Chromosomal Microarray Analysis in Children with Unexplained Developmental Delay/Intellectual Disability

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Abstract

Keywords

- ▶ chromosomal microarray analysis
- ▶ developmental delay
- ▶ intellectual disability
- ▶ microdeletion/microduplication

Chromosomal microarray (CMA) analysis for discovery of copy number variants (CNVs) is now recommended as a first-line diagnostic tool in patients with unexplained developmental delay/intellectual disability (DD/ID) and autism spectrum disorders. In this study, we present the results of CMA analysis in patients with DD/ID. Of 210 patients, pathogenic CNVs were detected in 26 (12%) and variants of uncertain clinical significance in 36 (17%) children. The diagnosis of well-recognized genetic syndromes was achieved in 12 patients. CMA analysis revealed pathogenic de novo CNVs, such as 11p13 duplication with new clinical features. Our results support the utility of CMA as a routine diagnostic test for unexplained DD/ID.

Introduction

Intellectual disability (ID) is a heterogeneous group of disorders, manifested by impaired cognitive functioning and difficulties with adaptive behaviors at the expected age. The incidence of developmental delay/intellectual disability (DD/ID) is approximately 3% in general population.^{1–3}

Chromosomal microarray (CMA) is a useful test for patients with diagnoses of DD/ID and autism spectrum disorders (ASD) with unknown etiology. CMA for discovery of copy number variants (CNVs) is now recommended as a first-line diagnostic tool in patients with unexplained DD/ID and ASD.^{4,5} CMA provides a high-resolution genome-wide scan of genomic CNVs such as microdeletions and microduplications. The ability of generating high-resolution genomic data also leads to difficulties in determining CNVs' clinical significance. To overcome this challenge, investigating CNVs in databases such as "Database of Genomic Variants" (DGV) and "Database of genomic variation and Phenotype in Humans using Ensembl Resources" (DECIPHER)

in the literature helps clinicians to get opinions and comments of the previous authors' about that variant and provides us insight on the nature of the CNV.

Over the past years, the widespread use of CMA has identified numerous diseases causing CNVs in patients with unexplained DD/ID, leading to higher diagnostic yields of abnormalities with corresponding increases in clinical value of CMA testing.^{6,7} Herein, we report the application of CMA in patients with DD/ID and describe new patients with microdeletions and microduplication syndromes.

Materials and Methods

Patients

We conducted a retrospective study on children with DD/ID of unknown cause, between January 2015 and June 2017. DD/ID was diagnosed by a pediatric neurologist after the completion of clinical assessments. Written informed consents were received from all guardians of the patients. The approval of the local ethics committee was obtained.

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The patients who presented with DD/ID and tested by CMA were included in the study. Patient demographics, clinical data, cranial magnetic resonance imaging (MRI), and electroencephalography (EEG) and CMA results of patients were obtained from the medical records and reviewed.

For the origin of the detected CNVs, parental CMA results were used, if they were available.

Chromosomal Microarray

Genomic DNA was isolated from peripheral blood samples according to the manufacturers' protocols (Magpurix Blood DNA Extraction Kit, Zinexts Life Science Corp., New Taipei, Taiwan). CytoScan 750K Array Kit and CytoScan Optima Array Kit from Affymetrix (Thermo Fisher Scientific, MA, United States) were used, microarray kits were covering a total number of 750,436 and 315,608 markers respectively. All microarray data were analyzed with Chromosome Analysis Suite (ChAS) 3.1 from Affymetrix, using GRCh37/hg19 libraries. For the samples tested with CytoScan 750K Array, duplications smaller than 100kb/50 markers and deletions smaller than 100kb/25 markers were filtered out. For CytoScan Optima Array Kit no filtering implemented.

Interpretation of Detected Copy Number Variants

For the interpretation of CNVs, variants were first checked in the DGV. If the CNV was not interpreted as benign using the data in DGV, CNV analysis continued with research on the DECIPHER. In addition, the clinical findings of the patient were compared with the literature and databases such as Online Mendelian Inheritance in Man (OMIM), European Cytogenetics Association Register of Unbalanced Chromosome Aberrations (ECARUCA), ORPHANET, and the association between the variant and the phenotype was tried to be established.

Results

Chromosomal microarray was performed in 210 children (116 males and 94 females) with DD/ID. The size of CNVs ranged from 102 kb to 19.5 Mb. The detection rate for pathogenic CNVs was 12%. (26/210), and the variants of unknown significance (VOUS) rate was 17% (36/210).

Pathogenic Copy Number Variants

In 26 patients, 1 or 2 CNVs were considered as the probable cause of DD/ID. Among the 26 patients who had a pathogenic CNV, 5 patients had ASD, 2 patients had attention-deficit hyperactivity disorder (ADHD), and 7 patients had epilepsy. Cranial MRI was performed in 23 out of 26 patients (88%) and was normal in 12 of 23 (52%) patients. EEG was obtained in eight patients and was normal in three out of eight patients (37%). ► **Table 1** shows the summary of the patient characteristics and pathogenic CNV findings.

Variants of Unknown Significance

Variants of Unknown Significance were detected in 36 patients with DD/ID. Of 36 patients, 5 patients had ASD and 13 patients had epilepsy. Cranial MRI was performed in 32 out 36 patients (89%) and was normal in 20 of 32 (62%)

patients. EEG was obtained in 19 patients and was normal in 8 of 19 patients (42%). ► **Table 2** shows the summary of the patient characteristics and VOUS results.

Discussion

Chromosomal microarray detects CNVs in the whole human genome with a much higher resolution than G-banded chromosome analysis.^{4,8} In this study, we present the results of CMA in 210 children with DD/ID. Chromosome analysis does not have any advantage over CMA except the ability to detect balanced changes so we performed CMA as a first step test for our patients who had unexplained DD/ID without multiple congenital anomalies. In pathogenic CNVs group, only one patient presented with isolated ID, while there were seven patients presented with isolated ID in VOUS group. Our results showed that patients in the pathogenic CNVs group presented with more complex clinical features.

The diagnosis of well-recognized genetic syndromes was achieved in 12 patients. We report two patients with Angelman syndrome, two patients with DiGeorge syndrome, two patients with neurofibromatosis type 1 microduplication syndrome, one patient with holoprosencephaly type 2, one patient with lissencephaly type 1, one patient with Williams-Beuren syndrome, one patient with branchio-otorenal syndrome 1, one patient with Primrose syndrome, and one patient with Cri du Chat syndrome.

Patient 2 presented with severe mental retardation and epilepsy. A 12 Mb deletion was detected at 2q23 including *CACNB4* gene. Heterozygous mutations in *CACNB4* have been reported in individuals with idiopathic generalized epilepsy type 9 (OMIM 607682) and episodic ataxia type 5 (OMIM 613855). Cranial MRI of the patient revealed cerebellar atrophy. An EEG showed generalized spike-and-wave activity. A 7.9-Mb deletion on chromosome 2q37 was identified in patient 3 with features of DD/ID, facial dysmorphism, and epilepsy. 2q37 deletion syndrome is associated clinically with mild-to-moderate intellectual disability, short stature, brachydactyly, and dysmorphic facial features.⁹ The patient had similar features with previously reported case, but he had no brachydactyly.

The 3q29 duplication syndrome was identified in patient 5 with mild mental retardation and ASD. Individuals with 3q29 duplication syndrome have widely variable phenotypes including mild-to-severe mental retardation, epilepsy, and ASD.^{10,11} 5q14.3 deletion syndrome was detected in patient 7 involving the *MEF2C* gene. The patient presented with severe mental retardation, stereotypic movements, and epilepsy. Cranial MRI showed focal cortical dysplasia in right parietal lobe. An EEG revealed sharp-and-slow wave patterns in the right temporal lobe. Overexpression of *MEF2C* in mice cardiomyocytes has been considered as a possible cause of cardiomyopathy.¹² However, echocardiogram of this patient revealed normal myocardial function.

Patient 10 presented with DD/ID and facial dysmorphism, carrying a 19Mb duplication at 9p24 and a 10Mb duplication at 10q26. Both duplications could have contributed to the features of DD/ID and facial dysmorphism.

Table 1 Clinical and molecular features of patients with pathogenic copy number variations

Patient no.	Sex	Age (years)	Known microdeletion syndrome	CMA results	Size	Number of genes	Origin	Clinical features	MRI	EEG
1	f	5 months	Holoprosencephaly type 2	arr[hg19] 2p21 (43,921,488_45,247,770)x1	1.3 Mb	10	N/A	DD/ID, microcephaly, facial dysmorphism, diabetes insipidus	Semilobar holoprosencephaly	N/A
2	m	14	2q23 deletion syndrome	arr[hg19] 2q23.3q24.2 (151,596,595_163,643,407)x1	12 Mb	37	N/A	DD/ID, epilepsy	Cerebellar atrophy	Generalized spike-and-wave activity
3	m	11	2q37 deletion syndrome	arr[hg19] 2q37.1q37.3 (234,835,169_242,783,384)x1	7.9 Mb	44	dn	DD/ID, epilepsy, facial dysmorphism, short stature	N/A	N/A
4	m	7	Primrose syndrome	arr[hg19] 3q13.13q13.33 (110,222,795_121,364,236)x1	11.1 Mb	46	N/A	DD/ID, epilepsy, ASD, facial dysmorphism, macrocephaly	Partial agenesis of corpus callosum	Sharp-and-slow wave discharges in the right temporal lobe
5	f	9	3q29 duplication syndrome	arr[hg19] 3q29 (195,703,615_197,356,334)x3	1.6 Mb	14	mat	DD/ID, ASD	Normal	N/A
6	m	7	Cri du Chat syndrome	arr[hg19] 5p15.33p14.3 (113,576_19,028,318)x1 8q24.12q24.3 (121,521,226_146,295,771)x3	19 Mb 24.8 Mb	45 99	N/A	DD/ID, microcephaly, facial dysmorphism	Normal	N/A
7	f	4	5q14.3 deletion syndrome	arr[hg19] 5q14.3 (87,976,675_88,654,001)x1	0.6 Mb	1	dn	DD/ID, epilepsy, stereotypic movements	Focal cortical dysplasia in right parietal lobe	Sharp-and-slow wave discharges in the right temporal lobe
8	m	5	Williams-Beuren syndrome	arr[hg19] 7q11.23 (72,608,514_74,090,648)x3	1.4 Mb	23	N/A	DD/ID, ASD	N/A	N/A
9	m	3	Branchiootorenal syndrome-1	arr[hg19] 8q13.2q13.3 (69,836,398_72,533,772)x1	2.7 Mb	6	dn	DD/ID, facial dysmorphism, bilateral hearing loss	Normal	N/A
10	f	8		arr[hg19] 9p24.3p22.1 (203,861_19,705,636)x3 10q26.13q26.3 (125,145,920_135,427,143)x3	19 Mb 10 Mb	51 35	N/A	DD/ID, facial dysmorphism	Cerebral atrophy	N/A
11	m	3		arr[hg19] 11p13p12 (31,866,288_41,160,136)x1	9.3 Mb	31	dn	DD/ID	Normal	N/A
12	f	1		arr[hg19] 14q32.31_q32.33 (103,029,072_107,285,437)x1	4.2 Mb	31	dn	DD/ID, microcephaly, facial dysmorphism, congenital heart disease	Normal	N/A
13	m	9 months	Angelman syndrome	arr[hg19] 15q11.2q13.1 (22,770,421_28,934,990)x1	6.2 Mb	23	N/A	DD/ID, facial dysmorphism	N/A	N/A
14	m	1	Angelman syndrome	arr[hg19] 15q11.2q13.1 (22,770,421_28,828,168)x1	6.1 Mb	23	N/A	DD/ID, facial dysmorphism	Posterior periventricular T2 hyperintensities	High amplitude rhythmic 2-3 Hz activity over the frontal regions
15	m	11	15q11.2 duplication syndrome	arr[hg19] 15q11.2q13.1 (22,770,421_28,516,084)x3	5.7 Mb	23	N/A	DD/ID, facial dysmorphism	Normal	N/A
16	m	7	15q11.2 duplication syndrome /1q43 microdeletion syndrome	arr[hg19] 15q13.2q13.3 (31,073,668_32,914,239)x3 1q43q44 (243,239,138_244,406,046)x1	1.8 Mb 1.1 Mb	7 4	dn	DD/ID, ASD	Thinned corpus callosum	N/A

(Continued)

Table 1 (Continued)

Patient no.	Sex	Age (years)	Known microdeletion syndrome	CMA results	Size	Number of genes	Origin	Clinical features	MRI	EEG
17	m	10	16p11.2 duplication syndrome	arr[hg19] 16p11.2 (29,622,813_30,165,725)x3	0.5 Mb	18	N/A	DD/ID, ADHD	Normal	N/A
18	m	12	16p11.2 deletion syndrome	arr[hg19] 16p11.2 (29,567,295_30,226,930)x1	0.6 Mb	20	N/A	DD/ID, ASD, epilepsy, obesity	Normal	Normal
19	f	1		arr[hg19] 16q21q23.2 (64,871,526_81,101,869)x3	16.2Mb	123	dn	DD/ID, facial dysmorphism, congenital heart disease	Normal	N/A
20	f	7	Lissencephaly type 1	arr[hg19] 17p13.3 (2,426,376_2,528,505)x1	0.1 Mb	1	dn	DD/ID, microcephaly, facial dysmorphism, bilateral sensorineural hearing loss	Agensis of corpus callosum and lissencephaly	N/A
21	f	3	Neurofibromatosis type 1 microduplication syndrome	arr[hg19] 17q11.2 (29,095,060_30,243,583)x1	1.1 Mb	10	N/A	DD/ID, facial dysmorphism, café au lait spots	Normal	N/A
22	m	2	Neurofibromatosis type 1 microduplication syndrome	arr[hg19] 17q11.2 (28,931,870_30,380,040)x1	1.4 Mb	11	dn	DD/ID, facial dysmorphism	T2 hyperintensities	N/A
23	f	10	18q deletion syndrome	arr[hg19] 18q21.2q23 (51,544,371_78,014,123)x1	26.4Mb	48	N/A	DD/ID, microcephaly, facial dysmorphism, epilepsy	Centrum semiovale T2 hyperintensities	Normal
24	f	1.5	22q11.2 duplication syndrome	arr[hg19] 22q11.21 (18,917,030_21,804,886)x3	2.8Mb	41	N/A	DD/ID, bilateral ptosis	Normal	Normal
25	m	8	DiGeorge syndrome	arr[hg19] 22q11.21 (18,972,449_20,716,877)x1	1.7 Mb	25	N/A	DD/ID, ADHD, facial dysmorphism, epilepsy, congenital heart disease	Normal	Generalized spike-and-wave activity
26	m	2	DiGeorge syndrome	arr[hg19] 22q11.21 (18,917,030_21,465,662)x1	2.5 Mb	38	N/A	DD/ID, facial dysmorphism, congenital heart disease	Cerebral and cerebellar atrophy	N/A

Abbreviations: ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; DD/ID, developmental delay/intellectual disability; dn, de novo; EEG, electroencephalography; f, female; m, male; mat, maternal; MRI, magnetic resonance imaging; N/A, not applicable.

Table 2 Clinical and molecular features of patients with variants of unknown significance

Patient no.	sex	Age (years)	CMA results	size	Number of genes	origin	Clinical features	MRI	EEG
27	m	4	arr[hg19] 1p35.2 (30,347,455_31,231,254)x3	0.8 Mb	2	pat	DD/ID, short stature	Normal	N/A
28	f	5	arr[hg19] 1q31.3 (196,741,353_197,011,636)x1	0.2 Mb	6	dn	DD/ID, epilepsy	Thinned corpus callosum and encephalomalacia in right temporoparietal lobe	Multifocal epileptiform discharges
29	f	5	arr[hg19] 1q43 (238,681,812_238,875,754)x1	0.1 Mb	–	N/A	DD/ID, microcephaly, facial dysmorphism	Normal	Generalized spike-and-wave activity
30	m	2	arr[hg19] 2p11.2 (83,983,334_84,216,361)x1/ Xp21.32 (91,890,834_92,290,604)x2	0.2 Mb/ 0.3 Mb	–/–	pat /dn	DD/ID	Cerebral atrophy	Normal
31	f	12	arr[hg19] 2q13 (110,458,409_111,388,468)x3/ Xq27.2 (140,313,608_140,563,347)x1	0.9 Mb/ 0.2 Mb	3	N/A	DD/ID, epilepsy	Normal	Normal
32	f	11	arr[hg19] 2q21.3q22.1 (136,719,172_142,015,586)x1	5.2 Mb	5	mat	DD/ID, facial dysmorphism, epilepsy	Normal	Sharp-and-slow wave discharges in the right temporal lobe
33	m	5	arr[hg19] 2q22.1 (137,111,384_137,314,018)x1/ Yq11.221 (15,711,576_16,211,527)x2	0.2 Mb/ 0.4 Mb	–/2	dn/pat	DD/ID, ASD	Normal	N/A
34	m	3	arr[hg19] 2q32.3 (194,592,290_194,866,146)x1	0.2 Mb	–	N/A	DD/ID, microcephaly, congenital heart disease	Cerebral atrophy	Normal
35	f	2	arr[hg19] 2q33.1 (198,727,625_198,940,251)x1	0.2 Mb	1	dn	DD/ID, microcephaly	Normal	N/A
36	m	5 months	arr[hg19] 4p13 (44,311,020_44,514,468)x1	0.2 Mb	–	N/A	DD/ID, epilepsy, macrocephaly	Dandy walker malformation	Normal
37	f	8	arr[hg19] 4q13.2 (69,320,945_69,615,068)x1	0.2 Mb	3	dn	DD/ID, facial dysmorphism, microcephaly, epilepsy	Cerebral atrophy and agenesis of corpus callosum	Multifocal epileptiform discharges
38	m	3	arr[hg19] 4q28.3 (133,589,730_133,884,885)x1	0.2 Mb	–	pat	DD/ID, ASD	Normal	N/A
39	f	2	arr[hg19] 5p12q11.1 (45,323,017_49,497,236)x3	4.1 Mb	1	mat	DD/ID	Normal	N/A
40	m	5	arr[hg19] 5p14.3 (20,896,606_21,409,572)x1	0.5 Mb	–	N/A	DD/ID, ASD	N/A	N/A
41	m	4	arr[hg19] 5q23.1 (120,208,191_120,536,562)x3	0.3 Mb	–	mat	DD/ID, facial dysmorphism, microcephaly, epilepsy	Pachygyria	Multifocal epileptiform discharges
42	m	9	arr[hg19] 6q14.1 (78,797,453_79,075,086)x1	0.2 Mb	–	N/A	DD/ID	Periventricular T2 hyperintensities	N/A

(Continued)

Table 2 (Continued)

Patient no.	sex	Age (years)	CMA results	size	Number of genes	origin	Clinical features	MRI	EEG
43	m	8	arr[hg19]7p14.3 (33,942,135_34,448,659)x3/1p11.12 (49,623,489_50,438,533)x3	0.5 Mb/ 0.8 Mb	2/-	pat/pat	DD/ID, facial dysmorphism, epilepsy	Normal	Generalized spike-and-wave activity
44	m	4	arr[hg19]7q11.21 (63,816,395_64,225,274)x0	0.4 Mb	1	mat + pat	DD/ID	Normal	Normal
45	m	2	arr[hg19]7q11.21 (63,816,395_64,225,274)x0	0.4 Mb	1	mat + pat	DD/ID, facial dysmorphism	Normal	Normal
46	m	15	arr[hg19]9p21.1 (28,442,769_29,119,535)x1	0.4 Mb	1	N/A	DD/ID, ASD	N/A	N/A
47	m	1	arr[hg19]10q21.3 (68,372,246_68,559,354)x1/arr[hg19]11q22.3 (107,198,838_107,419,341)x1	0.1 Mb/ 0.2 Mb	1/1	dn	DD/ID, facial dysmorphism	N/A	N/A
48	m	6	arr[hg19]13q31.2q31.3 (89,716,747_90,731,362)x1/arr[hg19]Xq26.3 (134,723,876_134,931,657)x2	1 Mb/ 0.2 Mb	-/4	mat/dn	DD/ID, facial dysmorphism	Normal	N/A
49	f	2	arr[hg19]15q11.2 (22,770,421_23,065,667)3	0.2 Mb	4	mat	DD/ID, facial dysmorphism	Normal	N/A
50	m	9	arr[hg19]15q11.2 (22,770,421_23,276,605)x1	0.5 Mb	4	N/A	DD/ID, epilepsy	Normal	Sharp-and-slow wave discharges in the left frontal lobe
51	m	5	arr[hg19]15q11.2 (22,770,421_23,276,605)x1	0.5 Mb	4	pat	DD/ID, facial dysmorphism, microcephaly	N/A	N/A
52	f	8	arr[hg19]15q13.3 (32,106,650_32,421,780)x4/arr[hg19]15q14 (34,621,751_35,032,856)x4	0.3 Mb/ 0.4 Mb	1/5	N/A	DD/ID, facial dysmorphism, epilepsy	Normal	Sharp-and-slow wave discharges in the right temporal lobe
53	f	14	arr[hg19]18p11.31 (6,592,675_6,834,651)x1	0.2 Mb	1	N/A	DD/ID, bilateral sensorineural hearing	Normal	N/A
54	m	4	arr[hg19]21q22.3 (47,895,306_48,097,372)x1	0.2 Mb	3	N/A	DD/ID, ASD	Normal	N/A
55	f	1	arr[hg19]xp22.1 (77,402,625_78,514,711)x3	1.1 Mb	3	pat	DD/ID, bilateral sensorineural hearing, epilepsy	Normal	Normal
56	f	1	arr[hg19]Xq28 (153,887,669_154,112,020)x3	0.2 Mb	4	dn	DD/ID, facial dysmorphism, microcephaly, epilepsy, brown patches on body	Thinned corpus callosum and unspecific leucoencephalopathy	Generalized spike-and-wave activity
57	m	3	arr[hg19]Xp22.33orYp11.32 (168,546_582,264o-r118,546_532,264)x4	0.4 Mb	2	dn	DD/ID facial dysmorphism, microcephaly, epilepsy	Cerebral and cerebellar atrophy	Generalized spike-and-wave activity in right hemisphere

Table 2 (Continued)

Patient no.	sex	Age (years)	CMA results	size	Number of genes	origin	Clinical features	MRI	EEG
58	f	6	arr[hg19] Xp22.33 (168,546_1,734,3979 × 3	1.5 Mb	19	mat	DD/ID	Normal	N/A
59	m	10 months	arr[hg19] Xq21.32 (91,890,834–92,434,453)×2	0.5 Mb	–	N/A	DD/ID facial dysmorphism, microcephaly, congenital heart disease	Thinned corpus callosum	N/A
60	m	5 months	arr[hg19] Yq11.221 (15,711,576_16,079,158)×2	0.3 Mb	1	N/A	DD/ID	Agnesis of corpus callosum	N/A
61	m	5	arr[hg19] Yp11.2(3,305,862–3,946,523)×2	0.6 Mb	1	N/A	DD/ID	Hypomyelination	Normal
62	m	2	arr[hg19] Yq11.23 (26,592,403_28,423,925)×0	1.8 Mb	4	N/A	DD/ID, epilepsy	Normal	Multifocal epileptiform discharges

Abbreviations: ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; DD/ID, developmental delay/intellectual disability; dn, de novo; EEG, electroencephalography; f, female; m, male; mat, maternal; MRI, magnetic resonance imaging; N/A, not applicable; pat, paternal.

Patient 11 presented with DD/ID. Cranial MRI was normal. He was found to carry a de novo 9.3 Mb deletion at 11p13. In the literature, such deletions have been commonly associated with the Wilms tumor-aniridia-genital anomalies-retardation (WAGR) syndrome. Candidate genes were suggested for the psychomotor retardation *SLC1A2*, *PRRG4*, and *BDNF* in WAGR syndrome.^{13,14} Our patient had mental retardation; however, he had no other clinical findings of WAGR syndrome.

Patient 12 presented with DD/ID and facial dysmorphism. Cranial MRI was normal. The echocardiogram demonstrated atrial septal defect. The patient showed a de novo 4.2 Mb deletion at 14q32.33. In the literature, common clinical characteristics of terminal 14q32.33 deletions include mental disability, facial malformation, hypotonia, congenital heart defects, genitourinary malformations, and ocular coloboma.¹⁵ Although the patient showed similar features of terminal 14q32.33 as the previously reported case, she had no ocular coloboma or urogenital anomalies.

Patient 15 presented with severe mental retardation and facial dysmorphism. The patient showed a 5.7 Mb duplication at 15q11.2. 15q11.2 duplication syndrome is associated with ASD, mental retardation, seizures, DDs, and behavioral problems.^{16,17}

Patient 16 was diagnosed with mental retardation and ASD. Cranial MRI showed thinned corpus callosum. A de novo 1.8 Mb duplication of 15q13 was detected in addition to a de novo 1.1 Mb deletion of 1q43 including *ZBTB18* gene. 15q13.3 duplication syndrome is associated with a highly variable phenotype, even among members of the families segregating the same deletion. Mild-to-moderate mental retardation and behavioral problems have been commonly reported.¹⁸ Microdeletions of 1q43 encompassing the candidate gene *ZBTB18* are associated with moderate-to-severe mental retardation and abnormalities of the corpus callosum.¹⁹ The patient's reported phenotype overlapped with clinical features seen in both the 15q13 duplication syndrome and the 1q43 microdeletion syndrome.

Patient 17 had mild mental retardation and ADHD. 16p11.2 duplication syndrome was detected including *KIF22*, *PRRT2*, *ALDOA*, and *TBX6* genes in this patient. Patient 18 was diagnosed with mild mental retardation, ASD, and epilepsy. CMA test revealed 16p11.2 deletion syndrome. Deletions and duplications at the 16p11.2 locus (29.5–30.1 Mb) are associated with neurodevelopmental disorders, including ASD, ID, ADHD, and seizures.^{20,21} Patient 19 had coarctation of aorta, facial dysmorphism, and DD/ID. Cranial MRI was normal. The patient was found to carry a de novo 16.2 Mb duplication at 16q21 including 123 *OMIM* genes.

Chromosomal microarray revealed a 26.4 Mb deletion at 18q21 in patient 23 with mental retardation, facial dysmorphism, and epilepsy. Clinical signs of the patient were consistent with the phenotype associated with 18q deletion syndrome, although she had no cardiac anomalies.²² Patient 24 showed delayed psychomotor development with poor speech ability. No dysmorphic features were noted but the patient had bilateral ptosis. CMA test revealed 22q11.2 duplication syndrome. 22q11.2 duplication syndrome is

associated with mild-to-severe mental retardation, ocular anomalies, and features similar to the DiGeorge Syndrome/Velo-cardio-facial-syndrome, including heart defects.^{23,24} However, echocardiogram of the patient was normal.

Patient 35 presented with DD/ID and microcephaly. Cranial MRI was normal. The patient was found to carry a de novo 0.2 Mb deletion at 2q33, which encompasses the part of 2q33 deletion syndrome. Rainger et al reported a 33-year-old man with severe ID, aggressive behavior, and dysmorphic features. They identified a de novo heterozygous t(2;3)(q33.1;q26.33) translocation with the breakpoint at 2q33.1 within the *PLCL1-SATB2* gene desert.²⁵ The deletion in our patient included *PLCL1* gene, and thus we thought that this deletion could contributed to her phenotype.

Patient 56 presented with DD/ID, facial dysmorphism, microcephaly, epilepsy, and brown patches on body. CMA revealed a de novo 0.2 Mb duplication at Xq28 which is close to *IKBKG* gene. *IKBKG* gene mutations are associated with incontinentia pigmenti. Incontinentia pigmenti is a disorder that affects the skin, eyes, and central nervous system.²⁶ The duplication at Xq28 is close to *IKBKG* gene and therefore it may affect expression levels of *IKBKG* gene. Thus, it could be associated with the patient's phenotype.

CMA also detects many CNVs of VOUS, which can be difficult to interpret. In nine patients, CMA results contain no *OMIM* gene, so the CNV is likely to have a benign nature. However, we interpreted this result as VOUS because the duplicated or deleted region has not been reported in databases including DGV, DECIPHER, and OMIM. Efforts to better identify the relevance of VOUS in these disorders have critical importance to understanding disease mechanisms and eventually help to determine the most appropriate treatment options.

Chromosomal microarray yields detection of pathogenic and VOUS results, which have clinical utility for the management of patients with neurodevelopmental disorders. Moreover, the identification of chromosomal abnormalities allows informed decisions about future pregnancies for the parents. Our study shows that CMA helps to expand the clinical and molecular spectrum of microdeletions/microduplications syndromes. Our results support the necessity of CMA as a routine diagnostic test for unexplained DD/ID.

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Conflict of Interest

None declared.

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