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ORIGINAL ARTICLE

Chromosomal Microarray Testing in 42 Korean Patients with Unexplained Developmental Delay, Intellectual Disability, Autism Spectrum Disorders, and Multiple Congenital Anomalies

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Chromosomal microarray (CMA) is a high-resolution, high-throughput method of identifying submicroscopic genomic copy number variations (CNVs). CMA has been established as the first-line diagnostic test for individuals with developmental delay (DD), intellectual disability (ID), autism spectrum disorders (ASDs), and multiple congenital anomalies (MCAs). CMA analysis was performed in 42 Korean patients who had been diagnosed with unexplained DD, ID, ASDs, and MCAs. Clinically relevant CNVs were discovered in 28 patients. Variants of unknown significance were detected in 13 patients. The diagnostic yield was high (66.7%). CMA is a superior diagnostic tool compared with conventional karyotyping and fluorescent *in situ* hybridization.

Keywords: autism spectrum disorder, chromosomal microarray, developmental delay, intellectual disability, multiple congenital anomalies

Introduction

Chromosomal microarray (CMA) analysis is widely recognized and recommended as the first-tier cytogenetic diagnostic test for patients with developmental delay (DD), intellectual disability (ID), autism spectrum disorders (ASDs), or multiple congenital anomalies (MCAs) [1, 2].

DD describes persons aged 5 years or younger who have demonstrated several significant delays in the following areas: cognitive, speech, social/personal, fine/gross motor, and daily activities. ID is diagnosed at or after age 5 years when one's intelligence quotient is deemed to be less than 70 and when permanent intellectual impairment results in a general deficit in adaptive behaviors [3]. ASD comprises a wide range of neurological and developmental disabilities, encompassing autism, Asperger syndrome, pervasive developmental disorders, and childhood disintegrative disorder [3]. MCAs refer to the presence of multiple major malformations, such as cardiac defects and missing limbs, or 3 or more minor malformations (e.g., syndactyly and club feet).

Combined, these disorders are highly prevalent (DD/ID, up to 3% [4]; ASDs, 1% to 2% [5]; MCAs, 0.16% [6]) and might have a genetic etiology, such as copy number variation (CNV) and loss of heterozygosity (LOH). The prevalence of pathogenic CNVs is high in those with autism (5% to 10%) and children with both ID and MCAs (20% to 25%) [7].

CMA is a high-resolution, high-throughput technique that detects submicroscopic CNVs that are not observable with traditional cytogenetic analysis tools, including karyotyping and fluorescence *in situ* hybridization (FISH) [8]. Further, CMA has a higher diagnostic yield (12.2% on average) than conventional tests [1, 2]. However, as with next-generation sequencing (NGS), interpreting microarray anomalies poses challenges for clinical laboratories and clinicians in establishing the clinical significance and pathogenicity of the detected CNVs.

Variability in the interpretation arises from internal

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databases that are limited by the number of cases that are analyzed; racial and socio-economic bias; and the complete lack of openly accessible centralized resources for sharing CMA data, patient phenotypes, and clinical interpretations between laboratories in South Korea and overseas. Moreover, disparate technology platforms with varying array designs, resolution, and coverage render the standardization of CMA and its uniform interpretation more difficult.

In this study, we attempted to establish the origin of DD, ID, ASDs, and MCAs in 42 Korean patients; demonstrate the value of CMA in determining the genomic etiology of unexplained DD, ID, and MCAs; and discuss the challenges facing CMA.

Methods

Patients

The parents and guardians of the probands were informed about the microarray study and given information about the risks, benefits, and limitations of CMA testing. Informed consent was obtained by a clinical geneticist or the researchers.

The study sample comprised 42 individuals (29 males, 13 females), ranging in age from newborns to 38 years, who had negative test results for metabolic disorders and other suspected disorders and did not present with any recognizable syndrome.

Clinical data, including medical history, were collected from the medical records and the parents and guardians of the probands. General observations for dysmorphic features were made, and the height and head circumference were measured by the clinician or the researchers.

DNA preparation

Blood samples were collected at the time of consent. Genomic DNA was extracted from peripheral blood lymphocytes using the AccuPure Cell/Blood DNA Mini Kit (AccuBioMed Co, Ltd, New Taipei City, Taiwan) according to the manufacturer's instructions. DNA concentration and quality were measured using a NanoDrop One Microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA, USA).

Chromosomal microarray

CMA tests were performed with Affymetrix CytoScan 750K arrays (Affymetrix, Santa Clara, CA, USA) and a GeneChip GCS3000dx V2 scanner (Affymetrix) according to the manufacturer's instructions. The array had 750,436 markers (550,000 non-polymorphic markers and 200,436 single nucleotide polymorphism markers), based on genome build hg19. The results were analyzed with Chromosome Analysis Suite ver. 3.2.0.1252 (Affymetrix).

Interpretation

CNVs were categorized as pathogenic, benign, and variants of uncertain significance (VUS) by considering gene content, size, inheritance pattern, and previously reported microdeletion or microduplication regions. University of California Santa Cruz Genome Browser was employed to check all of the information relevant to the locations of the CNVs. dbVar (https://www.ncbi.nlm.nih.gov/dbvar) and the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (https://decipher.sanger.ac.uk/) were consulted to check clinically relevant structural variations and determine whether the observed CNVs were of clinical significance. We considered CNVs to be causative if the variant was responsible for a known syndrome; encompassed a gene(s) of known function; or occurred de novo or, if inherited, the parent was variably affected or if the involved gene(s) had been reported to be associated with ID/DD/ASDs in public databases and the scientific literature. The term VUS was used if the variants affected a gene(s) of unknown clinical significance and if, at same time, the size was substantially less than 100 kb (the average resolution of the probes used) and when the family studies were inconclusive or unavailable [9].

Results

A total of 41 clinically relevant CNVs were detected in 28 (66.7%) patients: three (10.7%) of those presenting with MCAs, 23 (82.1%) with a variable degree of DD or ID, and two (7.1%) with varying degrees of ID and ASDs; 10 (35.7%) patients had multiple rearrangements.

Among 28 pathogenic cases, nine were diagnosed with microdeletion/microduplication syndromes, including 1p36 deletion syndrome, 5q14.3 deletion syndrome, 16p11.2 deletion syndrome, 16p11.2 duplication syndrome, 19p duplication syndrome, 9p duplication syndrome, 19p13.2 deletion syndrome, and 22q11.2 duplication syndrome.

In this study, more pathogenic duplications were observed than deletions (22 vs. 19). Also, 12 cases (28.6%) were VUS, and two cases (4.8%) were benign CNVs (Table 1).

Discussion

Genomic CNVs can contribute to the etiologies of DD, ID, ASDs, and MCAs. With its proven diagnostic yield, CMA has been recommended by a number of professional societies as part of the standard assessment for individuals in whom the underlying cause is unclear [10, 11].

Table 1. Chromosomal microarray results

Patient No.	Sex	Age	Medical history	Microarray result	Size (kbp)	Critical gene(s) or known syndrome	Classification
1	M	7 y	ID, ASD	arr[hg19] 9q34.3(139,486,767-140,141,288)x3	654.521	*THRB, GLB1, TRAF2, MAN1B1, GRIN1*	Pathogenic
				arr[hg19] 3p24.2(24,448,650-24,453,383)x1	4.733	*THRB*	
2	M	2 y	DD, dystonia, family history of hereditary spastic paraplegia	arr[hg19] 1q41q42.11(223,903,817-224,304,043)x3	400.226	*CAPN2*	Pathogenic
				arr[hg19] Xp21.1(32,535,197-32,544,026)x0	8.829	*DMD*	
3	M	8 y	ID	arr[hg19] 2q24.3(166,914,464-166,920,459)x1	6	*SCN1A*	Pathogenic
				arr[hg19] 3p26.3(282,750-283,756)x1	1	*CHL1*	
4	M	9 y	ID	arr[hg19] 2q24.3(166,914,464-166,920,459)x1	6	*SCN1A*	Pathogenic
				arr[hg19] Xp11.4(41,420,369-41,436,593)x0	16	*CASK*	
5	M	4 y	DD	arr[hg19] Xq22.2(103,022,505-103,032,637)x0	10	*PLP1*	Pathogenic
6	M	9 y	ID	arr[hg19] 8q23.3(116,594,180-116,597,777)x1	3.597	*TPRS1*	VUS
				arr[hg19] 19p13.3(1,377,665-1,712,859)x3	335.194	*NDUFS7*	
				arr[hg19] 20q13.33(61,914,225-62,223,211)x3	308.986	*KCNQ2*	
7	M	5 y	DD, dystonia	arr[hg19] 5q14.3q21.3(89,128,834-105,955,784)x1	16,826.95	5q14.3 deletion syndrome	Pathogenic
				arr[hg19] Xp21.1(32,685,839-32,696,594)x0	10.755	*DMD*	
8	M	17 y	Mild ID	arr[hg19] 2q21.2q21.3(134,274,063-135,144,454)x3	870.391	None	Benign
				arr[hg19] 7q11.21(64,612,879-65,162,169)x1	549.29	None	
9	M	7 y	ID, convulsion	arr[hg19] 9q21.13q21.31 (74804031-81810541)x1	7,006.51	*TRPM6*	Pathogenic
10	M	16 y	DD, MCA	arr[hg19] Xq28(152,927,530-153,002,877)x2	75.347	*SLC6A8*	Pathogenic
11	M	17 y	ID, ASD	arr[hg19]16p11.2(29,567,295-30,177,916x1	611	16p11.2 deletion syndrome	Pathogenic
12	M	10 mo	DD, hypotonia, failure to thrive	arr[hg19]16p11.2(29,657,192-30,192,347)x3	598	16p11.2 duplication syndrome	Pathogenic
13	M	NB	MCA	Normal (balanced de novo translocation later found)	-	N/A	Benign
14	F	5 y	MCA	arr[hg19] 1p36.33p36.23(849,466-7,637,060)x1	6,788	1p36 deletion syndrome	Pathogenic
15	M	6 y	ID, ASD	arr[hg19] 2q14.2(121,565,880-121,569,723)x1	3.843	*GLI2*	VUS
16	F	5 y	DD, mitochondrial disorder	arr[hg19] 7q36.2(153,965,792-153,987,424)x1	21.632	*DPP6*	Pathogenic
				arr[hg19] 9q34.3(139,015,355-139,425,340)x3	409.985	*NOTCH1*	
				arr[hg19] 12p13.33(1,953,989-2,306,966)x3	352.977	*CACNA2D4*	
17	M	7 y	ID, multiple neuromuscular problems	arr[hg19] 9q34.3(139,870,881-140,169,934)x3	299.053	*MAN1B1*	Pathogenic
				arr[hg19] 16p13.3(549,826-1,045,161)x3	495.335	*CCDC78*	
				arr[hg19] Xq28(153,167,262-153,189,112)x2	21.85	*AVPR2*	
				arr[hg19] Xp21.1(31,536,869-31,542,339)x0	5.47	*DMD*	
18	F	15 y	ID, seizure, brain anomaly	arr[hg19] 16p11.2(32,046,521-33,814,547)x1	1,768.026	16p11.1 deletion syndrome	Pathogenic
19	F	15 y	ID	arr[hg19] Xp21.2(29,368,422-29,383,438)x4	15.016	*IL1RAPL1*	Pathogenic
20	M	3 y	DD, cryptorchidism, dystonia	arr[hg19] 15q11.2(24,340,678-24,829,846)x3	489.168	*PWRN1*	Pathogenic
21	M	6 y	ID, dystonia, long limbs	arr[hg19] 15q21.1q21.3(46,413,347-53,769,553)x1	7356.206	*FBN1*	Pathogenic
22	M	3 y	DD, dystonia	arr[hg19] 16p13.3(571,141-910,738)x3	339.597	*CCDC78*	Pathogenic
23	M	14 y	DD, dystonia	arr[hg19] 16p13.3(1,002,394-1,007,394)x4	5.0	*LMF1*	Pathogenic
				arr[hg19] Xp21.1(31,536,869-31,536,878)x0	0.009	*DMD*	
24	F	31 y	Mild ID	arr[hg19] 15q26.3(99,183,519-99,204,934)x4	21.415	*IGF1R*	VUS
25	M	6 y	ID	arr[hg19] 22q11.21(18,916,842-19,024,659)x4	107.817	*PRODH*(22q11.2 duplication syndrome)	Pathogenic
26	F	27 y	ID	arr[hg19] 9p24.3q21.11(208,454-69,977,404)x3	69,768.95	9p duplication syndrome	Pathogenic
				arr[hg19] 19p13.3(669,306-1,725,991)x3	1,056.685	19p duplication syndrome	

Table 1. Continued

Patient No.	Sex	Age	Medical history	Microarray result	Size (kbp)	Critical gene(s) or known syndrome	Classification
27	F	10 y	DD, epilepsy	arr[hg19] Xq28(152,765,599-153,008,454)x3	242.855	*ATP2B3, SLC6A8*	Pathogenic
28	M	38 y	History of DD, hypotonia	arr[hg19] 7p21.2(16,438,181-16,443,839)x4	5.658	*ISPD*	VUS
29	F	7 y	History of DD, polydactyly	arr[hg19] 7q35(146,193,991-146,199,530)x1 arr[hg19] Xp11.22(53,225,023-53,240,693)x3	5.539 15.67	*CNTNAP2* *KDM5C*	VUS
30	M	5 y	DD, dystonia	arr[hg19] 9q34.3(139,381,821-140,086,032)x3 arr[hg19] Xp21.1(31,536,869-31,542,335)x0	704.211 5.466	*MAN1B1,GRIN1* *DMD*	Pathogenic
31	M	18 y	ID	arr[hg19] 9q34.3(139,897,180-140,086,032)x3	188.852	*MAN1B1,GRIN1*	Pathogenic
32	F	11 y	Mild ID, ASD	arr[hg19] 16p13.3(536,666-1,449,862)x3	913.196	*STUB1*	VUS
33	M	4 y	DD, iron-deficiency anemia	arr[hg19] 7p15.2(27,223,591-27,224,687)x1 arr[hg19] 16p13.3(1,129,251-1,517,728)x3	1.096 388.477	*HOXA11* *GNPTG*	VUS
34	F	16 y	ID, low bone density	arr[hg19] 1q21.3(151,331,913-151,409,973)x3	78.06	*POGZ*	VUS
35	M	15 y	ID, low bone density	arr[hg19] 1q21.3(151,331,913-151,409,973)x3	78.06	*POGZ*	VUS
36	F	5 y	DD, hypotonia, suspected Haddad syndrome	arr[hg19] 19p13.2p13.12(12,697,352-14,926,569)x1	2,229.217	19p13.2 deletion syndrome	Pathogenic
37	M	13 y	ID	arr[hg19] Xp11.22(53,428,070-53,477,879)x2	49.809	*SMC1A*	Pathogenic
38	F	20 y	History of DD, convulsion	arr[hg19] Xp11.22(53,428,070-53,477,882)x3	49.812	*SMC1A, HSD17B10*	Pathogenic
39	M	10 y	ID, ASD	arr[hg19] 5q31.2q31.3(137,500,664-140,552,558) hmz	3,051.894	*PURA*	VUS
40	M	6 y	ID	arr[hg19] Xq28(152,927,530-152,993,325)x2	65.795	*ABCD1*	VUS
41	M	4 y	DD	arr[hg19] 1p36.33(2,226,599-2,242,417)x4	15.818	*SKI*	Pathogenic
42	F	21 y	ID	arr[hg19] Xp22.32(5,844,864-5,872,572)x1	27.70	*NLGN4X*	VUS

ID, intellectual disability; ASD, autism spectrum disorders; DD, developmental delay; VUS, variantsof uncertain significance; MCA, multiple congenital anomalies.

Evaluating genomic variants identified by CMA is time-consuming and difficult. Variations in their interpretat ion are not uncommon due to platform differences (e.g., resolution and array design), lack of standardization, and differences in the resources that are available to and the expertise of the bioinformatics team and clinicians [12].

At the time of writing, South Korea's National Health Insurance (NHI) does not recognize CMA as a medically necessary test nor does it subsidize the cost of CMA testing. NHI allows only multiplex ligation-dependent probe amplification tests for four microdeletion syndromes: Angelman/Prader-Willi syndrome, DiGeorge syndrome, Miller-Dieker syndrome, and Williams syndrome [13]. Although subsidies for NGS genetic testing have received government approval this year, NGS tests are limited to a small number of pre-approved diagnostic gene panels. With NGS in the early stages of clinical application in this country due to government restrictions and due to insufficient human bioinformatics resources and lack of a central system for a nationwide interlaboratory QA program and data sharing, there is currently no affordable high-resolution, high-throughput diagnostic genetic test for CNVs available to the public in South Korea.

One major limitation of this study is that due to the costs of CMA, we were unable to investigate the families of the probands to confirm the clinical significance of some detected CNVs. Other limitations were associated with the sensitivity and specificity of the chosen CMA testing platform; low-level mosaicism and balanced rearrangements might not be detected [14]. For example, in patient 26, FISH revealed a derivative chromosome 15 resulting from a translocation between chromosomes 9 and 15.

This study illustrates the ability of CMA to greatly improve the diagnostic yield (66.7%) for patients with unexplained DD, ID, ASDs, and MCAs. However, it is also important to note that the clinical impact of CMA beyond the diagnosis is very limited. It is unlikely that any genomic genetic test will lead to better clinical outcomes for adults with DD, ID, ASDs, and MCAs, because the genetic diagnosis often does not lead to an intervention strategy for adult patients. Nevertheless, the diagnosis will influence the parents' reproductive planning, assist genetic counselors in

assessing recurrence risks and providing guidance to the patients' families, and ultimately help reduce childhood morbidity and mortality. Therefore, we recommend the introduction and recognition of CMA as the first-tier diagnostic genetic test in South Korea.

Authors' contribution

Conceptualization: SHL
Data curation: SHL, WJS
Formal analysis: SHL
Funding acquisition: SHL, WJS
Methodology: SHL
Writing - original draft: SHL
Writing - review & editing: SHL, WJS

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