Clinical Application of Chromosome Microarray Analysis in Han Chinese Children with Neurodevelopmental Disorders

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Abstract Chromosome microarray analysis (CMA) is a cost-effective molecular cytogenetic technique that has been used as a first-line diagnostic test in neurodevelopmental disorders in the USA since 2011. The impact of CMA results on clinical practice in China is not yet well studied, so we aimed to better evaluate this phenomenon. We analyzed the CMA results from 434 patients in our clinic, and characterized their molecular diagnoses, clinical features, and follow-up clinical actions based on these results. The overall diagnostic yield for our patients was 13.6% (59 out of 434). This gave a detection rate of 14.7% for developmental delay/intellectual disability (DD/ID, 38/259) and 12% for autism spectrum disorders (ASDs,

21/175). Thirty-three recurrent ($n \geq 2$) variants were found, distributed at six chromosomal loci involving known chromosome syndromes (such as DiGeorge, Williams Beuren, and Angelman/Prader-Willi syndromes). The spectrum of positive copy number variants in our study was comparable to that reported in Caucasian populations, but with specific characteristics. Parental origin tests indicated an effect involving a significant maternal transmission bias to sons. The majority of patients with positive results (94.9%) had benefits, allowing earlier diagnosis (36/59), prioritized full clinical management (28/59), medication changes (7/59), a changed prognosis (30/59), and prenatal genetic counseling (15/59). Our results provide information on *de novo* mutations in Chinese children with DD/ID and/or ASDs. Our data showed that microarray testing provides immediate clinical utility for patients. It is expected that the personalized medical care of children with developmental disabilities will lead to improved outcomes in long-term developmental potential. We advocate using the diagnostic yield of clinically actionable results to evaluate CMA as it provides information of both clinical validity and clinical utility.

Keywords Chromosome microarray analysis · Neurodevelopmental disorder · Autism spectrum disorder · Chromosome syndrome · Clinical management

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Introduction

Neurodevelopmental disorders (NDDs), including but not limited to intellectual disability (ID), global developmental delay, and autism spectrum disorders (ASDs), affect >15% of children [1]. The prevalence estimates of developmental delay (DD)/ID range from 1% to 3% [2], and the estimated

prevalence of ASDs is 1 in 68 [3]. Based on the worldwide prevalence and considering the population of China, 770,000–2,310,000 children in China suffer from DD/ID. NDDs are of great concern for public health and society since they require expensive care throughout the whole life of a patient.

Chromosome microarray analysis (CMA) is a molecular cytogenetic technique which allows genome-wide scanning to detect clinically significant copy number variants (CNVs), and deletions and duplications as small as 10 kb as reported recently [4]. CMA holds promise as an efficient and cost-effective approach to genetic testing because of its high throughput, high resolution, and affordability. In 2010, The American College of Medical Genetics and Genomics published guidelines for CMA as a first-line diagnostic test in the following groups of patients: (1) multiple anomalies not specific to a well-delineated genetic syndrome, (2) apparently nonsyndromic DD/ID, and (3) ASDs [4, 5]. In the same year, the CMA test was further supported for pediatric practice by the International Collaboration for Clinical Genomics [4] and the American Academy of Neurology. Although the diagnostic yields vary [patients with global developmental delay/ID and/or ASD (median, 13.6%; interquartile range IQR, 9.5%–17.2%), and primarily ASD (median, 8.4%; IQR, 7.2%–17.3%)] [6], this test has been used in developed countries for several years to help clinicians improve the medical service, and to understand the prognosis and future monitoring.

Large-scale whole-genome CNV studies have established the importance of *de novo* CNVs in NDDs, especially in Caucasian populations of European ancestry [7–9]. Importantly, the CMA results efficiently affect the treatment plan. Retrospective studies have shown that the change rate of clinical management based on CMA findings is 34.0%–75.7% [10–13] in all reviewed abnormal CMA cases including DD/ID, ASDs, seizures, dysmorphic features, and congenital anomalies. Application of the CMA test in China started relatively late. According to several genome-wide studies during the past three years [14–17], the CMA-based diagnostic rate of NDDs in Chinese children is comparable to that reported worldwide. However, all these studies have focused on significant CNVs and new disease genes, while the impact of CMA results on clinical practice in China is not well studied.

Therefore, in this study, we analyzed 434 patients in our clinic using CMA, and we characterized their molecular diagnoses, clinical features, and follow-up clinical action based on the CMA results. The objective of this study was to investigate the abnormal CNVs in a Chinese NDD cohort and evaluate the impact of CMA results on the medical management of developmental behavioral

pediatric patients, in order to advance personalized treatment in China.

Methods

Patients

The clinical cohort that underwent chromosomal testing was recruited from July 2014 to December 2016 in two clinics of the Department of Developmental Behavioral Pediatrics of Shanghai Children's Medical Center and Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine. A total of 434 patients with different degrees of DD/ID and/or ASDs (371 males and 63 females; average age 5.63 years, ranging from 4 months to 17 years) were enrolled. Among them, 175 (1.6–9.8 years, 81.14% male) were diagnosed with ASDs based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, and confirmed with the Chinese version of the Autism Diagnostic Observation Schedule and/or the Autism Diagnosis Interview-Revised. Patients diagnosed with DD/ID using the intellectual assessment DQ <75 assessed with the Gesell development scales, or IQ <70 assessed with the Wechsler Intelligence Scale for Children-Revised or the Wechsler Preschool and Primary Scale of Intelligence [17], were included. The exclusion criteria were: (1) neurological disorders, such as cerebral palsy; and (2) known chromosomal/genetic disorders, mainly trisomy 21, 18, or 13 syndromes. This study was approved by the Ethics Committee of both Shanghai Children's Medical Center (SCMCIRB-K2014051) and Xinhua Hospital (XHEC-C-2017-062). Informed written consent was given by parents.

CMA

Genomic DNA was extracted from the peripheral blood of patients and their parents. CMA was performed using the CytoScanTM HD system (Affymetrix, Thermo fisher, Santa Clara, CA) following the manufacturer's instructions. Data were visualized and analyzed with the Chromosome Analysis Suite software package (Affymetrix). The CNV calling threshold was set at 25 consecutive probes encompassing 25 kb or more in length.

Genetic Analyses

A qualified cytogeneticist made a clinical laboratory interpretation for each sample and assessed each of the chromosomal CNV regions reported in every sample, classifying them as benign, likely benign, pathogenic, likely pathogenic, or variant of uncertain significance.

Data Analysis

Data were analyzed using SPSS version 18.0 software (SPSS Inc., Chicago, IL). A one-way ANOVA test was used when comparing three groups and rates. $P < 0.05$ was considered significant.

Results

Demographics

Of the 434 patients, 59 were found to have pathogenic/likely pathogenic variants, accounting for 13.6% of all patients. The general demographic features of the patients are listed in Table 1. There was a higher percentage with pathogenic variants under 2 years of age (70%) than in older patients (6.4% at 2–5 years and 12.7% at >5 years, $P < 0.05$).

Molecular Diagnoses

We detected 51 pathogenic CNVs in 47 patients (10.8%) and 12 likely pathogenic CNVs in 12 patients (2.8%) (Table 2). The overall diagnostic yield of CMA testing for patients with DD/ID and/or ASDs was 13.6% when considering pathogenic and likely pathogenic CNVs as positive findings. This gave a detection rate of 14.7% for DD/ID (38/259) and 12% for ASDs (21/175) (Table 1). The ages of the probands with positive results ranged from 4 months to 17 years.

We found 33 recurrent ($n \geq 2$) variants distributed at six chromosomal loci (Table 3); they included imbalances involved in known chromosomal syndromes like DiGeorge syndrome, Williams Beuren syndrome (WBS) and Angelman/Prader-Willi syndrome (AS/PWS). Notably, some

classical syndromic loci were found with both deletions and duplications, including 7q11.23, 15q11-q13, and 1q21.2. We found 12 imbalances (11 deletions and 1 duplication) at 7q11.23 and five aberrations (4 deletions and 1 duplication) at 1q21.2 involving the *GJA5* and *GJA8* genes. We also identified 8 patients with a 15q11-q13 abnormality (4 duplications, 3 deletions, and 1 uniparental disomy), which ranged in length from 5764 kb to 79000 kb. Furthermore, mosaicism was found for both 17q11.2 deletion and 5p terminal deletion.

In addition, parents were tested for patients carrying non-recurrent pathogenic/likely pathogenic CNVs smaller than 10 Mb, when samples were available. Finally, the parental origin was determined in 12 affected individuals (Table 4). CNVs carried by 8 patients were identified as *de novo*, and four were inherited. Specifically, in one patient, two *de novo* CNVs were verified to be caused by imbalanced DNA translocation between chromosomes 8 and 9.

We identified a 10-Mb microdeletion at 1p33-p32.2 (Fig. 1) in a 3.6-year-old boy with global developmental delay and congenital anomalies. This interstitial microdeletion on the short arm of chromosome 1 is a rare aberration, which has not been reported elsewhere. This deletion region overlaps with the 1p32-p31 deletion syndrome, of which critical gene is *NFIA*. Of note, the *NFIA* gene was not included in the CNV region of this case, and three possible candidate genes were further pinpointed: *ORC1*, *SCP2*, and *DAB1*. These three genes may be responsible for the main phenotypes observed in the patient: microcephaly, growth retardation, short stature, leukoencephalopathy, and DD/ID. The spectrum of phenotypes caused by this 1p33-p32.2 deletion may represent a new microdeletion syndrome.

Table 1 Demographics of patients and CMA diagnostic yield.

	Total no.	Patients with pathogenic CNV (%)	Patients with likely pathogenic CNV (%)	Diagnostic yield (%)
Total	434	47 (10.8)	12 (2.8)	59 (13.6)
Sex				
Male	371	32 (8.6)	12 (3.2)	44 (11.8)
Female	63	15 (23.8)	0	15 (23.8)
Age (years)				
<2	20	14 (70)	1 (5)	15 (75)
2-5	312	20 (6.4)	7 (2.2)	27 (8.7)
>5	102	13 (12.7)	4 (3.9)	17 (16.7)
Clinical features				
DD/ID	259	31 (12.0)	7 (2.7)	38 (14.7)
ASD	175	16 (9.1)	5 (2.9)	21 (12)

Table 2 Summary of the CNVs identified in the proband.

No.	Proband	Coordinate	Copy number (CN)	Size (Kb)	Note
1	2q37.3	chr2:241494455-242340252	Loss	846	
2	16p13.11	chr16:15420069-16514368	Loss	1094	
3	5p15.33-p14.1	chr5:113576-25944592	Loss	25831	Cri du chat syndrome
4	15q26.3	chr15:100,384,057-102,429,112	Loss	2045	
5	1q21.1-q21.2	chr1:146043713-148513854	Loss	2470	
6	7q11.23	chr7:72718277-74142190	Loss	1424	Williams syndrome
7	7q11.23	chr7:72718277-74143240	Loss	1425	Williams syndrome
8	20q13.33	chr20:61579927-62915555	Loss	1336	
9	17q11.2	chr17: 28,464,942-30,528,569	Loss	2063	Neurofibromatosis type 1
10	7q11.23	chr7:72718277-74143240	Loss	1425	Williams syndrome
11	22q11.21	chr22:18916842-21465659	Loss	2549	DiGeorge syndrome
12	1q21.2	chr1:146498298-147823369	Gain	1325	
13	7q11.23	chr7:72700524-74147166	Gain	1447	7q11.23 duplication
14	18q22.2-q23	chr18:66840930-78014123	Loss	11173	
15	1q21.1-q21.2	chr1:145885645-147926347	Loss	2041	
	3q29	chr3:195703615-197344176	Loss	1641	
16	7q11.23	chr7:72718277-74142256	Loss	1424	Williams syndrome
17	5p15.33-p12	chr5:113,576-50,101,846	Mosaic gain (CN = 2.2)	49988	Cri du chat syndrome
18	2p16.3	chr2:50749598-50880967	Loss	131	<i>NRXNI</i> (exons 4-10)
19	10q11.22-q11.23	chr10:46206775-51812795	Gain	5606	
20	1q21.1-q21.2	chr1:145786360-147897962	Loss	2112	
21	11q22.1-q22.3	chr11:101484717-108657329	Gain	7173	
22	15q11.2-q26.3	chr15:22752398-102429049	Uniparental disomy (UPD)	79,000	Angelman/Prader-Willi syndrome
23	7q11.23	chr7: 72691242-74142190	Loss	1450	Williams syndrome
24	7q11.23	chr7:72718277-74143240	Loss	1425	Williams syndrome
25	7q11.23	chr7:72848720-74143060	Loss	1294	Williams syndrome
26	15q11.2-q13.1	chr15:22770421-28545355	Gain	5775	
27	22q11.21	chr22:18916842-21798907	Loss	2882	DiGeorge syndrome
28	Xp22.31	chrX:6458939-8135645	Loss	1677	
29	10q26.12-q26.3	chr10: 121,508,975 - 135,534,747	Mosaic copy-neutral loss of heterozygosity (LOH)		
30	7q11.23	chr7:72650240-74142190	Loss	1492	Williams syndrome
31	7q11.23	chr7:72611954-74286977	Loss	1675	Williams syndrome
32	1q21.1-q21.2	chr1:146043713-147926347	Loss	1883	
33	1p21.3-p21.1	chr1:96378051-103052282	Loss	6674	
34	17q11.2	chr17: 27386433-30622025	Mosaic loss (CN = 2.3)	3236	Neurofibromatosis type 1
35	8q13.3	chr8:71556059-73521359	Loss	1965	
	9p24.1-p23	chr9:7849231-12101640	Loss	4252	
36	7q11.23	chr7:72718277-74143240	Loss	1425	Williams syndrome
37	3p26.3-p24.1	chr3:285805-28206877	Gain	27596	Parental balanced translocation
	6p25.3	chr6:380684-883245	Loss	503	
38	15q11.2-q13.1	chr15:22,770,421-28,560,269	Loss	5789	Angelman syndrome
39	22q11.21	chr22:18644790-21798907	Loss	3154	DiGeorge syndrome
40	7q11.23	chr7:72718277-74143240	Loss	1425	Williams syndrome
41	15q11.2-q13.1	chr15:22770421-28534245	Gain	5764	
42	3q22.1-q22.3	chr3:133679690-136971562	Loss	3292	

Table 2 continued

No.	Proband	Coordinate	Copy number (CN)	Size (Kb)	Note
43	22q11.21	chr22:18644790-21465662	Loss	2821	DiGeorge syndrome
44	16p11.2	chr16:29412891-30191848	Loss	779	
45	15q11.2-q13.1	chr15:22770421-28545601	Loss	5775	Angelman/Prader-Willi syndrome
46	15q11.2-q13.1	chr15:22770421-28915864	Loss	6145	Angelman/Prader-Willi syndrome
47	9pter-9q21.13	chr9:203861-75444559	Gain	75600	Partial chromosome 9 trisomy
48	1p33-p32.2	chr1:47831383-58364913	Loss	10533	
49	15q11.2-q13.1	chr15: 22714985-28559437	Gain	5844	
50	17p11.2	chr17:16761814-20318253	Gain	3556	Potocki-Lupski syndrome
51	15q11.2-q13.1	chr15:22770421-28534245	Gain	5764	
52	3p26.1p11.1	chr3:5,464,640-90,485,635	LOH	23297	
	3q11.1q29	chr3:93,536,053-197,851,260	LOH	26247	
53	22q13.33	chr22:50,990,475-51,115,526	Loss	125	<i>SHANK3</i> gene
54	15q11.2	chr15:22,770,421-23,282,799	Loss	512	
55	17q13.1p11.2	chr17:8,235,947-18,992,506	Gain	10687	
56	4q31.21q33	chr4:145149737-170141221	Gain	24991	
57	13q34	chr13:112352171-115107733	Loss	2755	
58	14q31.1q32.12	chr14:82876271-92988797	Loss	10113	
	18q21.2q22.3	chr18:53637282-71890740	Loss	18253	
59	16q22.2		Gain	739	

Table 3 Recurrent CNVs identified in this study. UPD, uniparental disomy.

Loci	CN	Size range (Kb)	No. of patients	Syndrome involved
7q11.23	Loss	1294–1675	11	Williams syndrome
	Gain		1	
15q11.2-q13.1	Gain	5764–79000	4	Angelman/Prader-Willi syndrome
	Loss		3	
	UPD		1	
22q11.21	Loss	2821–3154	4	DiGeorge syndrome
1q21.2	Loss	1325–2470	4	1q21.2 microdeletion syndrome
	Gain		1	
17q11.2	Loss	2063–3236	2	Neurofibromatosis type 1
5pter	Loss	25831–49988	2	Cri du chat syndrome

Management Changes

Based on the previous reports [9] and our clinical practice, we applied five categories of clinical action after a positive CMA result: (i) allowing an earlier diagnosis, (ii) prioritizing full clinical assessment, including referral to other specialists and further diagnostic testing, (iii) changing

medication, such as discontinuation, starting a new medication, and a clinical trial, (iv) changing surveillance and prognosis, and (v) recommending prenatal genetic counseling. Our CMA results affected the clinical management in the above ways for 94.9% (56 out of 59) of the patients with abnormal CNVs (Table 5).

Table 4 Parental testing for 12 patients carrying non-recurrent pathogenic/likely pathogenic CNVs.

No.	Gender	Loci	CN	Size (Kb)	Inheritance	Note
1	Female	20q13.33	Loss	1336	<i>de novo</i>	
2	Male	1q21.2	Gain	1325	Maternal	
3	Male	2p16.3	Loss	131	<i>de novo</i>	<i>NRXN1</i> (exons 4–10)
4	Male	10q11.22–q11.23	Gain	5606	Maternal	
5	Male	11q22.1–q22.3	Gain	7173	<i>de novo</i>	
6	Female	1p21.3–p21.1	Loss	6674	<i>de novo</i>	
7	Male	8q13.3	Loss	1965	<i>de novo</i>	Imbalanced translocation
		9p24.1–p23	Loss	4252		
8	Female	3q22.1–q22.3	Loss	3292	<i>de novo</i>	
9	Male	15q11.2–q13.1	Gain	5775	Maternal	
10	Female	22q13.33	Loss	125	<i>de novo</i>	<i>SHANK3</i> gene
11	Male	15q11.2	Loss	512	Maternal	
12	Male	16q22.2	Gain	739	<i>de novo</i>

Fig. 1 Genotype comparison between our patient and the 1p31.3p32.2 deletion syndrome. Red bar: the 1p31.1–p32.2 deletion region detected in our case. Three possible candidate genes (red

boxes), including *ORC1*, *SCP2*, and *DAB1*, were pinpointed in this case. However, the *NFIA* gene (blue box), which is the core gene for 1p32–p31 deletion syndrome, was not involved in our case.

For 36 patients (61%), the results allowed us to give an earlier diagnosis: one for ASD (mosaic 17q11.2 deletion); 18 for known syndromes (including Cri du chat syndrome, Williams syndrome, AS/PWS, Neurofibromatosis type 1 and 1q21 microdeletion syndromes), and 17 for other microdeletion/microduplication syndromes caused by rare CNVs.

For patients with known symptoms presenting with atypical phenotypes, the CMA results allowed not only earlier diagnosis but also full clinical management and/or intervention changes. With these clinical actions, 30 patients (50.8%) had an improved prognosis. Referral and further clinical evaluation was done for almost half of the patients with pathogenic CNVs ($n = 29$; 49.2%). Eleven patients (18.6%) with WBS underwent full clinical assessment, such as serum calcium and thyroid function tests, and were further referred to cardiology and/or endocrinology clinics. In 4 patients (6.8%) with 15q11.2–q13 loss or uniparental disomy, the diagnosis was first confirmed (AS/PWS) and then clinical action was taken, including referral

to a neurologist and conducting an EEG and pituitary hormone test. Four patients (6.8%) with DiGeorge syndrome who visited our department were recommended by a cardiologist and/or an endocrinologist. They received full behavioral evaluation in addition to echocardiography, and serum calcium, immunology, and pituitary hormone tests. Two patients (3.4%) with 17q11.2 deletions involving the *NF1* gene linked to neurofibromatosis risk were given further evaluation and lifelong follow-up. Another two patients (3.4%) were recommended for a hearing test. Based on the molecular diagnoses, medication or intervention were changed in 7 patients (11.9%). In the patient with a 2p16.3 deletion involving *NRXN1* exons 4–10, who had mild autism symptoms and epileptic discharges, methylphenidate was replaced by atomoxetine [18]. Another patient with a CMA result of *SHANK3* loss (22q13.3 deletion syndrome) prompted a further clinical therapeutic trial with insulin-like growth factor. Two patients with 18q22 loss were confirmed with growth

Table 5 Clinical action for patients with pathogenic CMA results.

Case no.	Loci	Allowing earlier diagnosis	Prioritizing full clinical management	Changing medication	Changing prognosis	Parental genetic counselling
1	2q37.3					
2	16p13.11	+				
3	5p15.33-p14.1	+	+		+	
4	15q26.3	+				
5	1q21.1-q21.2	+				
6	7q11.23		+		+	
7	7q11.23		+		+	
8	20q13.33	+				+
9	17q11.2		+		+	
10	7q11.23		+		+	
11	22q11.21		+		+	
12	1q21.2					+
13	7q11.23		+		+	
14	18q22.2-q23	+	+	+	+	
15	1q21.1-q21.2 3q29	+				
16	7q11.23		+		+	
17	5p15.33-p12	+				
18	2p16.3	+		+	+	+
19	10q11.22-q11.23	+				+
20	1q21.1-q21.2	+				
21	11q22.1-q22.3					+
22	15q11.2-q26.3	+	+		+	
23	7q11.23	+	+		+	
24	7q11.23	+	+		+	
25	7q11.23	+	+		+	
26	15q11.2-q13.1	+				+
27	22q11.21		+		+	
28	Xp22.31	+				+
29	10q26.12-q26.3					
30	7q11.23		+		+	
31	7q11.23		+		+	
32	1q21.1-q21.2	+				
33	1p21.3-p21.1	+				+
34	17q11.2	+	+	+	+	
35	8q13.3	+	+		+	+
	9p24.1-p23					
36	7q11.23		+		+	
37	3p26.3-p24.1 6p25.3	+				+
38	15q11.2-q13.1		+		+	
39	22q11.21		+		+	

Table 5 continued

Case no.	Loci	Allowing earlier diagnosis	Prioritizing full clinical management	Changing medication	Changing prognosis	Parental genetic counselling
40	7q11.23		+		+	+
41	15q11.2-q13.1	+				
42	3q22.1-q22.3					+
43	22q11.21		+		+	
44	16p11.2		+		+	
45	15q11.2-q13.1	+	+	+	+	
46	15q11.2-q13.1	+	+	+	+	
47	9pter-9q21.13	+				
48	1p33-p32.2	+				
49	15q11.2-q13.1	+	+		+	
50	17p11.2	+	+		+	
51	15q11.2-q13.1	+				
52	3p26.1-p11.1 3q11.1-q29					
53	22q13.33	+		+		+
54	15q11.2	+				+
55	17q13.1-p11.2	+				
56	4q31.21-q33	+				
57	13q34	+				
58	14q31.1-q32.12 18q21.2-q22.3	+	+	+	+	
59	16q22.2					+

hormone deficiency and were given growth hormone treatment.

In 15 of these patients (25.4%), the results were essential for prenatal counseling to help the patients' parents to determine reproductive planning, especially in four patients who had abnormalities of maternal origin.

Discussion

The use of CMA to detect pathogenic CNVs underlying DD/ID or ASDs has improved a lot recently. At least two cohort studies have found that a CMA-first strategy may be the most cost-effective [19, 20]. In our study, the diagnostic

yield is comparable to those in previous publications on similar patient populations [21, 22]. The yield for patients under 2 years of age was significantly higher than that of older children, partly due to most of the younger patients having a medical history of other problems such as asphyxia of the newborn, neonatal jaundice, or nutritional diseases like malnutrition and anemia in addition to neurological abnormalities (such as microcephaly, macrocephaly, or hypotonia). These clinical findings suggest that NDD patients, especially at younger ages, may have comorbidity with other systemic abnormalities, reminding clinicians to consider genetic evaluation with CMA for young patients.

In our cohort, there were more male than female patients; the ratio was 5.89:1, which is higher than in studies from other countries [23]. There are three possible reasons for the difference. One is that the prevalence of DD/ID and ASDs is much higher in boys than in girls; the sex differentiation is 4:1 for ASDs in western countries [23]. According to statistics from the Institute of Mental Health, Peking University School of Medicine [24], the occurrence of autism is ~5–9 times higher in boys than in girls in China. This figure indicates that Asian males may be more susceptible to autism than Caucasians. Further statistical and epidemiological data are needed to clarify this. The second explanation may be a referral bias caused by the culture in China. Traditionally, Chinese parents value boys over girls [25]. The third possible cause comes from genetics, in that there is an effect of a higher female tolerance for additional mutations [26]. Generally, most pathogenic variations have been shown to be *de novo*. However, some recurrent genomic disorders like 15q11-q13 deletion/duplication and 22q11 deletion, represent a maternal bias [27]. This bias has been replicated and confirmed, revealing a highly significant maternal bias in the origin of the 22q11.2 deletion [28]. Krumm *et al.* [29] found that inherited truncating SNVs may be associated with an effect involving significant maternal transmission bias to sons. In the parental testing, we identified four inherited pathogenic CNVs which were all inherited from seemingly healthy mothers of male patients. This indicates that females are more tolerant to pathogenic/likely pathogenic CNVs, and tend to transmit them to male offspring.

Our results confirm the importance of CNVs underlying DD/ID and ASD, as reported in previous studies [30]. The profiles of abnormal CNVs in our study are mostly comparable to those reported previously [9, 31]. However, we did find some differences in the CNV spectrum between Chinese and Caucasian cohorts. Many studies have reported that the proximal 16p11.2 deletion, which has a population frequency of ~0.03% world-wide [32], is among the most frequent genetic etiologies of ASDs in Caucasian populations [33, 34] and accounts for ~1% of autism cases [35]. But there was only one 16p11.2 deletion in our cohort. Zhang *et al.* have demonstrated the potential involvement of the proximal 16p11.2 deletion in congenital scoliosis in a Han Chinese population [36], indicating a different genotype-phenotype association in this population. Our results highlight an urgent need for investment and extensive studies to establish a database and nationwide guidelines covering the Chinese population, considering low overall clinical application of CMA in China. Interestingly, we also found variability in the relationships between phenotypes and genotypes in the affected probands. For example, for 15q11.2-q13.1, the phenotypic

spectrum of both deletions and duplications appeared to be primarily neurological and included developmental and speech delays, and the deletion was recognized as classic AS/PWS, comparable to previously published work [4]. In this study, we also found that the phenotypes seen in patients with 7q11.23 microduplications were quite unlike those seen with the common microdeletion [5]. Those with WBS (7q11.23 microdeletion) are prone to have hyperverbal speech, a lack of stranger anxiety, and supravalvular aortic stenosis, while those with the 7q11.23 microduplication have speech delay, selective mutism (SM), and social anxiety, and are prone to aortic dilatation [37]. This variability of phenotypes caused by loss or gain mutation in the same region not only provides additional information on the interpretation of the effect of CNVs for counseling patient families but also sheds light on the underlying mechanism.

To date, all the CNV analysis studies on Chinese populations have focused on delineating the relationship between genotype and phenotype and explaining the pathogenic mechanism [14, 15, 17, 38]. But no studies have revealed the effects of positive CMA results on clinical practice in China. As developmental behavioral pediatricians, we are concerned about how to combine CMA results with clinical practice and how to use the positive results to optimize clinical management. Our study is the first report on using CNV analysis to guide the clinical management of a DD/ID and/or ASD cohort in a Chinese population. Based on the CMA tests, almost all patients (94.9%) with abnormal CMA results benefited from changed or optimized clinical actions, such as referral to specialists, further diagnostic tests, medication changes, and genetic consulting. Since the social challenges arising from the “one couple one child” policy, the Government of China announced that the policy was changed to encourage couples to have 2 children from January 1, 2016 [39]. Thus, there will be a baby boom in the coming years. Moreover, it seems probable that this population will include high-risk pregnancies associated with advanced paternal age and the use of assisted reproduction. Genetic consulting allows clinicians to estimate the recurrence risk and enable parents to make appropriate decisions on the second child or future pregnancy.

The clinical application of CMA in China is overall far behind, but increasing attention is being paid to this technology. In 2016, the Chinese expert consensus on clinical application of chromosomal microarray analysis in pediatric inherited disorders was released [40], and this has accelerated the spread of CMA in clinical practice.

A potential limitation of this study is the relatively limited follow-up time, which may underestimate the benefits of clinical management. Because of our study design, patients recruited more recently had a shorter

follow-up than those who had been tested earlier. This limitation may affect the effective rate of the clinical management in our study. Another limitation is that the sample size was not large enough, and this may undervalue the power of CMA in clinical management. Our next step is to carry out a larger study to determine recommendations or guidelines for proper medical management based on CMA results and follow up the health outcomes of affected patients.

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Compliance with Ethical Standards

Conflict of interest All authors claim that there are no conflicts of interest.

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