. Schematic view of deleted regions of cases and isoforms of *NRXN3* on chromosome 14q24.3q31.1. Case#13 is referred to as present case. Genomic coordinates are shown according to GRCh37/.

tion overlaps with the 5'-region of the *NRXN3* gene, including coding exons.

Cases 20 and 21

The patients were 10-year-old identical twin sisters, who suffered from rolandic epilepsy since the age of 3 years. They were born at term by caesarean section. Case 20 had a birth weight of 2,600 g (50th percentile) and case 21 had 2,800 g (50–75th percentile). Their parents were nonconsanguineous and had a healthy sibling. The mother had no history of miscarriages or intrauterine fetal deaths.

The girls could control their heads at 4, sit at 9, and unassistedly walk at 20 months. They could say a few words at 18 months. Seizure history started at 3 years of age, and they still had attacks despite medication. Electroencephalogram revealed centrottemporal spikes. Asymmetric dilatation of the lateral ventricle was shown on a brain MRI of case 20. Likewise, periventricular focal gliosis was detected in case 21.

Her measurements at 10 years of age were as follows: head circumference: 53 cm (50th percentile), height: 134 cm (25–50th percentile), and weight: 28 kg (25–50th percentile). They both had a high forehead and myopia. Their intelligence was normal, and the family history was unremarkable. Microarray analysis revealed the same de novo aberration in both patients: a 1.466-Mb loss at 18q21.31q21.32 (55090993_56556611). The deleted region spans *ONECUT2*, *FECH*, *NARS*, *ATP8B1*, *NEDD4L*, *ALPK2*, *MALT1*, and *ZNF532* (Fig. 1).

Discussion

ID/DD is a heterogeneous group of disorders, which requires an investigational approach to determine the etiology. CMA is the first step test in this patient group [Miller et al., 2010]. Finding the underlying causes helps in disease management, provides genetic counseling, and offers prenatal diagnosis for future pregnancies [Rauch et al., 2006].

In this study, genomic changes that could be associated with the phenotypes of the patients were identified at a rate of about 15.18% with CMA, which is consistent with the current literature: the diagnostic yield of CMA is in a wide range of 5–20% [Sagoo et al., 2009; Miller et al., 2010]. Patient number and selection criteria, interpretation criteria, and type of CMA platform used are considered effective factors in this difference [Miller et al., 2010].

A known microdeletion/microduplication syndrome was detected in 16 of the cases. It can be explained by specific syndromes that were not considered due to subtle clinical features.

A higher number of losses were responsible for the phenotype in this study (25 losses vs. 19 gains). Gains may present with milder phenotypes, which could result in a selection bias [attaglia et al., 2013].

Case 13 presented with subtle dysmorphic features and speech delay. A 1.198-Mb microdeletion at chromosome 14q24.3q31.1 spanning the *NRXN3* gene was responsible for the phenotype. Neurexin 1 (*NRXN1*), neurexin 2 (*NRXN2*), and neurexin 3 (*NRXN3*) are members of the human neurexin gene family of presynaptic

transmembrane proteins that alter the synaptic function and intervene with nerve signal conduction. Each of the genes produces alpha and beta isoforms [Vaags et al., 2012]. Alpha neurexins were shown to affect neurotransmitter release via voltage dependent Ca^{2+} channels [Zhang et al., 2005; Dudanova et al., 2006]. Mutations and deletions of *NRXN1*, *NRXN2*, and *NRXN3* are associated with a wide variety of neurodevelopmental conditions. Mutations of all 3 genes were identified in patients with ASD [Kasem et al., 2018]. The genomic region resembling our case was published by Yuan et al. [2018]. Exonic deletions of alpha isoform of *NRXN3* were shown in a 3-generation family. Although only the proband had dysmorphic facial findings, all individuals carrying the deletion had variable neurodevelopmental disorders. Also, CNVs of *NRXN3* were shown in ASD patients [Griswold et al., 2012; Vaags et al., 2012]. The aforementioned deleted regions of the cases are shown in Figure 2. Larger genomic losses were also defined in this region. Riegel et al. [2014] presented a case with a 13.11-Mb loss on 14q24.3q31.3 with dysmorphic traits, moderate developmental delay, and mild mental retardation. Cingoz et al. [2011] published another case with a 23-Mb loss on 14q24.3q32.2 in a patient with DD, language impairment, dysmorphic face, and congenital heart defect. Although an essential role of *NRXN3* in the phenotypic finding of patients with 14q24.3q31.1 deletions was discussed in several reports, its relation with speech delay is validated with this case.

Cases 20 and 21 were 10-year-old identical twin sisters diagnosed with rolandic epilepsy. They had a 1.466-Mb loss on chromosome 18q21.31q21.32. *ONECUT2*, *FECH*, *NARS1*, *ATP8B1*, *NEDD4L*, *ALPK2*, *MALT1* and *ZNF532* genes are located at this genomic region. *ONECUT2*, *NARS1*, *ALPK2*, and *ZNF532* have not been associated with a disease yet. Biallelic mutations of *FECH* are associated with erythropoietic porphyria [Schneider-Yin et al., 2000]. Although biallelic mutations of *ATP8B1* were identified in progressive familial intrahepatic cholestasis [Klomp et al., 2004], heterozygous mutations were shown to cause intrahepatic cholestasis of pregnancy [Müllensbach et al., 2005]. Homozygous *MALT1* mutations were shown to result in immunodeficiency [Jabara et al., 2013].

NEDD4L is highly expressed in the brain. It is an ubiquitin protein ligase that regulates ion channel internalization and turnover in the central nervous system. The *NEDD4L* protein contains an N-terminal C2 domain that binds to Ca^{2+} , 2–4 WW protein-protein interacting domains, and a HECT ubiquitin ligase domain at the C-ter-

minal end [roix et al., 2016]. Heterozygous mice models were found hyperactive, had increased basal synaptic transmission, and an enhanced sensitivity to inflammatory pain. Mutations of *NEDD4L* are suggested to be involved in neuropsychological deficits in humans [Yanpallewar et al., 2016]. Heterozygous mutations were reported in patients with neurodevelopmental disorders. HECT domain mutations cause periventricular nodular heterotopia 7 (MIM#607201), which is characterized by periventricular nodular heterotopia, cleft palate and toe syndactyly, developmental delay, and seizures in some patients [roix et al., 2016; Kato et al., 2017; Elbracht et al., 2018]. On the other hand, WW domain mutations were shown to cause photosensitive generalized epilepsy [Dibbens et al., 2007].

The patients had rolandic epilepsy and several findings in a brain MRI: case 20 had an asymmetric dilatation of the lateral ventricle and case 21 had periventricular focal gliosis. Although missense mutations were reported to date, our cases are remarkable being the first with a deletion. According to the current literature, *NEDD4L* has a revealing gene function that is relevant to referral reason. Hence, loss on chromosome 18q21.31q21.32 (55090993_56556611) was interpreted as likely pathogenic. Heterozygous *NEDD4L* deletions should also be considered in patients with epilepsy.

Case 28 was also remarkable. The patient was a 5-month-old girl with microphthalmia and subtle dysmorphic features. A 13.506-Mb microduplication at chromosome Xq25q27.2, spanning the *SOX3* gene, was responsible for her phenotype. Mutations of *SOX3* were reported as causative for microphthalmia with mental retardation. Also gains and losses containing *SOX3* have been reported several times in variable phenotypes [Jel-sig et al., 2018]. Gain of *SOX3* probably contributed to the phenotype of the patient via favorably skewed X-in-activation.

Conclusion

This study redemonstrated that CMA is a first step diagnostic tool in patients with developmental disabilities and/or MCA. However, the underlying cause of the phenotype cannot be determined in almost 50% of the patients [Miller et al., 2010]. Therefore, new CMA platforms should be developed accordingly and new diseases causing regions are discovered. Besides, next-generation techniques are alternatives in unsolved cases. With development in technology, next-generation sequencing can also

provide CNV information, but this technique is in necessity of specific bioinformatic data. For this reasons, CMA probably remains the first step in diagnostic approaches.

CMA also allows the identification of new CNVs by its genome-wide design [Miller et al., 2010]. This study provided new cases with *NRXN3* and *NEDD4L* deletions and expands the genotype-phenotype correlations on developmental disabilities.

Statement of Ethics

This work was approved by the Institutional Ethics Committee. Informed written consent was obtained from the patient's parents or guardians.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

A.H.Ç. obtained the patient information, analyzed the data, designed the work, and wrote the manuscript. Ş.A. analyzed the data and contributed to the manuscript. All authors read and confirmed the final manuscript.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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