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Chromosomal Microarray Analysis as a First-Tier Clinical Diagnostic Test in Patients With Developmental Delay/Intellectual Disability, Autism Spectrum Disorders, and Multiple Congenital Anomalies: A Prospective Multicenter Study in Korea

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Background: To validate the clinical application of chromosomal microarray analysis (CMA) as a first-tier clinical diagnostic test and to determine the impact of CMA results on patient clinical management, we conducted a multicenter prospective study in Korean patients diagnosed as having developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), and multiple congenital anomalies (MCA).

Methods: We performed both CMA and G-banding cytogenetics as the first-tier tests in 617 patients. To determine whether the CMA results directly influenced treatment recommendations, the referring clinicians were asked to complete a 39-item questionnaire for each patient separately after receiving the CMA results.

Results: A total of 122 patients (19.8%) had abnormal CMA results, with either pathogenic variants (N=65) or variants of possible significance (VPS, N=57). Thirty-five well-known diseases were detected: 16p11.2 microdeletion syndrome was the most common, followed by Prader–Willi syndrome, 15q11-q13 duplication, Down syndrome, and Duchenne muscular dystrophy. Variants of unknown significance (VUS) were discovered in 51 patients (8.3%). VUS of genes putatively associated with developmental disorders were found in five patients: *IMMP2L* deletion, *PTCH1* duplication, and *ATRNL1* deletion. CMA results influenced clinical management, such as imaging studies, specialist referral, and laboratory testing in 71.4% of patients overall, and in 86.0%, 83.3%, 75.0%, and 67.3% of patients with VPS, pathogenic variants, VUS, and benign variants, respectively.

Conclusions: Clinical application of CMA as a first-tier test improves diagnostic yields and the quality of clinical management in patients with DD/ID, ASD, and MCA.

Key Words: Chromosomal microarray analysis, Pathogenic, Variant of possible significance, Variant of unknown significance, Benign, Clinical management, Developmental delay, Intellectual disability, Autism spectrum disorders, Multiple congenital anomalies

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INTRODUCTION

Copy number variations (CNVs) have become increasingly recognized as significant contributors to human diseases [1], largely owing to technical progress of genome-wide analysis. Chromosomal microarray analysis (CMA) is a powerful tool for the genome-wide detection of invisible small chromosomal deletions or duplications.

In 2010, CMA was recommended as a first-tier diagnostic tool for patients with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), and multiple congenital anomalies (MCA) [2, 3]. CMA results have shown perfect concordance with results from FISH or multiplex ligation-dependent probe amplification (MLPA), and provide a much higher diagnostic yield than traditional karyotyping (15–20% vs 3%) [2, 4].

However, it is not always clear if and how physicians consider genomic medicine for patient care, which is another important issue with regard to the implementation of new genetic tests in routine clinical care. The Analytic validity, Clinical validity, Clinical utility and associated Ethical, legal and social implications (ACCE) model provides a framework for evaluating the clinical utility of emerging genetic tests for clinical practice [5]. Recently, a few proof-of-concept studies on how CMA results affect patient management demonstrated the overall clinical utility of CMA [6-8]. However, most of the research conducted in this field to date has been descriptive, using data from retrospective chart reviews. Therefore, we conducted a multicenter prospective study to assess the clinical application of CMA as the first-tier diagnostic test in Korean patients with DD/ID, ASD, and MCA, as well as the impact of CMA results on patient clinical management.

METHODS

Study population

A total of 712 individuals (617 patients and 95 family members) were referred from six Korean hospitals (Seoul St. Mary's Hospital and Yeouido St. Mary's Hospital in Seoul, Incheon St. Mary's Hospital and Inha University Hospital in Incheon, St. Vincent's Hospital in Suwon, and Daejeon St. Mary's Hospital in Daejeon) between February 2013 and January 2017 after providing informed consent. Patients were referred by physicians as part of clinical assessment for DD, ID, ASD, MCA, or a combination of those features with unexplained etiology. We performed both CMA and G-banding cytogenetics as the first-tier cytogenetic diagnostic tests. When available, the origin of any imbalance was determined through analysis of parental samples. The study protocol was approved by the Institutional Review Board of Seoul St Mary's Hospital, The Catholic University of Korea (KC17TESI0517).

Study design

The referring physicians were asked to complete a questionnaire to determine whether the CMA results had directly influenced their treatment recommendations. The questionnaire items focused on the clinicians' opinions of the following criteria: (1) demographic details and clinical features, such as neurodevelopmental disorders (DD, learning disability, seizures, ID, speech delay, and ASD), congenital anomalies, dysmorphic features, abnormal growth (failure to thrive and short stature) and hypotonia; (2) clinical management prompted by CMA results, including pharmacological management (indication and contraindication for drug treatment), specialist referral, diagnostic imaging studies, and laboratory tests. Developmental surveillance (i.e., ongoing monitoring of development, identification of risk factors, and elicitation of parental concerns) was not included as part of direct clinical management [9, 10]. Clinicians completed the questionnaire for each patient separately after receiving the CMA results. Follow-up periods ranged from six to 53 months. We did not include genetic counseling, confirmatory MLPA/FISH, or parental testing results performed to clarify the inheritance of CNVs as part of clinical management because these practices should be standard after abnormal CMA results.

Banding cytogenetics

Banding cytogenetics was performed on G-banded metaphase chromosomes of cultured peripheral blood lymphocytes using routine techniques. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016 [11].

Array comparative genomic hybridization and interpretation

Genomic DNA was extracted from a whole blood sample collected in an EDTA tube. Comparative genomic hybridization (CGH) array analysis was performed with the SurePrint G3 Human CGH Microarray 8X 60K kit (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions. Scanned images were quantified using Agilent Feature Extraction software (v. 10.0). Resulting data were imported into Agilent Genomic Workbench 7.0.4.0 software for visualization. CNVs were detected using the Aberration Detection Method-2 (ADM-2) algorithm. Genomic positions were defined according to the human reference genome hg19/GRCh37.

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CNVs were classified into four groups: pathogenic, variants of possible significance (VPS), variants of unknown significance (VUS), and benign [2, 12]. We used the DGV, DECIPHER, ClinGen, Online Mendelian Inheritance in Man (OMIM), and dbVar databases, and peer-reviewed literature to determine clinically significant CNVs. Pathogenic variants or VPS were considered abnormal. When available, the known deletion/duplication found via CMA was confirmed by FISH or MLPA. The term "VUS" was used when the imbalance was >200 kb for deletions and >500 kb for duplications involving multiple genes that had never or rarely been reported in normal population controls or candidate genes for an inherited disease, but the significance of the imbalance could not be determined based on available knowledge or family studies. CNVs were considered benign when reported as a normal variant in healthy controls or detected in ≥1% of our patient population.

Statistical analysis

Differences in the frequency of clinical features (DD, learning disability, seizures, ID, speech delay, ASD, congenital anomalies, dysmorphic features, failure to thrive, short stature, and hypotonia) and management (pharmacological management, specialist referral, imaging studies, and laboratory testing) between groups were investigated using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. Statistical analyses were performed using SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). $P<0.05$ (two-sided) indicated statistical significance.

RESULTS

Characterization of detected CNVs

Abnormal CNVs were detected in 122 of the 617 patients (pathogenic, N=65; VPS, N=57), representing overall diagnostic yield of 19.8%. VUS, excluding cases with abnormal CNVs, were found in 51 patients (8.3%), while benign CNVs were found in 444 patients (72.0%) (Supplemental Data Fig. S1). The diagnostic yields of CMA were higher than those obtained with banding cytogenetics (38/617, 6.2%, $P<0.001$). Aneuploidy accounted for 8.2% (10/122) of cases with abnormal results. Three patients showed an abnormal karyotype with normal CMA results, including one patient each with balanced translocation, low-level mosaicism, and marker chromosome. No incidental CNV results involving cancer predisposing genes were detected in patients with abnormal CNVs.

Altogether, 65 patients (10.5%) showed pathogenic variants associated with well-known genetic diseases. Rearrangements in 15q11-q13, 16p11.2, 1q21.1, 7q11.23, and 22q11.2 were

Table 1. Classification of pathogenic CMA results identified in 65 patients with pathogenic variants

Syndrome/Disease	OMIM No.	Patients (N)
1p36 deletion syndrome	607872	1
1q21.1 deletion syndrome	612474	2
1q21.1 duplication syndrome	612475	2
2q37 microdeletion syndrome	600430	1
3q29 deletion syndrome	609425	1
Sotos syndrome	117550	1
Reversed Sotos syndrome	-	1
Williams syndrome	194050	2
7q11.23 duplication syndrome	609757	2
8q21.11 deletion syndrome	614230	1
Warkany syndrome 2	-	1
10q22.3-q23.2 deletion syndrome	612242	1
Jacobsen syndrome	147791	1
Prader-willi syndrome	176270	5
15q11-q13 duplication syndrome	608636	5
15q13.3 microdeletion	612001	1
15q24 microdeletion syndrome	-	1
16p11.2 microdeletion	611913	6
16p11.2 duplication syndrome	614671	2
16p12.2 microdeletion	-	1
16p13.11 microduplication syndrome	-	3
Potocki-Lupski syndrome	610883	2
Smith-Magenis syndrome	182290	1
Charcot-Marie-Tooth disease	118220	1
17p13.3 duplication syndrome	613215	2
17q12 duplication syndrome	614526	1
Down syndrome	190685	4
DiGeorge syndrome	188400	2
22q11.2 duplication	608363	2
Phelan-McDermid deletion syndrome	606232	1
DMD	310200	3
Sex chromosome disorders		5
Turner syndrome	-	1
Triple X syndrome	-	1
Klinefelter syndrome	-	1
47, XYY syndrome	-	2

Abbreviations: CMA, chromosomal microarray analysis; OMIM, Online Mendelian Inheritance in Man; DMD, Duchenne muscular dystrophy.

Table 2. CMA results identified in 57 patients with variant of possible significance

Case	ISCN description	Imbalance	Size (Mb)
24m/F	arr[GRCh37] 1q22q24.1(156132786_166047765)x3	dup	9.9
5/M	arr[GRCh37] 1q25.2q31.3(177898011-198465186)x1	del	21
12/F	arr[GRCh37] 1q43q44(240039421_249212668)x1 mat, 18q21.31q23(54037167_77982126)x3 mat	del/dup*	9.2/23.9
4/M	arr[GRCh37] 2p25.3p25.1(42444_7304259)x3	dup	7.3
29m/M	arr[GRCh37] 2q22.1q22.3(142036895_145533609)x1	del	3.4
10/F	arr[GRCh37] 2q11.1q12.3(95529039_108083956)x3 mat, 18p11.32p11.31(142096_5853122)x1 dn	dup/del	12.6/5.7
neo/M	arr[GRCh37] 2q32.1(186763813_188960123)x3 dn	dup	2.2
3/M	arr[GRCh37] 3p26.3(270649_1125759)x3	dup	0.855
11m/F	arr[GRCh37] 3p26.3p26.1(93949_4994502)x1, 15q25.1q26.3(80190103_102465355)x3	del/dup	4.9/22
11m/M	arr[GRCh37] 3p11.2p13(76026268_90254062)x1	del	14.2
1m/F	arr[GRCh37] 4q35.1q35.2(185274461_190469337)x1 pat, 10p15.3p11.23(148206_29975521)x3 pat	del/dup*	5.2/30
35m/M	arr[GRCh37] 5q13.3(73470360_74032634)x1	del	0.562
14m/F	arr[GRCh37] 5q21.3(106716799_108175671)x3	dup	1.4
9/M	arr[GRCh37] 5q31.2(137260366_138206885)x3	dup	0.946
3/M	arr[GRCh37] 5q35.2(175437847_176491972)x1	del	1.1
5m/M	arr[GRCh37] 6p25.3p25.2(170426_2794740)x1 mat	del	2.6
26m/M	arr[GRCh37] 6p25.3p25.1(170426_5431448)x1	del	5.3
4/F	arr[GRCh37] 6q14.3q15(86185546_88051322)x1	del	1.9
9m/F	arr[GRCh37] 6q26q27(163357909_170890108)x1	del	7.5
5/F	arr[GRCh37] 6q27(166754981_167569353)x1	del	0.814
19m/F	arr[GRCh37] 6q12(66205374_67257639)x1 pat	del	1.1
6/M	arr[GRCh37] 7q36.1q36.3(149128443_159088636)x3 dn, 9p24.3(271257_2183334)x1 dn	dup/del	10/1.9
5/F	arr[GRCh37] 7q36.2q36.3(153933437_158909738)x1	del	5
20m/F	arr[GRCh37] 8p23.3p23.1(221611_6914076)x1, 8p23.1p12(12583259_29936174)x3	del/dup	6.7/17.4
18m/M	arr[GRCh37] 8p23.3p23.1(221611_7753583)x1 dn, 12p13.33p13.31(230421_8238072)x3 dn	del/dup	7.5/8.0
8m/M	arr[GRCh37] 8q21.11q21.13(76069471_81532974)x1 dn	del	5.5
42/F	arr[GRCh37] 8q23(113498500_114173066)x1, 12p13.33p13.32(230421_3394129)x1	del/del	0.674/3.2
23m/M	arr[GRCh37] 9p24.3p13.3(271257_35163255)x3	dup	35
15m/M	arr[GRCh37] 9p13.3p13.1(33414184_39156954)x1 dn	del	5.7
18m/F	arr[GRCh37] 9q33.2q33.3(124664562_127176303)x1 dn	del	2.5
9m/M	arr[GRCh37] 10p15.3p15.1(193492_6135095)x3	dup	5.9
4/M	arr[GRCh37] 11p14.3p14.1(24063998_30323839)x1	del	6.3
16/F	arr[GRCh37] 11q24.2q24.3(126830381_128391970)x3, 11q24.3q25(106396480_106513022)x1	dup/del	1.6/6.4
12/F	arr[GRCh37] 12p13.33p13.32(230421_3394129)x1	del	3.2
2m/M	arr[GRCh37] 12p13.33p11.1(450479_34345585)x2-3	dup	34
26/M	arr[GRCh37] 12p13.33p11.23(230421_27768451)x3, 18p11.32(142096_1038964)x1	dup/del	27.5/0.897
3/M	arr[GRCh37] 13q12.3(30656355_31905182)x3	dup	1.2
4/M	arr[GRCh37] 13q31.1q31.2(85888171_87980615)x1 mat	del	2.1
4/F	arr[GRCh37] 13q33.3q34(109683987_115059020)x1	del	5.4
6m/M	arr[GRCh37] 14q13.2q13.3(35316655_37777710)x1 dn	del	2.5

(*Continued to the next page*)

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Table 2. Continued

Case	ISCN description	Imbalance	Size (Mb)
4/F	arr[GRCh37] 14q13.3q21.1(36747497_42447650)x1 mat	del	5.7
17m/M	arr[GRCh37] 14q13.2q21.3(35316655-48123507)x1 dn	del	12.8
16/F	arr[GRCh37] 14q32.11q32.33(90043558_107258824)x3	dup	17
23/M	arr[GRCh37] 14q32.11q32.33(90017463_107240869)x3	dup	17.2
16m/F	arr[GRCh37] 16q21q23.1(62705632_75960327)x3	dup	13.3
4/M	arr[GRCh37] 16q23.1(74176768_74966776)x1 mat	del	0.790
22m/M	arr[GRCh37] 18p11.32p11.22(142096_8536828)x1	del	8.3
18m/M	arr[GRCh37] 18p11.32p11.23(198111_7290232)x1 mat	del	7.1
5/M	arr[GRCh37] 20p13(439387_1227535)x3	dup	0.788
20m/M	arr[GRCh37] 20q13.33(61986322_62382463)x1	del	0.396
13/F	arr[GRCh37] 21q11.2q21.3(15170361_29447105)x1	del	14
22m/M	arr[GRCh37] 21q21.1(20090068_22116178)x1	del	2
4m/F	arr[GRCh37] Xp22.33(154062_1464218)x3 dn	dup	1.3
6/F	arr[GRCh37] Xp22.33p22.2(61091_10125133)x1	del	10
15/F	arr[GRCh37] Xp22.31(6552712_8115153)x1	del	1.6
6/F	arr[GRCh37] Xp11.4p.11.3(41306936_45980483)x1	del	4.7
11m/M	arr[GRCh37] Xq27.1q27.3(138231171_142763942)x0	del	4.5

*Two patients had a concurrent deletion and duplication in two different chromosomal regions inherited from parents with a balanced translocation.
Abbreviations: ISCN, International System for Human Cytogenetic Nomenclature; m, months; M, Male; F, Female; neo, neonate; CMA, chromosomal microarray analysis; Mb, megabase; mat, maternal origin; dn, de novo; pat, paternal origin; del, deletion; dup, duplication.

frequently found (Table 1 and Supplemental Data Fig. S2A). Although cancer was not present at diagnosis, four patients were diagnosed as having syndromes in which cancer is a reported feature (Sotos, Warkany, and DiGeorge syndromes). The 67 aberrations classified as VPS, detected in 57 patients, did not overlap with the CNVs previously identified to be related to known syndromes, but they were large in size and found in gene-rich areas, implicating their contribution to the abnormal phenotype (Table 2 and Supplemental Data Fig. S2B). VPS were mutually exclusive except for two siblings with a 14q32.11-q32.33 duplication. With the exception of the 10 aneuploidy cases, the size of the pathogenic variants ranged from 142 kb (exons 45–57 of the *DMD* gene) to 10.2 Mb (supernumerary marker chromosome containing a duplication of 15q11.1-q13.2), and the majority (45/55, 81.8%) were less than 5 Mb. The size of the VPS ranged from 396 kb to 35 Mb, and approximately a half of the VPS (36/67, 53.7%) were larger than 5 Mb (Supplemental Data Table S1).

Excluding cases with abnormal CNVs, VUS were discovered in 51 patients (8.3%, 51/617), including 47 with one VUS and four with two VUS. Another three patients with VUS also had a concurrent pathogenic variant (15q11.1q13.1 duplication) or VPS (4q35.2 duplication and 5q13.3 deletion) (Table 3). The most promising result was five patients with gene dose alterations associated with putatively developmental disorders. Three patients showed a microdeletion in 7q31.1 encompassing the *IMMP2L* (MIM 605977) gene. Among them, one patient had a maternally inherited small supernumerary marker chromosome of 15q11.1-q13.1 as well as a VUS. The other two patients had a microduplication, including the *PTCH1* (MIM 601309) gene and a microdeletion in the *ATRNL1* (MIM 612869) region, respectively.

Patient characteristics and clinical features according to the detected CNVs

The general demographic features of the patients are summarized in Supplemental Data Table S2. Overall, 77% (472/617) of the patients were 0–5 years old, and the percentage of males was greater than that of females (60.3% vs 39.7%, $P<0.001$). At least one symptom of neurodevelopmental disorders was detected in most patients (95.1%), and DD and speech delay were common (91.2% and 78.7%, respectively).

The mean±SD number of clinical features was 4.4±1.7 among patients with pathogenic variants, and was 4.8±1.8, 4.0±1.9,

Table 3. CMA results identified in patients with variants of unknown significance

Cytoband	Genes	Deletion/ duplication (N)
1p36.33-p36.31	*MORN1, LOC100129534, RER1, PEX10, PLCH2, PANK4, HES5, LOC115110, TNFRSF14, C1orf93, MMEL1, ACTRT2, FLJ42875, PRDM16, ARHGEF16, MEGF6, MIR551A, TPRG1L, WDR8, TP73, KIAA0495, CCDC27, LOC388588, LRRC47, KIAA0562, DFFB, C1orf174, LOC100133612, LOC284661, AJAP1*	-/1
1p36.23	*SLC45A1, RERE*	1/-
1p32.3	*SSBP3, ACOT11, FAM151A, C1orf175, TTC4, PARS2, TTC22, C1orf177, DHCR24, TMEM61, BSND*	-/1
q42.12	*DNAH14*	1/-
2p12	*REG3G, REG1B, REG1A, REG1P, REG3A, CTNNA2*	-/2
2q21.1	*CCDC115, IMP4, PTPN18, CFC1B, CFC1, LOC150527, C2orf14, GPR148, FAM123C*	-/3
2q23.1-q23.2	*EPC2, KIF5C, MIR1978, LYPD6B*	-/1
3p14.2	*FHIT*	1/-
3q13.31	*QTRTD1, DRD3, ZNF80, TIGIT, MIR568, ZBTB20*	1/-
3q26.31	*NLGN1, NAALADL2*	-/1
4p13	*KCTD8*	1/-
4q28.3	*PCDH10, PABPC4L*	-/4
5q21.2	*RAB9P1*	1/-
6p21.33	*TCF19, POU5F1, PSORS1C3, HCG27, HLA-C, HLA-B, MICA*	1/-
6p21.32	*HLA-DRB6, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DQA2, HLA-DQB2, HLA-DOB, TAP2*	5/-
6q12	*EYS, MCART3P*	1/-
6q13	*COL19A1*	1/-
6q16.1	*EPHA7, TSG1*	-/1
7p21.3p-p21.2	*ETV1, DGKB*	-/2
7p21.2	*DGKB*	1/-
7q11.21	*INTS4L1, ZNF92*	2/-
7q11.23	*UPK3B, LOC100133091, POMZP3, PMS2L11, LOC100132832, CCDC146, FGL2*	1/-
7q31.1	*IMMP2L*	3/-
9p23	*TYRP1*	1/-
9p21.2	*MOBKL2B, IFNK, C9orf72*	1/-
9q22.32	*PTCH1, C9orf130, C9orf102*	-/1
10p15.3-p15.2	*PFKP, PITRM1*	-/1
10q11.22	*PPYR1, LOC728643, ANXA8, ANXA8L1, FAM25B, FAM25C, FAM25G, LOC642826, FAM35B2*	1/2
10q23.31	*RNLS, LIPJ, LIPF, LIPK, LIPN, LIPM, ANKRD22, STAMBPL1, ACTA2, FAS, CH25H, LIPA, IFIT2, IFIT3, IFIT1L, IFIT1, IFIT5, SLC16A12, PANK1, MIR107*	1/-
10q25.3	*ATRNL1*	1/-
11p11.12	*FOLH1, LOC440040, OR4C13, OR4C12*	-/1
12q14.1	*FAM19A2*	-/1
15q11.2	*LOC727924, OR4M2, OR4N4, OR4N3P, GOLGA8D, GOLGA6L1*	1/-
15q26.2-q26.3	*LOC91948, ARRDC4*	-/1
15q26.3	*ADAMTS17*	1/-
16p13.3-p13.2	*A2BP1*	-/1
16p12.3	*XYLT1*	-/1
16q21	*CDH8*	-/3
16q23.1-q23.2	*WWOX, MAF, DYNLRB2, CDYL2*	-/1
21q11.2	*POTED*	1/-

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and 3.9±1.8 in the VPS, VUS, and benign groups, respectively (pathogenic vs VPS, P=0.167; pathogenic vs VUS, P=0.478; pathogenic vs benign, P=0.054; VPS vs VUS, P=0.086; VPS vs benign, P=0.001, and VUS vs benign, P=0.451). Patients with pathogenic variants or VPS were considered a single group in our analysis because no significant differences were found in the rate of clinical features and management after CMA between these two groups. The frequency of clinical features associated with developmental disorders, except for ASD, were the highest in the patients with abnormal variants, followed by those with VUS and those with benign variants. Frequencies of ID, dysmorphic features, and hypotonia differed among the three groups (P=0.029, P<0.001, and P=0.006, respectively). These features were more common in patients carrying abnormal variants than in those with benign variants (ID, 77.3% vs 66.1%, P<0.001; dysmorphic features, 31.0% vs 14.6%, P=0.016; hypotonia,

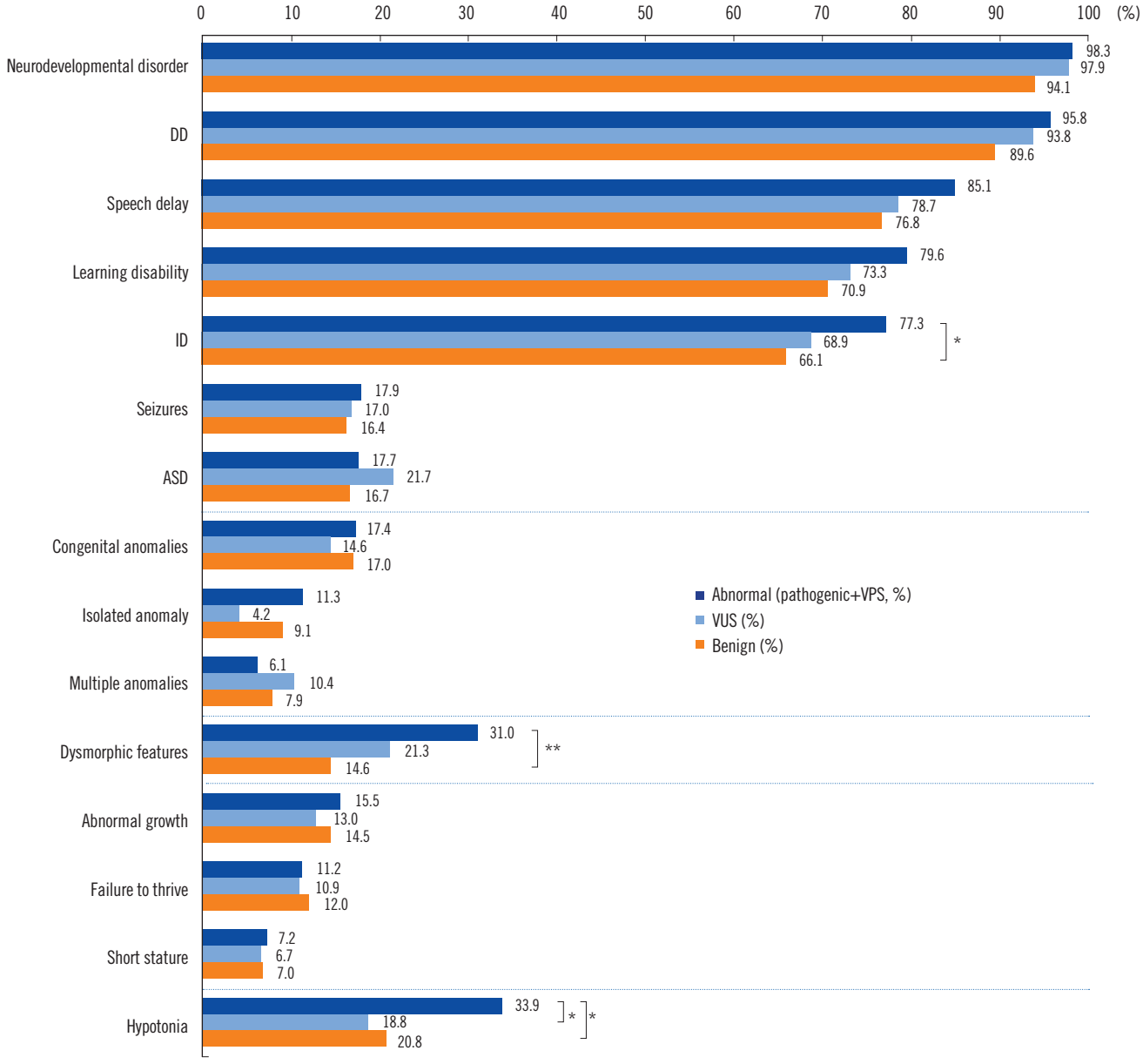


Fig. 1. Evaluation of clinical features in patients with DD/ID, ASD, and MCA. Significant differences in the frequencies of ID, dysmorphic features, and hypotonia were found among the three groups (P=0.029, P<0.001, and P=0.006, respectively).
*P<0.05; **P<0.001.
Abbreviations: DD, developmental delay; ID, intellectual disability; ASD, autism spectrum disorders; MCA, multiple congenital anomalies; VUS, variants of unknown significance.

33.9% vs 20.8%, $P=0.003$) (Fig. 1).

Clinical management following CMA

Among the 581 patients available for follow-up, 415 (71.4%) were given at least one recommendation of clinical management (Table 4). A total of 1,663 new management strategies were recommended, demonstrating that a mean of 2.9 new recommendations per patient were prompted by CMA results.

Table 4. Summary of recommendations of clinical management in response to CMA results

	Patients, N (%)				
	Total (N=581)*	Pathogenic (N=60)	VPS (N=57)	VUS (N=48)	Benign (N=416)
Pharmacologic management	20 (3.4)	4 (6.7)	3 (5.3)	2 (4.2)	11 (2.6)
Pharmacologic treatment	18 (3.1)	2 (3.3)	3 (5.3)	2 (4.2)	11 (2.6)
Synthyroxine	2 (0.3)§	1 (1.7)	1 (1.8)	-	-
Growth hormone	4 (0.7)	-	2 (3.5)	-	2 (0.5)
Antiepileptic drugs	12 (2.1)	1 (1.7)	1 (1.8)	1 (2.1)	9 (2.2)
Vitamin D, calcium	1 (0.2)	-	-	1 (2.1)	-
Contraindication	2 (0.3)§	2 (3.3)**	-	-	-
Avoid excess calcium and vitamin D	2 (0.3)§	2 (3.3)**	-	-	-
Specialist referral	306 (52.7)§	41 (68.3)**	36 (63.2)††	28 (58.3)	201 (48.3)
Cardiology	37 (6.4)§	10 (16.7)**	8 (14.0)††	2 (4.2)‖	17 (4.1)
Audiology	104 (17.9)	15 (25.0)	9 (15.8)	8 (16.7)	72 (17.3)
Ophthalmology	108 (18.6)	12 (20.0)	15 (26.3)	10 (20.8)	71 (17.1)
Immunology	3 (0.5)	1 (1.7)	1 (1.8)	-	1 (0.2)
Neurology	187 (32.2)§	25 (41.7)**	20 (35.1)	20 (41.7)	122 (29.3)
Endocrinology	67 (11.5)§	12 (20.0)**	9 (15.8)	10 (20.8)	36 (8.7)
Musculodystrophic clinic	26 (4.5)§	11 (18.3)**	6 (10.5)††	1 (2.1)‖,¶	8 (1.9)
Psychiatry	55 (9.5)	3 (5.0)	6 (10.5)	7 (14.6)	40 (9.6)
Orthopedics	28 (4.8)	5 (8.3)	3 (5.3)	2 (4.2)	18 (4.3)
Otolaryngology	5 (0.9)§	4 (6.7)**	-	1 (2.1)	-
Nephrology	1 (0.2)	-	1 (1.8)	-	-
Gastroenterology	2 (0.3)	-	1 (1.8)	-	1 (0.2)
Hematology	3 (0.5)	-	1 (1.8)	-	2 (0.5)
Other†	3 (0.5)	1 (1.7)	-	-	2 (0.5)
Imaging studies	351 (60.4)	38 (63.3)	42 (73.7)††	31 (64.6)	240 (57.7)
Ultrasonography	42 (7.2)	5 (8.3)	6 (10.5)	6 (12.5)	25 (6.0)
X-ray	169 (29.1)	15 (25.0)	17 (29.8)	17 (35.4)	120 (28.8)
Skeletal survey	159 (27.4)	20 (33.3)	19 (33.3)	13 (27.1)	107 (25.7)
CT/MRI	322 (55.4)§	37 (61.7)	39 (68.4)††	29 (60.4)	217 (52.2)
Laboratory testing	302 (52.0)	33 (55.0)	35 (61.4)	26 (54.2)	208 (50.0)
Overall impact on clinical management	415 (71.4)§	50 (83.3)**	49 (86.0)††	36 (75.0)	280 (67.3)
Total number of clinical managements (mean)	1,663 (2.9)§	215 (3.6)**	203 (3.6)††	156 (3.3)	1,089 (2.6)
Developmental surveillance‡	166 (28.6)§	10 (16.7)**	8 (14.0)††	12 (25.0)	136 (32.7)

*Follow-up was available for 581 patients (follow-up periods: six–53 months); †Other: Urology for one patient with abnormal variants, and dermatology and general surgery for two patients with benign variants; ‡Developmental surveillance indicates ongoing monitoring of development, identification of risk factors, and elicitation of parental concerns; §$P<0.05$ among the four groups; ‖$P<0.05$, pathogenic vs VUS; ¶$P<0.05$, VPS vs VUS; **$P<0.05$, pathogenic vs benign; ††$P<0.05$, VPS vs benign.

Abbreviations: CMA, chromosomal microarray analysis; VPS, variants of possible significance; VUS, variants of unknown significance; CT, computed tomography; MRI, magnetic resonance imaging.

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Computed tomography (CT)/magnetic resonance imaging (MRI) studies were recommended for more than half of all patients (55.4%), and the most common CT/MRI types were of the brain. Ultrasonography examination was recommended for 42 patients,

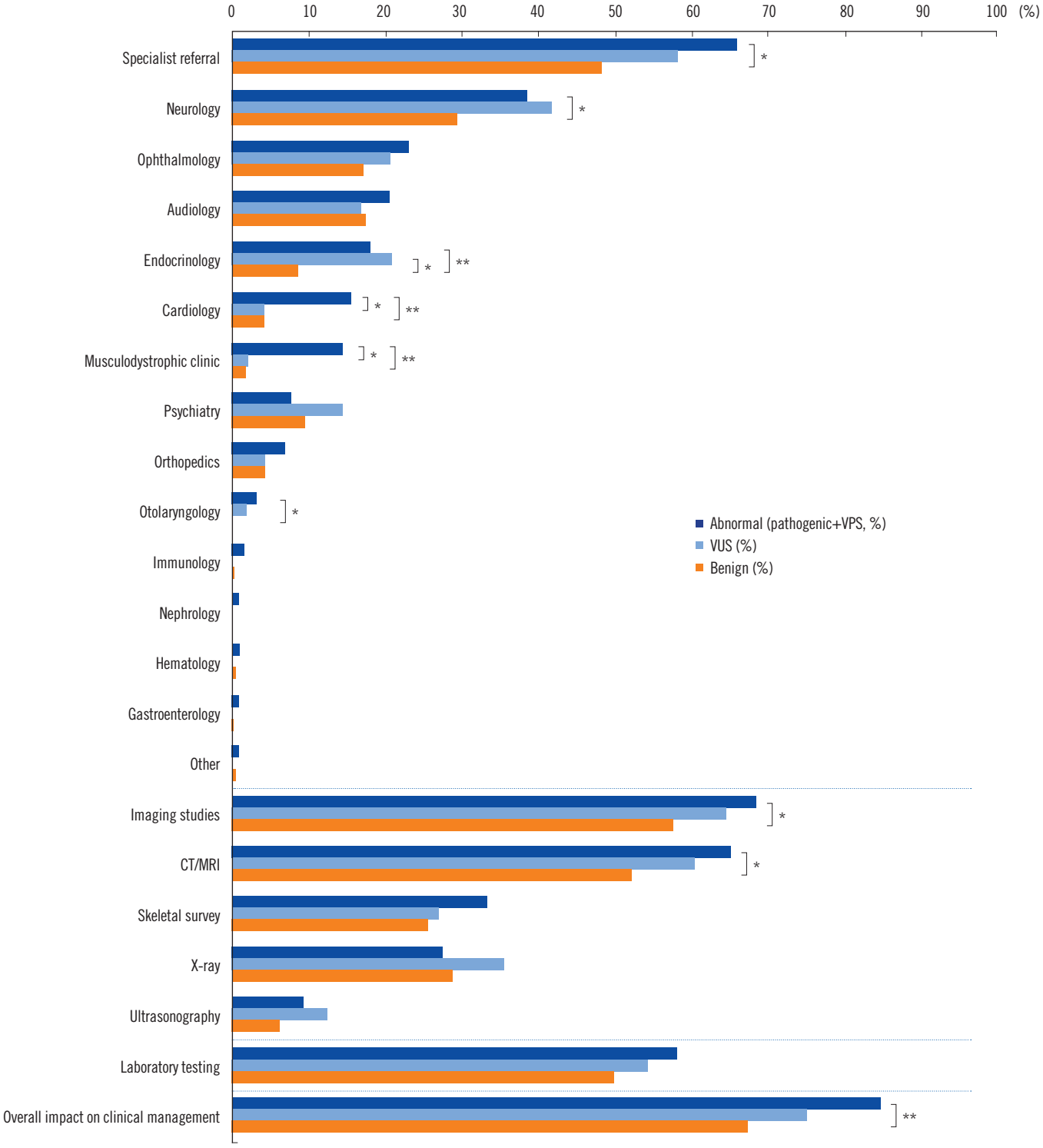


Fig. 2. Rate of clinical management recommendations following CMA.
*$P<0.05$; **$P<0.001$.
Abbreviations: CMA, chromosomal microarray analysis; VUS, variants of unknown significance; CT, computed tomography; MRI, magnetic resonance imaging.

80% of which included echocardiogram and 20% included abdomen and kidney ultrasound. Clinical management was not recommended after a benign CMA result in 23.4% (136/581) of all patients. Patients with abnormal variants consulted with specialists for cardiology, neurology, endocrinology, and musculodystrophy more frequently than patients with benign CNVs did ($P<0.001$, $P=0.040$, $P=0.005$, and $P<0.001$, respectively). CT/MRI imaging was more frequently recommended for patients with abnormal variants than for those with benign variants ($P=0.009$) (Fig. 2). Pharmacological management was recommended for 20 patients (3.4%). Thyroid hormone medication was recommended for treating hypothyroidism in two patients with 1p36 deletion syndrome and 13q31.1q31.2 deletion, respectively. Two patients with Williams syndrome were advised to avoid taking multivitamins with extra calcium or vitamin D to prevent hypercalcemia, while one patient was treated with vitamin D and calcium supplements after excluding Williams syndrome.

DISCUSSION

The translation of research results to public health applications has been unexpectedly slow in many countries, including Korea, although CMA has an enhanced diagnostic yield compared with standard karyotype analysis for patients with developmental disabilities [2, 3]. One of the main barriers to the clinical adoption of CMA is the lack of standardization for reporting results. However, guidelines for the three- to five-level interpretative categories of CNVs and the expansion of open-access databases of patient cohorts or healthy controls allow for the provision of precise information with high reproducibility [2, 8, 11]. In our study, CMA revealed clinically relevant chromosomal imbalances in 19.8% of patients, which is similar to the previously reported diagnostic yield of 15–20% [2]. When CMA was used as a first-tier diagnostic test [13-15], the detection rate was much higher than that obtained using CMA as a second-tier test after standard karyotyping (18–30% vs 7–14%) [16, 17].

The classification and reporting of VUS remains a challenge. Although parental testing is recommended to clarify the clinical significance of a variant detected, most disorders associated with CNVs show no clear genotype-phenotype correlation, even within the same family. In this study, 8.3% of the patients had chromosomal imbalances of still unclear clinical relevance, which is consistent with rates reported in earlier studies (5–14%) [13]. However, classification of VUS varies considerably across studies [17-19]. Recent analyses of variant classifications reported in ClinVar showed that among the 11% of variants with more than one submitter, 17% showed different interpretations [20]. For example, the duplication of 8p23.2, including the *CSMD1* gene has been detected in patients with speech delay, autism, and learning difficulties [18] as well as in normal individuals [19]. Duplication of the *CSMD1* gene was reported as a VUS with a frequency of 3.1% (3/96) in a previous study [17], while we classified such variants as benign according to our laboratory's current variant classification criteria (≥1% of the patient population), that is, 1.8% (11/617) of patients and 3.2% (3/95) of normal family members in our cohort.

Despite these limitations, VUS may be a good candidate gene and pathway marker for rare developmental disorders. We detected a 7q31.1 deletion, including *IMMP2L* in three unrelated patients with DD, learning disability, ID, and speech delay. Indeed, microdeletion in 7q31.1 encompassing the *IMMP2L* gene has been suggested as a susceptibility factor for neurodevelopmental disorders, such as Tourette syndrome [21, 22]. Duplication of the *PTCH1* gene has also been reported in a family with microcephaly and DD [23]. Our patient with a *PTCH1* duplication exhibited not only DD and microcephaly but also polydactyly, tongue papilloma, and corpus callosum dysgenesis. In addition, 10q25.3 deletion, including the *ATRNL1* gene has been reported in a patient with cognitive impairment, autism, and dysmorphic facial features [24]. A female neonate with 10q25.3 deletion in our cohort had congenital heart defects, including an atrial septal defect and ventricular septal defect. However, because of the very young age of the patient, we were not able to determine whether cognitive impairment or autism is present. Results obtained through a "reverse genetics" approach and further collaborative efforts will help definitively characterize the role of candidate genes in pathogenesis.

Assessment of the patients' clinical features revealed a tendency for a higher frequency of clinical abnormalities in the group with abnormal variants, which is consistent with the aforementioned studies [6, 25]. In our study, the frequency of ID, dysmorphic features, and hypotonia were significantly higher in patients with abnormal variants than in those with benign variants. Developmental disorders and congenital anomalies or dysmorphic features have been reported in most patients with abnormal CMA results [6]. Other studies reported that facial abnormalities [26], heart defects [25] and ID and a family history of ID/MCA/ASD [14] were more common in patients with abnormal CMA results.

Another obstacle hindering the widespread clinical application of CMA as the first-tier cytogenetic test is related to the uncertainty of whether the testing will directly influence medical

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management. Although recent studies have correlated abnormal CMA results with predicted clinical impact [6-8, 27], most of them were performed in a single institution, based solely on medical records [6-8]. Moreover, an appropriate control was not applied to prove that such intervention would not have occurred with patients who had not received a positive CMA result [6, 8]. Thus, we queried the referring clinicians regarding follow-up clinical management to assess the impact of CMA results. Approximately 85% of patients with clinically relevant variants received more direct clinical management in our study. These results support those of earlier studies [6, 8, 27, 28], demonstrating that abnormal CMA results contributed to medical management in a substantial proportion (34–94%) of patients with DD/ID, MCA, and ASD. Differences in the extent of clinical management might be attributed to the different health care systems among countries as well as the patient heterogeneity across studies. Only one other study [27] has assessed medical recommendations following benign CMA results, finding that patients with benign CNVs received a mean of 2.7 medical recommendations. Similarly, clinical management was recommended for patients with benign variants (mean: 2.6 recommendations) in our study. Specifically, compared with benign CMA results, abnormal CMA variants were a significant driver of medical recommendations; VUS results also drove recommendations, but to a lesser extent. These results suggest that some additional diagnostic tests can be avoided in patients with negative CMA results, which could lead to tangible savings in healthcare expenditures.

Even when no specific cure is available or when some genetic diagnoses may have minimal impact on patient management, establishing a clear diagnosis through genetic testing may lead to earlier initiation of medical care and consequently improve outcomes for patients and their families who have endured a "diagnostic odyssey." In addition, along with the development of whole-genome analysis using genome-wide arrays, recurrent CNVs associated with ID/DD, ASD, and MCA have been labeled as novel microdeletion/duplication syndromes [29, 30]. There is now published literature supporting specific clinical management implications for at least 146 conditions potentially diagnosable by CMA [7]. Medical knowledge regarding pathogenic CNVs will also continue to progress.

Although CMA has a higher resolution than conventional karyotyping, polyploidy, balanced translocations, inversion, low-level mosaicism, and marker chromosomes may be missed [3]. A benign CMA result does not exclude all genetic diseases. Therefore, for these patients, a next-generation sequencing approach as a subsequent diagnostic test may aid in establishing the diagnosis [31, 32].

Overall, this prospective multicenter study highlights the clinical application of CMA as a first-tier testing in patients with DD/ID, ASD, and MCA. CMA results directly affect the subsequent clinical management strategy, and the impact is not limited to patients with abnormal or negative results. Thus, the widespread use of CMA in clinical settings has potential to improve the efficiency and quality of clinical management for these patients.

Authors' Disclosures of Potential Conflicts of Interest

The authors declare that they have no competing interests.

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Supplemental Data Table S1. Size distribution of CNVs found in patients

Size (Mb)	Number of CNVs (%)		
	Number of pathogenic variants (N=65)	Number of VPS (N=67)	Number of VUS (N=58)
<0.5	4 (6.2)	1 (1.5)	23 (39.7)
0.5–1	6 (9.2)	8 (11.9)	16 (27.6)
1–5	35 (53.8)	22 (32.8)	18 (31.0)
5–10	9 (13.8)	20 (29.9)	1 (1.7)
>10	11 (17.0)	16 (23.9)	0 (0.0)

Abbreviations: CNV, copy number variation; VPS, variants of possible significance; VUS, variants of unknown significance.

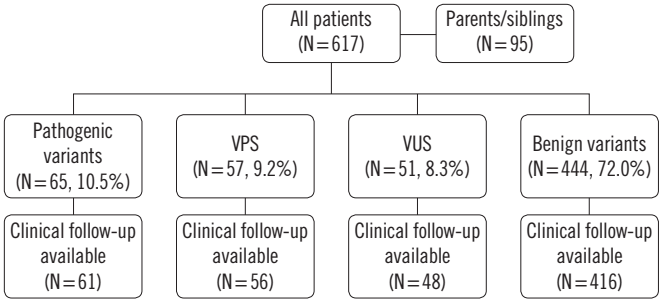
Supplemental Data Table S2. Demographic and clinical features of patients according to CMA results

	Total N (%)	Pathogenic N (%)	VPS N (%)	VUS N (%)	Benign N (%)
Gender					
Male	372/617 (60.3)	34/65 (52.3)	33/57 (57.9)	23/51 (45.1)	282/444 (63.5)
Female	245/617 (39.7)	31/65 (47.7)	24/57 (42.1)	28/51 (54.9)	162/444 (36.5)
Age (yr)					
<2	218/617 (35.3)	22/65 (33.8)	25/57 (43.9)	20/51 (39.2)	151/444 (34.0)
2–5	254/617 (41.2)	29/65 (44.6)	18/57 (31.6)	17/51 (33.3)	190/444 (42.8)
5–10	66/617 (10.7)	7/65 (10.8)	4/57 (7.0)	6/51 (11.8)	49/444 (11.0)
>10	79/617 (12.8)	7/65 (10.8)	10/57 (17.5)	8/51 (15.7)	54/444 (12.2)
Clinical features					
Neurodevelopmental disorder	561/589 (95.2)	59/61 (96.7)	57/57 (100)	47/48 (97.9)	398/423 (94.1)
Developmental delay	536/588 (91.2)	56/61 (91.8)	57/57 (98.2)	45/48 (93.8)	378/422 (89.6)
Learning disability or behavioral or psychiatric disorder	390/535 (72.9)	44/57 (77.2)	42/51 (82.4)	33/45 (73.3)	271/382 (70.9)
Seizures	98/584 (16.8)	9/61 (14.8)	12/56 (21.4)	8/47 (17.0)	69/420 (16.4)
Intellectual disability	372/542 (68.6)	42/57 (73.7)	43/53 (81.1)	31/45 (68.9)	256/387 (66.1)
Speech delay	435/553 (78.7)	49/60 (81.7)	48/54 (88.9)	37/47 (78.7)	301/392 (76.8)
Autism spectrum disorder	95/549 (17.3)	12/58 (20.7)	8/55 (14.5)	10/46 (21.7)	65/390 (16.7)
Congenital anomalies	97/580 (16.7)	9/58 (15.5)	11/57 (19.3)	7/48 (14.6)	71/418 (17.0)
Isolated anomaly	52/580 (9.0)	8/58(13.8)	5/57 (8.8)	2/48 (4.2)	38/418 (9.1)
Multiple anomalies	45/580 (7.8)	1/58 (1.7)	6/57 (10.5)	5/48 (10.4)	33/418 (7.9)
Dysmorphic features	107/580 (18.4)	17/59 (28.8)	19/57 (33.3)	10/47 (21.3)	61/417 (14.6)
Abnormal growth	82/563 (14.6)	9/60 (15.0)	9/56 (16.1)	6/46 (13.0)	58/401 (14.5)
Failure to thrive	66/562 (11.7)	6/60 (10.0)	7/56 (12.5)	5/46 (10.9)	48/400 (12.0)
Short stature	37/529 (7.0)	4/58 (6.9)	4/53 (7.5)	3/45 (6.7)	26/373 (7.0)
Hypotonia	136/584 (23.3)	19/61 (31.1)	21/57 (36.8)	9/48 (18.8)	87/418 (20.8)

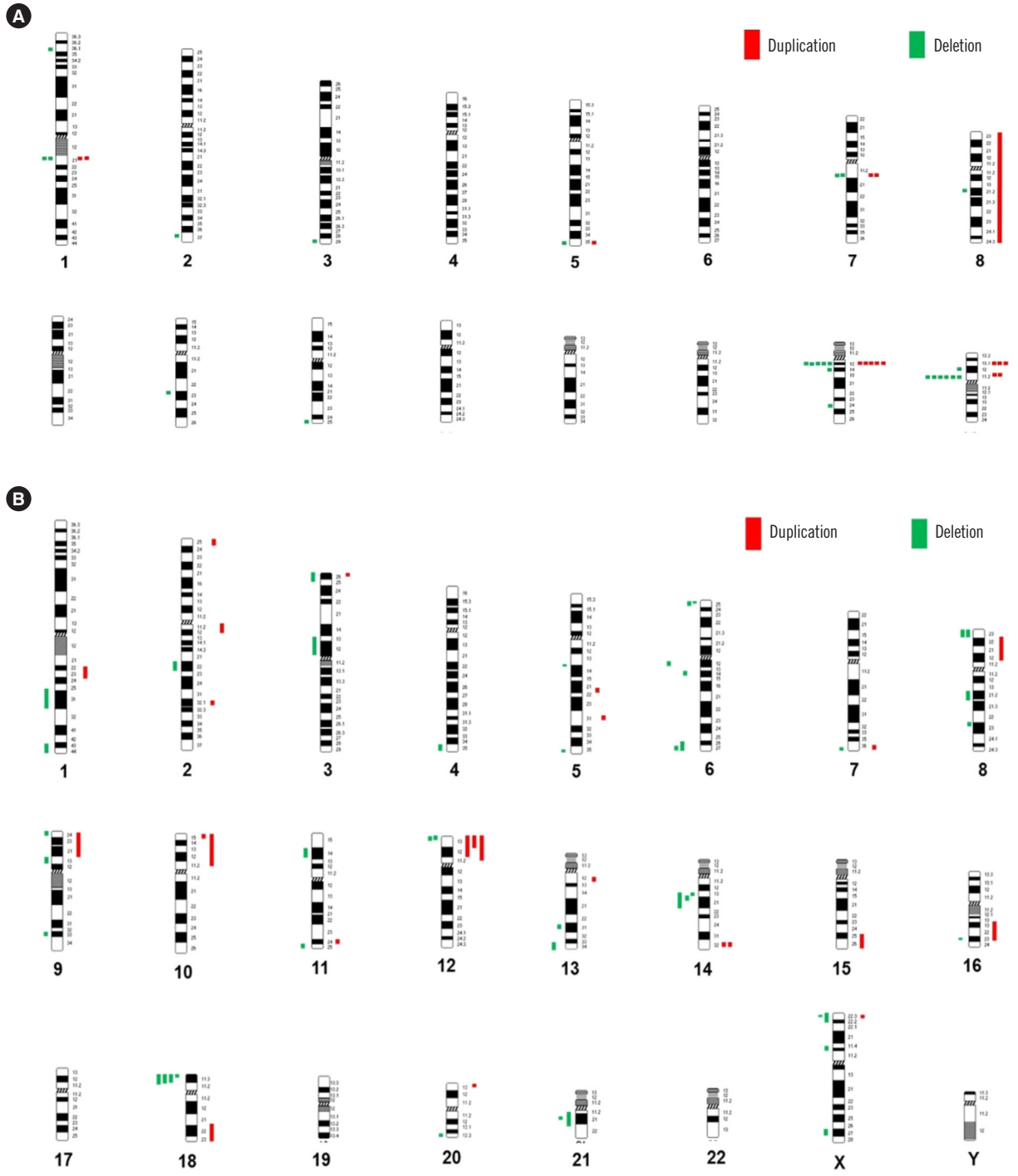
Abbreviations: CMA, chromosomal microarray analysis; VPS, variants of possible significance; VUS, variants of unknown significance.

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Supplemental Data Fig. S1. Overview of patient enrollment, chromosomal microarray analysis results, and clinical follow-up.
Abbreviations: VPS, variants of possible significance; VUS, variants of unknown significance.



Supplemental Data Fig. S2. Regions of chromosomal duplication and deletion in patients with (A) pathogenic variants and (B) VPS. Red bars indicate specific regions of duplication, and green bars indicate deletion of chromosomes for each patient.
Abbreviation: VPS, Variants of possible significance.

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