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Research Paper

Chromosomal Aberrations in Pediatric Patients With Moderate/Severe Developmental Delay/Intellectual Disability With Abundant Phenotypic Heterogeneities: A Single-Center Study

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ABSTRACT

Background: This study aimed to examine the clinical usefulness of chromosome microarray (CMA) for selective implementation in patients with unexplained moderate or severe developmental delay/intellectual disability (DD/ID) and/or combined with different dysphonic features in the Han Chinese population.
Methods: We retrospectively analyzed data on 122 pediatric patients with unexplained isolated moderate/severe DD/ID with or without autism spectrum disorders, epilepsy, dystonia, and congenital abnormalities from a single-center neurorehabilitation clinic in southern China.
Results: A total of 46 probands (37.7%) had abnormal CMA results among the 122 study patients. With the exclusion of aneuploidies, uniparental disomies, and multiple homozygotes, 37 patients harbored 39 pathogenic copy number variations (pCNVs) (median [interquartile range] size: 3.57 [1.6 to 7.1] Mb; 33 deletions and 6 duplications), enriched in chromosomes 5, 7, 15, 17, and 22, with a markedly high prevalence of Angelman/Prader-Willi syndrome (24.3% [nine of 37]). Three rare deletions in the regions 5q33.2q34, 17p13.2, and 13q33.2 were reported, with specific delineation of clinical phenotypes. The frequencies of pCNVs were 18%, 33.3%, 38.89%, 41.67%, and 100% for patients with 1, 2, 3, 4, and 5 study phenotypes, respectively; patients with more concomitant abnormalities in the heart, brain, craniofacial region, and/or other organs had a higher CMA diagnostic yield and pCNV prevalence ($P < 0.05$).

Ethics statement: This study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the Second Affiliated Hospital of SUMC, China (2020-4). Written informed consent to participate in this study was provided by the participants' legal guardian.

Consent for publication: All participants' legal guardians gave written informed consent for the publication of their medical information.

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Availability of data and material: The data that support the findings of this study are available from the corresponding author, Hongwu Wang, upon reasonable request.

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Conclusions: Clinical application of CMA as a first-tier test among patients with moderate/severe DD/ID combined with congenital structural anomalies improved diagnostic yields and the quality of clinical management in this series of patients.

Introduction

Developmental delay/intellectual disability (DD/ID) is the most common childhood neurodevelopmental abnormality, accounting for ~3% of cases worldwide.[1-3] In mainland China, 11,820,000 people were diagnosed with DD/ID in 2007, of whom 954,000 were younger than six years.[4] DD/ID is the failure to achieve certain developmental milestones at the appropriate age, involving physical, cognitive, communication, social, emotional, and/or adaptive skills.[5] This failure to achieve normal life skills potentiates substantial economic and emotional burdens on families and society.

Neurodevelopment is a transcriptionally orchestrated process. Genetic etiology, including chromosomal abnormalities (e.g., trisomies, microdeletions, and microduplications), has been proposed to account for approximately 40% of DD/ID cases.[6] Copy number variations (CNVs), encompassing insertions, deletions, and duplications of genomic sequences, are known to be important contributors to human genetic diversity[7] and are sources of genetic mutations associated with these disorders.[8,9] Large CNVs >400 kb are estimated to account for the clinical manifestations in ~25.7% of affected individuals.[10] As such, microarray-based genomic copy number analysis enabling the detection of submicroscopic chromosomal aberrations has been recommended as a standard practice for the diagnosis of patients with unexplained DD/ID, autism spectrum disorders (ASD), and multiple congenital anomalies (MCA) since 2010[11] and has been implemented as the first-tier diagnostic tool among the Chinese population since 2016.[12]

According to prior literature and consensus, the average diagnostic yield of chromosome microarray (CMA) for DD/ID was 15% to 20%.[11,13,14] Notably, the types and prevalence rates of CNVs and cytogenetic diagnosis rates in constitutional disorders varied between populations, races/ethnicities, and even regions.[6,13,15] Moreover, the positive CNVs detected were highly dependent on specific phenotypes and the severity and complexities of the concomitant morbidities.[16,17] For example, patients with craniofacial anomalies and cardiovascular defects have greater CNV enrichment than those with epilepsy or ASD.[10,13]

To date, limited studies have focused on the genetic architecture of patients with DD/ID in the Chaoshan area in China and the frequencies of pathogenic CNVs (pCNVs) in patients with unexplained moderate/severe DD/ID or DD/ID accompanied by abundant phenotypic heterogeneities are understudied. We therefore conducted a retrospective analysis based on prospectively collected data from a single clinic center and performed comprehensive comorbidity stratification. Together, we delineated the specific genotypes, phenotypes, and CMA diagnostic yields with heterogeneous manifestations.

Material and Methods

Subjects

This is a retrospective study conducted in the Neurorehabilitation Clinic of Second Affiliated Hospital of Shantou University Medical College (SUMC), China. CMA results from 122 patients with DD/ID who visited the clinic from September 2013 through June 2017 were reviewed. The inclusion criteria for the CMA analysis were as follows: (1) moderate to severe DD/ID diagnosed in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, with total intelligence quotient <55 assessed by the Chinese version of Gesell Child Development Scale or Wechsler Intelligence Scale for Children Revised in China, as applicable; (2) retrogression of neurodevelopment or progressive deterioration of clinical symptoms; (3) neither obvious causal structural abnormalities in their neuroimaging studies nor evident metabolic conditions that could explain their symptoms; and (4) complete phenotypic data. The exclusion criteria were as follows: (1) definite perinatal brain injury, premature encephalopathy, or postnatal bilirubin encephalopathy; and (2) individuals who had central nervous system infection or histories of toxication, hypoxia, and cranial trauma. This study was carried out with approval from the Institutional Review Board of Second Affiliated Hospital of SUMC (2020-4). All subjects' parents or legal guardians gave informed consent for the data analysis. All data were presented and analyzed anonymously.

Data collection

The phenotypic information of the patients was assessed routinely in the clinic. Neurodevelopmental specialists performed anthropometric measures and formal neuropsychologic evaluations. Instrumental evaluations (including brain magnetic resonance imaging [MRI], electroencephalogram, and ultrasound) were also conducted. The Chinese version of Gesell Child Development Scale (GDS-C2) and Wechsler Intelligence Scale for Children Revised in China (WISC-CR) were used to assess DD/ID. Information on family history, prenatal/perinatal/postnatal history, growth and development history, and social demographics was collected via a standard questionnaire. These clinical records, including CMA results, were prospectively collected, categorized, and accessed electronically by experienced experts. To evaluate the comorbidities, we categorized the patients into eight phenotype types: epilepsy/seizure, ASD, dystonia, craniofacial deformities ([CFD] mainly special face and microcephaly), congenital heart disease (CHD), brain MRI abnormalities (BMA), abnormalities in other organs (mainly in genitourinary organs and cleft lip and palate), and MCA (defined as two or more phenotypes of CHD, CFD, BMA, abnormalities in other organs).

CMA

The CMA tests were performed by the "KingMed Diagnostics" company, a well-known qualified company in China, with the qualification certificate for this company provided. DNA samples were obtained from the peripheral blood of the patients. CMA was performed using the Affymetrix CytoScanHD Array (Affymetrix, Santa Clara, CA, USA; includes 2.7 million probes [1.95 million copy number probes and 750,000 single-nucleotide polymorphism probes]) according to the manufacturer's protocols. The report principle was as follows: ≥50 kb (marker ≥20 kb) for deletion, ≥100 kb (marker ≥50 kb) for duplication, >3 Mb (marker ≥50 kb) and contained ≥2% autosomal genome, or two or more

chromosomes had two or more homozygous fragments larger than 8 Mb for homozygosity. Genomic positions were defined according to the human reference genome hg19/GRCh37. The CNVs detected were referred to as known CNVs available in well-known databases, including the database of genomic variants (DGV, http://dgv.tcag.ca/dgv/app/home), online Mendelian Inheritance in Man (OMIM, http://www.Omim.org), and database of Genomic Variation and Phenotype in Humans Using Ensemble Resources (DECIPHER, https://decipher.sanger.ac.uk/) to determine clinically significant CNVs. The pathogenicity of CNVs was predicted based on their size and gene content in accordance with the American College of Medical Genetics guidelines for the interpretation of CNV results.[18] CNVs predicted to be clinically relevant, pathogenic, or likely pathogenic were reported. Affymetrix Chromosome Analysis Suite Software was used for analysis if all the experimental quality control criteria were met. Normal human DNA provided by Affymetrix was used as a control standard.

Statistical analysis

We calculated the total CMA diagnostic fields and the pCNV prevalence rates after excluding aneuploidies, uniparental disomy (UPD), and homozygote(s). The Pearson chi-square or Fisher exact test was used to compare the diagnostic yields of CMA and pCNVs between the isolated DD/ID and the abovementioned eight categories of comorbidities and between sexes, when appropriate. CNV size differences between duplications and deletions were assessed by the Wilcoxon rank-sum test. In addition, we compared the diagnostic yields of CMA and pCNVs among patients with an increasing number of comorbidities by Cochran-Mantel-Haenszel analysis. All statistical analyses were performed with SAS software (version 9.4; SAS Institute, Cary, NC, USA). A two-sided $P < 0.05$ was considered statistically significant.

Results

We analyzed CMA results from 122 unrelated patients with unexplained moderate and severe DD/ID with or without dysmorphic features (Table 1). The investigated patients' ages ranged from 3 to 120 months (median [interquartile range (IQR)], 14.5 [7 to 24] months), and 76 (62.3%) were male. Young children (≤36 months) accounted for the majority of the patients (104 of 122; 85.2%). The specific phenotypes were divided into 10 categories: total DD/ID (122, 100%), isolated DD/ID (50, 40.98%), DD/ID with epilepsy/seizure (23, 18.5%), DD/ID and ASD (10, 8.2%), DD/ID and dystonia (8, 6.56%), DD/ID with CFD (39, 31.97%), DD/ID and CHD (12, 9.84%), DD/ID and BMA (mainly myelin retardation; 18, 14.75%), DD/ID with abnormalities in other organs (13, 10.66%), and DD/ID and MCA (56, 45.9%). Patients with ASD had the oldest average age, with a median of 45 (19 to 96) months.

The CMA diagnostic field was 37.7% (46 of 122) in the entire population and was 39.5% (30 of 76) for males and 34.8% (16 of 46) for females; *P*-difference: 0.6044. With the exclusion of seven aneuploidies (five of Down syndromes, one of Turner syndrome, one of trisomy 12 syndrome), one UPD of chromosome 14 (Kagami syndrome), and two multiple homozygotes (one combined with 0.421-Mb deletion in chr15q11.2), 37 patients (30.3%) had one or more clinically relevant pCNVs. The prevalence of pCNVs in males versus females was 31.6% vs 28.3%; $P = 0.6992$. The diagnostic yield of CMA was 26% for isolated DD/ID, 60.87% for DD/ID with seizure/epilepsy, 40% for DD/ID with ASD, 37.5% for DD/ID with dystonia, 48.72% for DD/ID with CFD, 50% for DD/ID with CHD, 55.56% for DD/ID with BMA, 53.85% for DD/ID with abnormities in other organs, 48.21% for DD/ID with at least one organ abnormality, and 55% for DD/ID combined with MCA. The prevalence of pCNVs was 18% for isolated DD/ID, 56.52% for DD/ID with seizure/epilepsy, 30% for DD/ID with ASD, 25% for DD/ID with dystonia, 46.15% for DD/ID with CFD, 41.67% for DD/ID with CHD, 50% for DD/ID with BMA, 50.7% for DD/ID with abnormities in other organs, 39.29% for DD/ID with at least one organ abnormality, and 50% for DD/ID combined with MCA. Collectively, DD/ID with epilepsy, CFD, BMA, at least one organ abnormality, or MCA had both higher CMA and pCNV diagnostic yields than isolated DD/ID (all $P < 0.05$; Table 1).

We further examined the association between CMA and pCNV diagnostic yields with phenotype complexities (Table 2). As expected, patients with DD/ID with more concomitant abnormalities

TABLE 1.
Characteristics and Phenotypes of the Study Participants

Phenotypes	Cases (n, %)	CMA Diagnostic Yield (n, %)	pCNVs Diagnostic Yield (n, %)	Males (n, %)	Age Median [IQR] (months)	*P* for CMA	*P* for pCNVs
Total DD/ID	122 (100)	46 (37.70)	37 (30.33)	76 (62.30)	14.5 (7.0-24.0)	0.1420	0.0972
Isolated DD/ID	50 (40.98)	13 (26.00)	9 (18.00)	30 (60.00)	13.0 (5.0-120.0)	-	-
DD/ID + seizure/epilepsy	23 (18.50)	14 (60.87)	13 (56.52)	14 (60.87)	15.0 (10.0-19.0)	0.0041	0.0009
DD/ID + ASD	10 (8.20)	4 (40.00)	3 (30.00)	7 (70.00)	45.0 (24.0-60.0)	0.4478	0.4027
DD/ID + dystonia	8 (6.56)	3 (37.50)	2 (25.00)	6 (75.00)	14.0 (6.0-21.5)	0.6716	0.6391
DD/ID + CHD	12 (9.84)	6 (50.00)	5 (41.67)	7 (58.33)	12.5 (7.5-22.5)	0.1616	0.1206
DD/ID + BMA	18 (14.75)	10 (55.56)	9 (50.00)	12 (66.67)	14.0 (10.0-24.0)	0.0230	0.0131
DD/ID + CFD	39 (31.97)	19 (48.72)	18 (46.15)	28 (71.28)	13.0 (6.0-24.0)	0.0267	0.0041
DD/ID + abnormalities in other organs	13 (10.66)	7 (53.85)	4 (30.70)	8 (61.54)	14.0 (5.0-21.0)	0.0917	0.4410
DD/ID + MCA	20 (16.40)	11 (55.00)	10 (50.00)	14 (70.00)	14.0 (5.0-27.0)	0.0209	0.0065
DD/ID + organ abnormalities	56 (45.90)	27 (48.21)	22 (39.29)	38 (67.87)	13.5 (7.0-24.0)	0.0185	0.0162

Abbreviations:
ASD = Autism spectrum disorder
BMA = Brain magnetic resonance imaging abnormality
CFD = Craniofacial deformities
CMA = Chromosome microarray analysis
CHD = Congenital heart disease
DD/ID = Developmental delay/intellectual disability
IQR = Interquartile range
MCA = Multiple congenital anomalies
n = Number
pCNVs = Pathogenic copy number variations
MCA: ≥2 of (CFD, CHD, BMA, and abnormities in other organs).
DD/ID with organ abnormalities: ≥1 of (CHD, BMA, CFD, and abnormities in other organs).

had a higher CMA diagnostic yield and pCNV prevalence ($P < 0.05$). The prevalence of genetic aberrances detected by CMA was 26%, 38.5%, 50%, 50%, and 100% for phenotypes 1, 2, 3, 4, and 5, respectively, among the mentioned phenotypic categories. The prevalence rates of pCNVs were 18%, 33.3%, 38.89%, 41.67%, and 100% for patients with phenotypes 1, 2, 3, 4, and 5, respectively.

Of the 37 patients who harbored 39 pCNVs (33 deletions and six duplications; Table 3), the majority (35 of 37) carried a single clinically relevant pCNV and only two patients (two of 37) carried two CNVs; the median [IQR] size was 3.57 [1.6 to 7.1] Mb. The CNV sizes in deletions were generally smaller than those in duplications. Among the 33 deletions, the median [IQR] CNV size was 3.2 [1.6 to 6.2] Mb, ranging from 0.421 to 33.9 Mb, whereas in the six duplications, the median [IQR] size was 5.6 [3.4 to 12.3] Mb, ranging from 2.8 to 18.3 Mb (*P* for the size difference between deletion and duplication: 0.1019). Twenty-four patients (19.7%, 24 of 122) had *de novo* pCNVs, and the rest were of unknown inheritance patterns. Among the abnormal variants, 21 of 39 (53.8%) were below 5 Mb (the size range routinely detectable by karyotype) in size. These CNVs were enriched on chromosomes 5, 7, 15, 17, and 22. Of these 39 pCNVs, 36 were common syndromes, including nine associated with Angelman/Prader-Willi syndrome (AS/PWS), four associated with Williams-Beuren syndrome (WBS), three associated with 22q11.2 microdeletion/microduplication syndrome, two associated with DiGeroge syndrome, two associated with cri-du-chat syndrome, two associated with 17p11.2 microdeletion/microduplication syndrome, two associated with 1p36 microdeletion syndrome, two associated with Wolf-Hirschhorn syndrome, one associated with 2q37 microdeletion syndrome, one associated with Waardenburg syndrome, one associated with Sotos syndrome, one associated with 17p13.1 microdeletion syndrome, one associated with 17q21.31 microdeletion syndrome, one associated with 16p11.2 microdeletion syndrome, one associated with 12q24.31 microdeletion syndrome, and one associated with 4q32.2 duplication syndrome. There were three rare syndromes, including 5q33.2q34 microdeletion syndrome, 17p13.2 microdeletion, and 13q33.2 deletion syndrome. The specific phenotypes of the 37 patients with pCNVs are reported in Table 4. In brief, we documented a 19-month-old male proband with a *de novo* 7.8-Mb deletion in the 5q33.2q34 region (Supplementary eFigure 1). The patient was born at term without a notably abnormal prenatal or perinatal history and positive family history. The proband manifested with moderate DD/ID, epilepsy, and ASD, with a combination of facial deformities (wide nose, epicanthus). Brain MRI showed that the bilateral ventricles were widened and the hydrops were dilated. We also detected a 0.882-Mb 17p13.2 microdeletion in a male proband who presented at 33 months with severe DD and microcephaly, with brain MRI suggesting myelin retardation. In terms of the 13q33.2 deletion, an 18-month boy carrying a 9.6-Mb deletion with unknown inheritance presented with severe DD and epilepsy, albeit lacking notable abnormalities in other organs.

Twenty-three pCNVs were within the known recurrent microdeletions and microduplications (Table 5), including 5q35 Williams-Beuren syndrome (four), AS/PWS (nine), 16p11.2 microdeletion syndrome (one), Smith-Magenis syndrome (one), Potocki-Lupski syndrome (one), Koolen-de Vries syndrome (17q21.31 microdeletion syndrome) (one), and 22q11.2 microdeletion/microduplication syndrome (three). Among the 16 nonrecurrent regions (Table 6), the top two genomic disorders were 1p36 deletion and Wolf-Hirschhorn syndromes.

We further delineated the epilepsy phenotype of the detected pCNVs (Table 7). There were 14 patients with DD/ID with epilepsy phenotypes. Among them, except for one UPD, 13 had deletions located on chromosomes 1, 2, 4, 5, 7, 13, 15, 16, 17, and 22, ranging from 0.555 Mb to 7.8 Mb in size. Notably, most DD/ID combined with epilepsy probands had phenotypic complexities (76.9% [10 of 13] had additional phenotypic abnormalities). Of these pCNVs, 7q11.23, 1p36, 15q11.2, 16p11.2, 22q11.2, 4p16.3p, 17q 21.31, and 5q14.3q15 deletions were among known epilepsy-associated syndromes or in epilepsy hotspots and 5q33.2q34 and 13q33.2 deletions were among the rare CNVs.

Discussion

A growing number of studies have collectively confirmed the role of the CMA array in diagnosing neurodevelopmental disorders with unknown etiology.[13,19] In this study, we performed retrospective analyses on CMA and clinical data among 122 unrelated patients (76 males vs 46 females) with unexplained moderate/severe DD/ID or DD/ID combined with more complex morphologic phenotypes. The CMA diagnostic yield was 37.7%, and the prevalence of pCNVs accounting for their abnormal phenotypes was 30.3% after excluding aneuploidies, UPD, and multiple homozygotes. There was an overwhelming prevalence of copy number loss

TABLE 2.
Phenotype Complexities and Genotypes

Phenotype Counts	Cases (n)	CMA Diagnostic Field (n, %)	pCNVs Diagnostic Field (n, %)	Age (Median [IQR]); months	≤36 Months (n, %)	Males (n, %)
1	50	13 (26.00)	9 (18.00)	13 (7.0-31.0)	42 (84.00)	30 (60.00)
2	39	15 (38.46)	13 (33.33)	16 (6.0-27.0)	34 (87.18)	22 (56.41)
3	18	9 (50.00)	7 (38.89)	15 (10.0-24.0)	16 (88.89)	13 (72.22)
4	12	6 (50.00)	5 (41.67)	15 (5.0-40.5)	9 (75.00)	10 (83.33)
5	3	3 (100.00)	3 (100.00)	13 (10.0-19.0)	3 (100.00)	1 (33.33)
6	0	-	-	-	-	-
7	0	-	-	-	-	-
8	0	-	-	-	-	-
Total	122	46 (37.70)	37 (30.30)	14.5 (7.0-24.0)	104 (85.25)	76 (62.20)
P value	-	0.0043	0.0025	-	-	-

Abbreviations:
ASD = Autism spectrum disorder
BMA = Brain magnetic resonance imaging abnormality
CFD = Craniofacial deformities
CHD = Congenital heart disease
CMA = Chromosome microarray analysis
DD/ID = Developmental delay/intellectual disability
IQR = Interquartile range
n = Number
pCNVs = Pathogenic copy number variations
Phenotypes: DD/ID, seizure/epilepsy, ASD, dystonia, CFD, CHD, BMA, and abnormalities in other organs.

TABLE 3.
Pathogenic CNVs Associated With DD/ID

Pathogenic CNVs Associated with DD/ID	Number	Genomic Coordinates (NCBI 37, hg 19)
1p36 deletion syndrome	2	Chr1: 849,466-3,743,391 Chr1: 948,409-4,523,376
2q37 deletion syndrome	1	Chr2:233,291,336-242,783,384
2q23.1 microdeletion syndrome	1	Chr2:148,720,994-149,655,641
Wolf-Hirschhorn syndrome	2	Chr4: 1,537,622-8,594,759 Chr4: 68,345-2,133,290
Cri-du-chat syndrome[b]	2	Chr5: 113,576-34,098,585 Chr5: 113,576-5,213,074[#]
Sotos syndrome	1	Chr5: 172,533,407-197,514,609
5q33.2q34 deletion	1	Chr5: 153,179,494-160,966,394
5q14.3 deletion syndrome	1	Chr5: 85,554,714-92,651,412
Williams-Beuren syndrome	4	Chr7: 72,612,042-74,287,433 Chr7: 72,718,277-74,142,256 Chr7: 72,654,781-74,143,060 Chr7: 72,723,370-74,141,494
10p15.3p13 duplication[b]	1	Chr10: 100,047-12,395,881
11q23.3 duplication[a]	1	Chr11:116,681,007-134,938,470
12q24.31 microdeletion syndrome	1	Chr12: 122,266,560-123,951,064
13q33.2 deletion syndrome	1	Chr13: 105,474,272-115,107,733
14q32.2 duplication syndrome	1	Chr14: 99,666,211-107,285,437
Angelman or Prader-Willi syndrome	9	Chr15: 22,770,421-30,386,398 Chr15: 23,290,862-28,545,459 Chr15: 23,288,336-28,545,355 Chr15: 22,770,421-28,928,730 Chr15: 22,770,421-28,723,454 Chr15: 22,770,421-23,191,651 Chr15: 22,770,421-28,823,722 Chr15: 23,462,543-28,769,426 Chr15: 22,770,421-29,081,921
16p11.2 microdeletion syndrome	1	Chr16: 29,622,757-30,177,999
Potocki-Lupski syndrome	1	Chr17: 16,741,411-20,339,795
17p11.2 microdeletion syndrome	1	Chr17: 16,579,803-18,483,206
17p13.2 microdeletion syndrome	1	Chr17: 6,386,880-7,268,846
17q21.31 microdeletion syndrome	1	Chr17: 44,195,806-44,784,639
DiGeorge syndrome	2	Chr22: 18,916,842-21,800,797 Chr22: 18,636,748-21,798,907
Waardenburg syndrome	1	Chr22: 36,430,787-39,019,853
22q11.21 microduplication syndrome[a]	2	Chr22: 18,640,299-21,465,659 Chr22: 16,888,899-20,311,858

Abbreviations:
Chr = Chromosome
CNV = Copy number variations
DD/ID = Developmental delay/intellectual disability
Two patients harbored 2 pathogenic CNVs; "a", "b" indicates the CNV presented in one patient.

(33 deletions vs six duplications). The diagnostic yields of CMA and pCNVs were highly dependent on the types and complexities of the concomitant morbidities; the diagnostic yields of CMA in isolated DD/ID were much lower than those in DD/ID combined with other comorbidities, especially congenital malformations of solid organs. The more phenotypic complexities there were, the higher the CMA diagnostic yield. In addition, recurrent CNVs were more commonly observed than nonrecurrent CNVs (23 vs 16) in the study cohort. AS/PWS (nine of 37) and WBS (four of 37) had a markedly high prevalence among the study participants and were positive for comorbidity enrichments. Furthermore, we also reported three rare deletions accounting for DD/ID in the regions of 5q33.2q34, 17p13.2, and 13q33.2.[16]

The average diagnostic rate of CMA is commonly reported to be 15% to 20%.[6,16,20] It is known that the diagnostic yield of a cohort involves multiple influential factors, including but not limited to sociodemographic characteristics (e.g., recruiting age and sex), referring physician specialty, and referring indication (or combination of indications) for testing.[21] The application of the CMA array to the selected study patients may underline this overall higher contribution of pCNVs in the study DD/ID cohort. Emerging data have suggested that the diagnostic yields of CMA in patients with DD/ID are highly associated with comorbid features.[13,20,22,23] Our study comprised patients with DD/ID with moderate/severe disorders, and a substantial proportion of these patients had additional neurodevelopmental disorders and/or congenital abnormalities (31.97% with CFD and 45.9% with at least one structural abnormality). Rare CNVs were most commonly identified in patients with DD/ID with CFD and MCA1[13,16,19]; thus, the large proportion of patients with DD/ID with comorbid features yielded a high diagnostic rate.

Syndromic patients (those exhibiting two or more phenotypes) had a higher yield of pCNVs than nonsyndromic patients. In subgroup analyses among the phenotypic heterogeneities, we observed a 26% CMA diagnostic yield and 18% pCNV prevalence rate for patients with isolated DD/ID, comparable to the findings reported in other studies.[11,13] As expected, higher diagnostic yields of CMA and prevalence rates of pCNVs were documented in patients with congenital malformations in the craniofacial region, heart, and other organs, similar to previous findings among the Chinese population.[13,16,19] A large-scale population-based study in China suggested that neurodevelopmental disorders, including attention-deficit/hyperactivity disorder (ADHD), ASD, and myopathy, had lower CMA yields than congenital structural abnormalities.[13] Consistent with these findings, the combination of other neurodevelopment disorders, for example, ASD and dystonia, failed to increase the diagnostic yield of CMA in our study. Notably, the combination of epilepsy phenotypes in our study had a significantly high prevalence of pCNVs, differing from the results reported in other studies.[16,24] Indeed, meta-analysis studies indicated that CMA may not be the most cost-effective test in a generic case of epilepsy of unknown etiology due to the low diagnostic field.[24,25] We interpreted the difference to result from more substantial phenotypic complexities among the probands diagnosed with epilepsy with DD/ID; over 75% had three phenotypic comorbidities. Consistently, we observed a higher prevalence rate of pCNVs with increasing aberrant phenotype frequencies. A previous study also documented a pCNV diagnostic yield of 53.62% among Chinese patients with four or more phenotypes.[13] The CMA diagnostic yield appeared to be positively associated with phenotypic complexities; the increased number of comorbidities was associated with a higher prevalence of pCNVs.

In addition, population-specific CMA profiles allowed for an overall comparative analysis of clinical presentations in a list of genomic disorders and aided in interpreting the genotype-phenotype associations. Although it has been reported that nonrecurrent genomic disorders are more prevalent in patients of Chinese descent than in those of Western descent,[26] in our study participants we detected 23 recurrent CNVs among the 39 detected. DD/ID, ASD, and MCA associated with recurrent CNVs are considered novel microdeletion/duplication syndromes.[27] Notably, our study probands had an overwhelming prevalence of AS/PWS (nine of 37). As a well-established genomic disorder, AS/PWS has been reported to have high penetrance among the Chinese DD/ID population.[16,19,23,28] For example, a multicenter study in southern China reported 42 patients with AS/PWS among 489 patients with DD/ID.[19] Further studies are warranted to investigate whether the high incidence of PWS/AS among the Chinese population is an incidental result or a result of specific influences from the patients' environment and genetic backgrounds. Conversely, cases of recurrent 17q12.31 deletion were infrequently reported in the Chinese

TABLE 4.
Characteristics of Probands Carrying Pathogenic CNVs

Probands	Related Syndromes	Types	Sizes	Inheritances	Genomic Coordinates	Dystonia	DD/ID	Epilepsy	ASD	CFA	MCA	BMA
23 m/F	Williams-Beuren syndrome	Del	1.6 Mb	*De novo*	7q11.23 (72,612,042-74,287,433)	-	+	-	-	+	-	N
16 m/M	Williams-Beuren syndrome	Del	1.4 Mb	*De novo*	7q11.23 (72,718,277-74,142,256)	-	+	-	-	+	CHD (aortic valve stenosis)	Myelin retardation
21 m/F	Williams-Beuren syndrome	Del	1.4 Mb	*De novo*	7q11.23 (72,654,781-74,143,060)	-	+	-		+	-	N
84 m/M	Williams-Beuren syndrome	Del	1.4 Mb	*De novo*	7q11.23 (72,723,370-74,141,494)	-	+	+	-	+	CHD (aortic stenosis)	N
19 m/M	5q33.2q34 deletion	Del	7.8 Mb	*De novo*	5q33.2q34 (153,179,494-160,966,394)	-	+	+	+	+	-	Bilateral ventricles were widened and hydrops were dilated
13 m/F	5q14.3 deletion syndrome	Del	7.1 Mb	*De novo*	5q14.3q15 (85,554,714-92,651,412)	-	+	+	-	-	-	Bilateral paraventricular white matter malacia
13 m/M	Cri-du-chat syndrome/ 10p15.3p13 duplication	Del/ Dup	5.1 Mb/ 12.3 Mb	Unknown	5p15.33p15.32 (113,576-5,213,074) and 10p15.3p13 (100,047-12,395,881)	-	+	-	-	-	-	N
3 m/M	Cri-du-chat syndrome	Del	33.9 Mb	*De novo*	5p15.33p13.2 (113,576-34,098,585)	-	+	-	-	+	-	N
6 m/F	Wolf-Hirschhorn syndrome	Del	7.1 Mb	*De novo*	4p16.3p16.1 (1,537,622-8,594,759)	-	+	+	-		-	N
42 m/M	Wolf-Hirschhorn syndrome	Del	2.1 Mb	*De novo*	4p16.3 (68,345-2,133,290)	-	+	-	-	+	Cleft palate	N
23 m/M	2q37 deletion syndrome	Del	9.5 Mb	*De novo*	2q37.1q37.3 (233,291,336-242,783,384)	-	+	-	+	-	-	N
24 m/M	2q23.1 microdeletion syndrome	Del	0.935 Mb	Unknown	2q23.1 (148,720,994-149,655,641)	-	+	+	+	-	-	N
9 m/M	Waardenburg syndrome	Del	2.6 Mb	*De novo*	22q12.3 (36,430,787-39,019,853)	-	+	-	-	+	+	N
13 m/M	22q11.21 microduplication syndrome	Dup	2.8 Mb	Unknown	22q11.21 (18,640,299-21,465,659)	-	+	-	-	-	+	N
13 m/F	DiGeorge syndrome	Del	2.9 Mb	*De novo*	22:(18,916,842-21,800,797)	-	+	-	-	+	-	Myelin retardation
4 m/M	DiGeorge syndrome	Del	3.2 Mb	*De novo*	22q11.21 (18,636,748-21,798,907)	-	+	+	-	+	Cleft uvula with soft and hard palate	N
7 m/F	1p36 deletion syndrome	Del	2.9 Mb	*De novo*	1p36.33 (849,466-3,743,391)	-	+	-	-	-	-	Myelin retardation
33 m/M	17p13.2 microdeletion syndrome	Del	0.882 Mb	Unknown	17p13.2 (6,386,880-7,268,846)	-	+	-	-	+	-	Myelin retardation
16 m/M	Potocki-Lupski syndrome	Dup	3.6 Mb	*De novo*	17p11.2 (16,741,411-20,339,795)	+	+	-	-	-	-	N
7 m/M	Angelman syndrome	Del	7.6 Mb	*De novo*	15q11.2 (22,770,421-30,386,398)	-	+	-	-	+	-	N
10 m/F	Angelman syndrome	Del	5.3 Mb	*De novo*	15q11.2 (23,290,862-28,545,459)	-	+	+	-	+	-	N
9 m/M	Angelman syndrome	Del	5.3 Mb	*De novo*	15q11.2 (23,288,336-28,545,355)	-	+	+	-	+	-	N
3 m/F	Angelman or Prader-Willi syndrome	Del	6.2 Mb	Unknown	15q11.2 (22,770,421-28,928,730)	-	+	-	-	+	-	N
14 m/M	Angelman or Prader-Willi syndrome	Del	6.0 Mb	Unknown	15q11.2 (22,770,421-28,723,454)	-	+	+	-	+	-	Myelin retardation
36 m/F	Angelman or Prader-Willi syndrome	Del	0.421 Mb/ 204 Mb	Unknown	15q11.2 (22,770,421-23,191,651)	-	+	-	-	+	-	N
6 m/M	Angelman or Prader-Willi syndrome	Del	6.0 Mb	Unknown	15q11.2 (22,770,421-28,823,722)	+	+	-	-	+	cryptorchidism	N

(*continued on next page*)

Table 4. (*continued*)

Probands	Related Syndromes	Types	Sizes	Inheritances	Genomic Coordinates	Dystonia	DD/ID	Epilepsy	ASD	CFA	MCA	BMA
18 m/M	13q33.2 deletion syndrome	Del	9.6 Mb	Unknown	13q33.2 (105,474,272-115,107,733)	-	+	+	-	-	-	N
24 m/M	12q24.31 microdeletion syndrome	Del	1.6 Mb	Unknown	12q24.31 (122,266,560-123,951,064)	-	+	-	-	-	-	N
6 m/M	11q23.3 and 22q11.1-q11.21 duplication	Dup/Dup	11-18.3 Mb; 22-3.4 Mb	Unknown	11q23.3 (116,681,007-134,938,470)/22q11.1 (16,888,899-20,311,858)	-	+	-	-	+	-	N
10 m/F	17q21.31 microdeletion syndrome	Del	0.589 Mb	*De novo*	17q21.31 (44,195,806-44,784,639)	-	+	+	-	+	CHD (Patent ductus arteriosus)	Myelin retardation and supratentorial hydrocephalus
12 m/M	16p11.2 microdeletion syndrome	Del	0.555 Mb	*De novo*	16p11.2 (29,622,757-30,177,999)	-	+	+	-	-	-	Myelin retardation
48 m/F	14q32.2 duplication syndrome	Dup	7.6 Mb	*De novo*	14q32.2 (99,666,211-107,285,437)	-	+	-	-	-	-	N
36 m/F	1p36 microdeletion syndrome	Del	3.57 Mb	*De novo*	1p36.33 (948,409-4,523,376)	-	+	+	-	-	-	N
23 m/M	17p11.2 microdeletion syndrome	Del	1.9 Mb	Unknown	17p11.2 (16,579,803-18,483,206)	-	+	-	-	-	-	N
7 m/M	Prader-Willi syndrome	Del	5.3 Mb	*De novo*	15q11.2 (23,462,543-28,769,426)	-	+	-	-	-	-	N
1 m/M	Sotos syndrome	Del	2.5 Mb	Unknown	5q35.2 (172,533,407-197,514,609)	-	+	-	-	-	-	N
2 m/F	Angelman or Prader-Willi syndrome	Del	6.3 Mb	*De novo*	15q11.2 (22,770,421-29,081,921)	-	+	-	-	-	-	N

Abbreviations:
ASD = Autism spectrum disorders
BMA = Brain magnetic resonance imaging abnormalities
CFA = Craniofacial deformities
CHD = Congenital heart disease
DD/ID = Developmental delay/intellectual disability
Del = Deletion
Dup = Duplication
F = Female
m = Month
M = Male
MCA = Multiple congenital anomalies
Detailed descriptions of brain MRI images are displayed in Supplementary eTable 2.

TABLE 5.
Recurrent pCNVs (n = 23)

Pathogenic CNVs Associated with DD/ID	Number	Genomic Coordinates (NCBI 37, hg 19)
Cri-du-chat syndrome	2	Chr5: 113,576-34,098,585 Chr5: 113,576-5,213,074
Williams-Beuren syndrome	4	Chr7: 72,612,042-74,287,433 Chr7: 72,718,277-74,142,256 Chr7: 72,654,781-74,143,060 Chr7: 72,723,370-74,141,494
Angelman or Prader-Willi syndrome	9	Chr15: 22,770,421-30,386,398 Chr15: 23,290,862-28,545,459 Chr15: 23,288,336-28,545,355 Chr15: 22,770,421-28,928,730 Chr15: 22,770,421-28,723,454 Chr15: 22,770,421-23,191,651 Chr15: 22,770,421-28,823,722 Chr15: 23,462,543-28,769,426 Chr15: 22,770,421-29,081,921
16p11.2 microdeletion syndrome	1	Chr16: 29,622,757-30,177,999
Potocki-Lupski syndrome	1	Chr17: 16,741,411-20,339,795
17p11.2 microdeletion syndrome	1	Chr17: 16,579,803-18,483,206
17q21.31 microdeletion syndrome	1	Chr17: 44,195,806-44,784,639
DiGeorge syndrome	2	Chr22: 18,916,842-21,800,797 Chr22: 18,636,748-21,798,907
22q11.21 microduplication syndrome	2	Chr22: 18,640,299-21,465,659 Chr22: 16,888,899-20,311,858

Abbreviations:
Chr = Chromosome
CNV = Copy number variations
DD/ID = Developmental delay/intellectual disability
pCNVs = Pathogenic CNVs

population associated with DD/ID,[13,23] with only one deletion in 17q21.31 associated with DD/ID and epilepsy reported thus far.[16] Aside from the 17q21.31 deletion, other CNVs on chromosome 17 were also proposed to be less prevalent in Chinese descendants.[13,23] Nevertheless, our study observed three pCNVs accounting for DD/ID in southern China, including a 10-month girl carrying a *de novo* 0.589-Mb deletion in the 17q21.31 region who manifested with DD, epilepsy, CHD (patent ductus arteriosus), CFD, and a brain MRI displaying myelin retardation and supratentorial hydrocephalus. Furthermore, compared with the results from a large-scale study in China, WBS was less positively associated with the MCA phenotypes among the Chinese population. However, our study documented four patients with WBS, with deletion sizes ranging from 1.4 Mb, manifesting with CHD (two of four, aortic stenosis) and CFD (four of four), but not moderate/severe DD/ID. In summary, despite a small sample size, our study provided valuable delineations of clinical phenotypes and genetic architectures among a DD/ID cohort from the Chaoshan area, adding to the current understanding of specific disease heterogeneities.

TABLE 6.
Nonrecurrent pCNVs (n = 16)

Pathogenic CNVs Associated with DD/ID	Number	Genomic Coordinates (NCBI 37, hg 19)
1p36 deletion syndrome	2	Chr1: 849,466-3,743,391 Chr1: 948,409-4,523,376
2q37 deletion syndrome	1	Chr2:233,291,336-242,783,384
2q23.1 microdeletion syndrome	1	Chr2:148,720,994-149,655,641
Wolf-Hirschhorn syndrome	2	Chr4: 1,537,622-8,594,759 Chr4: 68,345-2,133,290
Sotos syndrome	1	Chr5: 172,533,407-197,514,609
5q33.2q34 deletion	1	Chr5: 153,179,494-160,966,394
5q14.3 deletion syndrome	1	Chr5: 85,554,714-92,651,412
10p15.3p13 duplication	1	Chr10: 100,047-12,395,881
11q23.3 duplication	1	Chr11:116,681,007-134,938,470
12q24.31 microdeletion syndrome	1	Chr12: 122,266,560-123,951,064
13q33.2 deletion syndrome	1	Chr13: 105,474,272-115,107,733
14q32.2 duplication syndrome	1	Chr14: 99,666,211-107,285,437
17p13.2 microdeletion syndrome	1	Chr17: 6,386,880-7,268,846
Waardenburg syndrome	1	Chr22: 36,430,787-39,019,853

Abbreviations:
Chr = Chromosome
CNV = Copy number variations
DD/ID = Developmental delay/intellectual disability
pCNVs = Pathogenic CNVs

Moreover, our study also documented rare pCNVs that potentially contribute to the etiology of DD/ID. We observed a boy harboring a 7.8-Mb *de novo* deletion in the 5q33.2q34 region who manifested with severe DD, ASD, and epilepsy. To the best of our knowledge, existing studies which have reported the same deletion and linked it to neurodevelopmental disorders (NDDs) are sparse. Comparing the clinical features of patients with overlapping deletions favors the discovery of potential disease-relevant genes.[29] We then searched the DECIPHER database for overlapping CNVs and available clinical delineations of the 5q33.2q34 deletion and found 11 fully characterized 5q33.2 deletions overlapping the deletion in our study proband. A minimum 3.4-Mb region that probably contains the dosage-sensitive element(s) was narrowed down to underlie the cardinal features of the disorder. In addition, a 5q33-q34 deletion has been associated with epilepsy encephalopathies.[30] Nevertheless, future additional studies and comparisons with similar cases are required to evaluate the effects of deletions that overlap specific genes.

DD/ID and its symbolic comorbidities are a substantial financial and emotional burden on the patient's family and society. The etiologic diagnosis of DD/ID remains challenging in clinical practice due to the substantial involvement of diverse genetic and environmental factors.[31-33] Early discoveries relied on a common clinical presentation, the ability to detect chromosomal abnormalities by specific assays, and a professional clinical evaluation. Although no specific cure is available and 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 consequent financial and emotional improvements for patients and their families, especially for those who have endured a “diagnostic odyssey.” For example, one of the parents in our study was convinced to try the “alien therapy,” which costs 100,000 yuan per treatment. Without a clear diagnosis and correct treatment expectations, the caregivers of the patients are likely to be duped by individuals who take advantage of their insufficient understanding, their desire to improve their children's health, and even their sense of guilt. Providing an early and scientific diagnosis in time is critical. Our study indicates that CMA is an excellent diagnostic tool for unexplained DD/ID in southern China, especially for those combined with dysmorphic features. Nevertheless, a great proportion of probands remained undiagnosed with CMA. Recently, next-generation sequencing (including exome and/or genome sequencing [ES/GS]) has further exposed the complex genetic architecture of NDDs and outperformed CMA for their shorter turnaround times and/or higher diagnostic yields. Several studies comparing the clinical utility or cost-effectiveness of ES/GS with that of CMA have collectively highlighted the higher molecular diagnosis value and the maximized cost-efficiency by early application of ES/GS.[34,35] Indeed, in 2021, the American College of Medical Genetics and Genomics developed an evidence-based clinical guideline for the clinical practice of genetic tests and strongly recommended ES/GS as a first- or second-tier test for

TABLE 7.
Pathogenic CNVs in Patients With DD/ID With Epilepsy Phenotypes

Probands	Syndromes	Regions	Types	Sizes	Inheritances	Genomic Coordinates (NCBI 37, hg 19)	Phenotypes Counts
84 m/M	Williams-Beuren syndrome	chr7q11.23	Del	1.4 Mb	*De novo*	Chr 7: 72,723,370-74,141,494	4 (CHD + CFA)
19 m/M	5q33.2q34 deletion syndrome	chr5q33.2q34	Del	7.8 Mb	*De novo*	Chr 5: 153,179,494-160,966,394	5 (BMA + ASD + CFA)
13 m/F	5q14.3q15 deletion syndrome	chr5q14.3q15	Del	7.1 Mb	*De novo*	Chr 5: 85,554,714-92,651,412	3 (BMA)
6 m/F	Wolf-Hirschhorn syndrome	chr4p16.3p16.1	Del	7.1 Mb	*De novo*	Chr 4: 1,537,622-8,594,759	2
24 m/M	2q23.1 microdeletion syndrome	chr2q23.1	Del	0.935 Mb	Unknown	Chr 2: 148,720,994-149,655,641	3 (ASD)
10 m/F	Angelman syndrome	chr15q11.2q13.1	Del	5.3 Mb	*De novo*	Chr 15: 23,290,862-28,545,459	4 (CHD + abnormalities in other organs)
9 m/M	Angelman syndrome	chr15q11.2q13.1	Del	5.3 Mb	*De novo*	Chr 15: 23,288,336-28,545,355	3 (CFA)
14 m/M	Angelman or Prader-Willi syndrome	chr15q11.2q13.1	Del	6.0 Mb	Unknown	Chr 15: 22,770,421-28,723,454	3 (CFA)
18 m/M	13q33.2 deletion syndrome	Chr13q33.2-ter	Del	9.6 Mb	Unknown	Chr13: 105,474,272-115,107,733	4 (BMA + CFA)
10 m/F	17q21.31 microdeletion syndrome	ch17q21.31	Del	0.589 Mb	*De novo*	Chr 17: 44,195,806-44,784,639	2
12 m/M	16p11.2 microdeletion syndrome	ch16p11.2	Del	0.555 Mb	*De novo*	Chr 16: 29,622,757-30,177,999	5 (CHD + BMA + CFA)
4 m/M	DiGeorge syndrome	chr22q11.21	Del	3.2 Mb	*De novo*	Chr 22: 18,636,748-21,798,907	3 (BMA)
36 m/F	1p36 microdeletion syndrome	Chr1p36.33	Del	3.57 Mb	*De novo*	Chr1: 948,409-4,523,376	2

Abbreviations:
ASD = Autism spectrum disorder
BMA = Brain magnetic resonance imaging abnormality
CFD = Craniofacial deformities
CHD = Congenital heart disease
CMA = Chromosome microarray analysis
DD/ID = Developmental delay/intellectual disability
MCA = Multiple congenital anomalies
pCNVs = Pathogenic copy number variations
n = Number

patients with congenital anomalies or DD/ID.[36] At present, the consensus on pediatric genetic diseases in China recommends a stepwise approach starting with CMA, if negative ES/GS is thereafter recommended.[12] For reasons mainly including knowledge gap regarding the genetic tests, family disharmony, or financial burdens, only 11 of the undiagnosed probands in this study underwent a further "clinical exome" test (Illumina TruSight One "clinical exome" 4811 genes panel)[37] at that time. Six of them (six of 11) had pathogenic or likely pathogenic variants and one (one of 11) had a variant of unknown clinical importance. The high diagnostic value by further exome sequencing supported ES/GS as an important alternative for exploring genetic etiology (the characteristics of the probands and detected variants are displayed in Supplementary eTable 1).

Collectively, our study revealed new findings with certain clinical significance. First, syndromic patients had a higher yield of pCNVs than nonsyndromic patients. Second, DD/ID with congenital structural aberrances had a more stable and increased diagnostic yield of pCNVs than DD/ID with manifestations of other NDDs. For DD/ID with additional NDDs, the pCNV contribution was highly dependent on the age of the study participants, as the inconclusive properties of the developing phenotypes increase over time. Third, the mechanisms underlying the highly variable expression of neurocognitive and neuropsychiatric disorders in similar CNVs, including different types and severities of DD/ID, epilepsy, and ASD, require further exploration. Fourth, although the sizes of the deletions and duplications among the observed pCNVs were not significantly different, there was a marked predominance in copy number loss in the study population (36:3). This CNV distribution is consistent with the phenomenon that the human genome is less tolerant of deletion than duplication.[38] Furthermore, given the developing nature of NDDs (as the majority of the study participants were under age three years, when the disease phenotypes may be immature), ongoing monitoring is needed. Developmental surveillance (i.e., ongoing monitoring of development, identification of risk factors, and elicitation of parental concerns) is critical.[39,40] For example, probands with a deletion in the 12q24.31 region were reported to be at risk for depressive disorders.[41] Probands with a deletion in the 17p13.2 region are at risk for developing cancers.[42]

Our study has several strengths. As there is a possible difference in genomic architecture among populations, races/ethnicities, and regions,[13] our study enriches the phenotypic diversities of the observed CNVs. In addition, this is the first study focusing on the genetic architecture of DD/ID combined with a high diversity of phenotypic disorders in the Chaoshan area, potentially extending the region-specific etiologic understanding. Furthermore, overlapping clinical features may indicate common neurophysiological pathways. Our detected syndromes with syndromic DD/ID extend the growing list of multiorgan abnormalities observed with aberrant CNVs and underscore the causative roles of chromatin modifiers in cognitive and craniofacial development. As pre- and postnatal genetic testing by array comparative genomic hybridization has emerged in genetic counseling,[43] more medical knowledge of pCNVs is required to keep up with technological advances. Our study revealed rare CNVs associated with DD/ID and specifically delineated clinical phenotypes, aiding in the interpretation of rare CNVs and further identifying the candidate genes involved.

Limitations of this study should be noted. First, the relatively small sample size inevitably led to the underpowering of statistical significance in some subgroup analyses. Second, a single-center study design among the Han Chinese population in the Chauhan area may potentially limit the generalizability of the results to individuals in the rest of the country or other races/ethnicities; however, the Chaoshan area is commonly considered a representative population for the Han population as it migrated from the ancient Han area. Third, it is a retrospective analysis; nonetheless, the prospectively collected data and single-center measurements ensure relatively high-quality data. In addition, the relative homogeneity of the study population in terms of environmental exposure enhanced the internal validity and the analysis of the region-specific genetic background of patients with DD/ID. Fourth, partly resulting from the developing nature of neurological disorders and the specific age limit for the diagnosis of certain diseases, the phenotypes described here were not conclusive, as most of the probands were under age 36 months. In addition, we failed to

obtain the genotypes from some of the probands' parents, as they believed that delving into the source of a child's abnormal genetic information would lead to complex family problems and, therefore, declined further examination.

Conclusions

Our study describes our implementation of a CMA array for patients with moderate/severe DD/ID with unknown etiology combined with other phenotypic heterogeneities. The difference in CMA diagnostic yield differed by specific comorbidities, and phenotypic complexities are clinically informative in the practice of CMA among these kinds of patients. Overall, exploring region-specific genetic architecture is instrumental for an etiologic understanding of a particular population. Furthermore, delineating phenotype-genotype associations resulting from rare CNVs contributes to understanding the range of phenotypes associated with pCNVs.

Declaration of competing interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2023.06.001.

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