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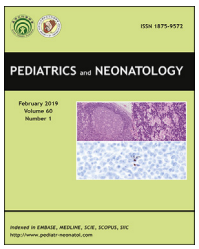

Original Article

Application of chromosome microarray analysis in patients with unexplained developmental delay/intellectual disability in South China

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Key Words
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Background and methods: Chromosome microarray analysis (CMA) is currently the first-tier diagnostic assay for the evaluation of developmental delay (DD) and intellectual disability (ID) with unknown etiology. Here, we present our clinical experience in implementing whole-genome high-resolution single nucleotide polymorphism (SNP) arrays to investigate 489 patients with unexplained DD/ID in whom standard karyotyping analyses showed normal karyotypes. This study aimed to assess the usefulness of CMA for clinical diagnostic testing in the Chinese population.

Results: A total of 489 children were classified into three groups: isolated DD/ID (n = 358), DD/ID with epilepsy (n = 49), and DD/ID with other structural anomalies (n = 82). We identified 126 cases (25.8%, 126/489) of pathogenic copy number variants (CNVs) by CMA, including 89 (24.9%, 89/358) with isolated DD/ID, 13 (26.5%, 13/49) with DD/ID with epilepsy, and 24 (29.3%, 24/82) with DD/ID with other structural anomalies. Among the 126 cases of pathogenic CNVs, 79 cases were identified as microdeletion/microduplication syndromes, among which 76 cases were classified as common syndromes, and 3 cases were classified as rare syndromes, including 15q24 microdeletion syndrome, Xq28 microduplication syndrome and Lowe syndrome. Additionally, there were forty-seven cases of non-syndromic pathogenic CNVs. The *ABAT*, *FTSJ1*, *DYNC1H1*, and *SETBP1* genes were identified as DD/ID candidate genes.

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Conclusion: Our findings suggest the necessity of CMA as a routine diagnostic test for unexplained DD/ID in South China.

1. Introduction

Developmental delay (DD)/intellectual disability (ID) is a variable manifestation of central nervous system dysfunction, and its incidence rate approaches 3% in the general population.[1] Approximately 40% of cases of DD/ID have a genetic etiology, including chromosomal abnormalities (e.g., Trisomies, microdeletions and microduplications) and monogenic causes (e.g., fragile X syndrome).[2–4] Chromosome abnormalities are detected in 25% of patients with DD/ID.[2,5] Conventional cytogenetics has been the standard first-tier test for the detection of genetic anomalies in patients with DD/ID for more than 35 years, and it allows for the detection of numerical and structural chromosomal abnormalities present in the entire genome but has a limited resolution of 5–10 Mb. Fluorescence in situ hybridization (FISH) can detect specific cytogenetic aberrations with a higher sensitivity than conventional cytogenetics; however, FISH cannot cover entire regions of chromosomes. In addition, only a relatively small fraction of patients (~6%) can be diagnosed by conventional cytogenetics and FISH.

Chromosome microarray analysis (CMA) is now recommended worldwide as the first-tier clinical diagnostic test for patients with DD/ID of unknown etiologies.[6] CMA detects copy number variants (CNVs) in the entire genome at a much higher resolution than does conventional cytogenetics. Studies using genome-wide array comparative genomic hybridization (aCGH) analyses or single nucleotide polymorphism (SNP) arrays to investigate cytogenetically normal patients with unexplained DD/ID have shown a potential diagnostic yield of 12–15%.[6] In our study, we used the CytoScan™ HD array platform provided by the Affymetrix Corporation to analyze 489 DD/ID individuals with normal karyotypes. We aimed to evaluate the utility of CMA as a routine clinical diagnostic test in patients with DD/ID in South China.

2. Materials and methods

2.1. Patients

A total of 489 unexplained DD/ID patients were recruited from the Department of Neurological Rehabilitation in Guangzhou Women and Children's Medical Center between January 2011 and October 2015. Written informed consent was obtained from all guardians of the patients. The study was approved by the Institutional Review Board of the Ethics Committee at Guangzhou Women and Children's Medical Center. The age of the patients ranged from 12 months to 9 years and 7 months old. All patients were required to have normal karyotypes and uncomplicated perinatal histories (no viral infection and no exposure to other teratogens). The 489 patients were classified into 3 groups: isolated DD/ID (n = 358), DD/ID with epilepsy (n = 49), and DD/ID with other structural anomalies (n = 82) (Table 1).

2.2. Genomic DNA

Genomic DNA was extracted from the peripheral blood of the patients and their parents using QIAamp® DNA Blood mini kits (Qiagen, Dusseldorf, Germany) following the manufacturer's protocol. At least 250 ng of genomic DNA was applied to the CytoScan™ Array.

2.3. CMA

CMA was performed using the Affymetrix CytoScan™ array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocols, and the reporting threshold of the CNVs was set at 100 kb with a marker count of ≥50. The CNVs detected were aligned with known CNVs that were listed in databases, such as the database of genomic variants (DGV,

Table 1 Summary of the study cohort of 489 patients with DD/ID.

Group	Total cases	Pathogenic CNVs (%)	VOUSs (%)	Benign CNVs (%)
Isolated DD/ID	358	89 (24.9)	1 (0.3)	268 (74.9)
DD/ID + epilepsy	49	13 (26.5)	3 (6.1)	33 (67.3)
DD/ID + other structural anomalies	82	24 (29.3)	11 (13.4)	47 (57.3)
Central nervous system	34	8 (22.9)	6 (17.6)	20 (58.8)
Cardiovascular system	15	7 (46.7)	1 (6.7)	7 (46.7)
Skeletal system	12	3 (21.4)	2 (16.7)	7 (58.3)
Urogenital system	4	2 (50.0)	0 (0)	2 (50)
Oral clefts	2	1 (33.3)	0 (0)	1 (50)
Ear deformity	1	0 (0)	0 (0)	1 (100)
Multiple congenital anomalies	10	3 (30.0)	2 (20.0)	5 (50.0)
Total	489	126 (25.8)	15 (3.1)	348 (71.1)

Table 2 The 79 patients with common microdeletion/microduplication syndromes.

Syndromes	Isolated DD/ID	DD/ID + other abnormalities	Total
Angelman/Prader—Willi syndrome	33	9	42
Williams-Beuren syndrome	7	2	9
22q11.2 microdeletion/microduplication syndrome	3	3	6
Wolf-Hirschhorn syndrome	3	2	5
16p11.2 microdeletion/microduplication syndrome	4	1	5
1p36 microdeletion syndrome	2	2	4
17p11.2 microdeletion/microduplication syndrome	2	1	3
2q37 deletion syndromes	1	0	1
Rubinstein-Taybi syndrome	0	1	1
15q24 microdeletion syndrome	1	0	1
Xq28 microduplication syndrome	1	0	1
Lowe syndrome	1	0	1
Total	58	21	79

http://dgv.tcag.ca/dgv/app/home), Database of Genomic Variation and Phenotype in Humans Using Ensembl Resources (DECIPHER, https://decipher.sanger.ac.uk/), Online Mendelian Inheritance in Man (OMIM, http://www.omim.org), University of Santa Cruz (UCSC, http://genome.ucsc.edu/) and International Standards for Cytogenomic Arrays Consortium (ISCA, https://www.iscaconsortium.org/). According to the literature,[7] the CNVs identified using CMA were classified as pathogenic CNVs, variants of uncertain significance (VOUSs) and benign CNVs. The DNA from both parents was assessed by CMA to confirm the pathogenic CNVs and whether the VOUSs were de novo or inherited. All pathogenic CNVs identified by CMA were further confirmed by real-time qPCR according to standard procedures.

2.4. Statistical analysis

Statistical analyses were performed using the statistical package SPSS (version 17.0.1 Chicago, IL, USA). The Pearson Chi—Square test was used to compare the pathogenic CNV detection rates between the isolated DD/ID, DD/ID with epilepsy and DD/ID with other structural anomalies groups.

3. Results

A total of 489 patients were found to have genomic CNVs, with a range of 1—10 CNVs per sample. The size of the detected CNVs varied from 152 kb to 9.99 Mb. We divided the detected CNVs into three groups. The first group contained CNVs that were considered to be pathogenic, and we identified 126 cases (25.8%, 126/489) of pathogenic CNVs, including 89 (24.9%, 89/358) with isolated DD/ID, 13 (26.5%, 13/49) with DD/ID with epilepsy and 24 (29.3%, 24/82) with DD/ID with other congenital anomalies (Table 1). There were no significant differences between the detection rates in any two groups [$P = 0.064$ (24.9% vs. 26.5%), $P = 0.679$ (24.9% vs. 29.3%) and $P = 0.113$ (26.5% vs. 29.3%); χ^2-test]. However, the sample size of each subgroup was too small to draw a conclusion.

Among the 126 cases of pathogenic CNVs, 79 were identified to have microdeletion/microduplication syndromes (Table 2). Of these syndromes, 76 were common syndromes, including Angelman/Prader—Willi syndrome, Williams-Beuren syndrome, 22q11.2 microdeletion/microduplication syndrome, Wolf-Hirschhorn syndrome, 16p11.2 microdeletion/microduplication syndrome, 1p36 microdeletion syndrome, 17p11.2 microdeletion/microduplication syndrome, 2q37 deletion syndrome and Rubinstein-Taybi syndrome, and 3 were rare syndromes,[4,8] including 15q24 microdeletion syndrome, Xq28 microduplication syndrome and Lowe syndrome (Table 3, Fig. 1). There were forty-seven (37.3%, 47/126) cases of non-syndromic pathogenic CNVs. The size of the CNVs varied from 153 kb to 7.93 Mb. Additionally, the genes *ABAT*, *FTSJ1*, *DYNC1H1*, and *SETBP1* (Fig. 2) were identified as DD/ID candidate genes (Table 3). The copy numbers for the sequences of genes in these regions were determined using real-time qPCR to confirm the de novo deletions and duplications in the proband DNA.

The second group consisted of CNVs that were considered benign, and these CNVs were identified in 348 (71.1%, 348/489) patients. In 288 patients, we could find identical CNVs in the online DGV/Children's Hospital of Philadelphia (CHOP) database; in the other 60 cases, the CNVs were inherited from a normal parent (Table 1).

In the third group, we first classified 75 deletions and duplications of uncertain clinical significance. However, after analyzing the parental samples by CMA, we found that 60 of them were inherited from a normal parent and were thus considered benign (shown in the second group). Thus, the detection rate of the VOUSs was 3.1% (15/489) (Table 1, Table 4).

4. Discussion

In the 2010 American College of Medical Genetics (ACMG) practice guidelines, CMA testing for CNVs was recommended as a first-line test in the initial postnatal evaluation of individuals with apparent DD/ID.[9] However, the CMA platforms were not the same in all studies, and the pathogenic CNV detection rate also differed. Xiang et al.[10]

Table 3 Pathogenic CMA results of seven DD/ID patients.

Case ID	Age	Gender	Clinical feature	CMA result	Size (Mb)	OMIM morbid gene (OMIM)	DECIPHER patient/Known syndrome
BY445	9m	M	DD, congenital heart disease	Del 15q24.1q24.2 (72,969,435—76,071,744) x 1	3.10	Contiguous genes	15q24 microdeletion syndrome
BY630	8y8m	F	ID	Dup Xq28 (153,282,927—153,624,603) x 3	0.342	*L1CAM* (308840), *MECP2* (300005)	Xq28 (*MECP2*) microduplication syndrome
BY653	6y6m	F	ID, cataract of the eyes, nystagmus	Del Xq25q26.1 (128,652,372—128,901,629) x 1	0.249	*OCRL* (300535)	Lowe syndrome
BY169	2y3m	F	DD, epilepsy	Dup 16p13.2 (8,806,673—8959,401) x 3	0.153	***ABAT*** (137150), *PMM2* (601785)	—
BY1403	27m	F	DD	Dup Xp11.23p11.22 (48,307,474—52,801,080) x 3	4.49	***FTSJ1*** (300499)	Patient 2500, 4483; nssv582182, nssv577124
BY1420	6y	F	ID, speech delay	Del 14q32.31q32.33 (102,394,766—106,246,786) x 1	3.85	***DYNC1H1*** (600112)	Patient 251,327, 2826; nssv577487, nssv577488
BY935	6y1m	F	ID, facial dysmorphism	Del 18q12.3q21.1 (39,747,201—45,955,757) x 1	6.21	***SETBP1*** (611060), *EPG5* (615068), *KATNAL2* (614697)	Patient 254,553, 249,099, 867, 259,788; nssv582149, nssv1608656

y: year, m: month; M: male, F: female; Del: deletion, and Dup: duplication.

screened 50 postnatal children with DD/ID using the Oligo-Agilent 44k technology platform and showed that only 3 cases were associated with pathogenic CNVs and exhibited a detection rate of 6%. Coutton et al.[11] used the Human Genome CGH Microarray Kit 180k to test 66 patients with DD, and the pathogenic CNV detection rate was 21%. Miller et al.[6] used high-resolution microarray analysis to test 21,698 patients with DD/ID and showed that the detection rate of pathogenic CNVs ranged from 15% to 20%. In our study, we used SNP arrays to investigate 489 patients with normal karyotypes and unexplained DD/ID in South China. One hundred twenty-six pathogenic CNVs were identified, which resulted in a detection rate of 25.8% (126/489). The diagnostic yield was comparable with a similar recent study including 36,325 patients.[1,12] Our findings support the necessity of implementing CMA as a routine diagnostic test in the Chinese population. Moreover, considering the cost-effectiveness of CMA compared with that of current conventional cytogenetics and multiplex ligation-dependent probe amplification (MLPA) or FISH, CMA as the first choice in the clinical genetic evaluation of unexplained DD/ID could be beneficial for patients.[13]

Among the 126 cases of pathogenic CNVs, 79 cases were identified as common microdeletion/microduplication syndromes, and 3 cases were rare syndromes, including 15q24 microdeletion syndrome, Xq28 microduplication syndrome and Lowe syndrome. 15q24 microdeletion syndrome is a rare genomic disorder that is characterized by mental retardation, growth retardation, and other structural anomalies, such as congenital heart defects and hypospadias. To date, only 20 patients have been diagnosed with this microdeletion syndrome.[14] Patients with 15q24 microdeletions should receive a thorough neurodevelopmental evaluation; physical, occupational, and speech therapies; and regular audiological and ophthalmological screening.[15,16] Case BY445 in this study is a nine-month-old male child with DD and congenital heart disease. CMA revealed a de novo 3.10-Mb deletion at the 15q24.1q24.2 region, which overlaps with the critical region for the 15q24 microdeletion syndrome (OMIM 613406). The finding provides genetic evidence for the patient's treatment and prognosis.

Xq28 microduplication syndrome is characterized by a small duplication at Xq28 that ranges in size from 0.4 to 0.8 Mb and harbors the critical pathogenic mental retardation-related *L1CAM* and *MECP2* genes. The *MECP2* gene is required for the maturation of neurons and is developmentally regulated.[17] Mutations in MECP2 can cause Rett syndrome, while increased dosage of MECP2 results in the mental retardation phenotype.[18] In general, duplication of the MECP2 region frequently manifests as a severe form of mental retardation in male patients, and females with the duplication are often asymptomatic carriers. Case BY630 is an eight-year-and-eight-month-old female child with mental retardation, poor learning ability, and slow reactions. CMA revealed a 342-kb microduplication in the Xq28 region that contains the *MECP2* gene (OMIM 300005). The patient is considered to have Xq28 microduplication syndrome potentially due to the abnormal inactivation of the normal X chromosome. However, more evidence is needed to support this hypothesis.

Lowe syndrome is an X-linked recessive genetic disease caused by mutations (e.g., point mutations) in the *OCRL*

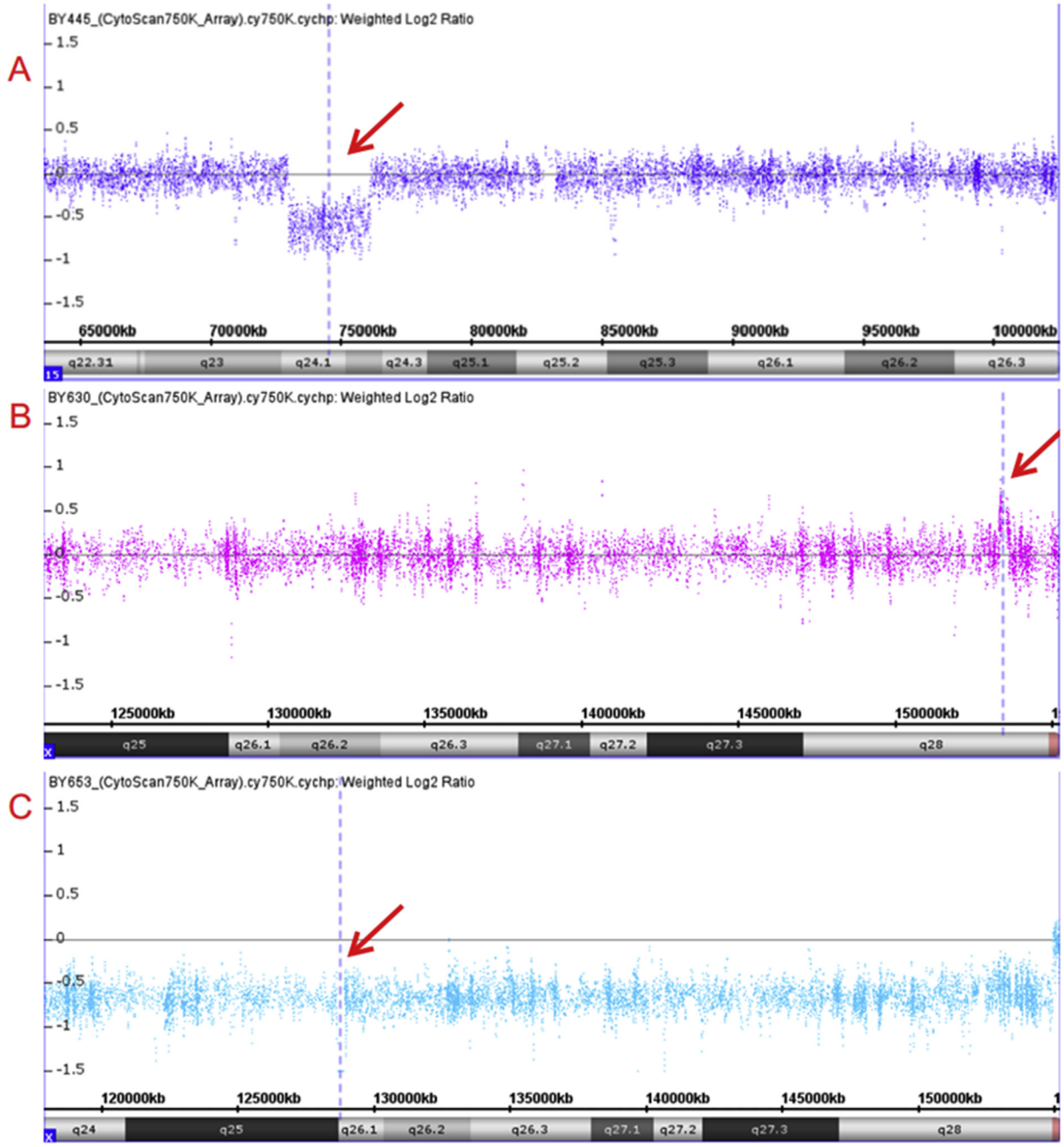

Figure 1 (A) CMA image showing a 3.1-Mb deletion at 15q24.1q24.2 (chr15: 72,969,435—76,071,744, hg19) from Case BY445. (B) CMA image showing a 342-kb microduplication at Xq28 (chrX: 153,282,927—153,624,603, hg19) from Case BY630. (C) CMA image showing a 249-kb microdeletion at Xq25q26.1 (chrX: 128,652,372—128,901,629, hg19) from Case BY653.

gene. Clinical manifestations involve the eye, brain, and kidney. Brain symptoms include mental retardation, hypotonia, and the weakening or disappearance of tendon reflexes. Eye symptoms include congenital cataracts and congenital glaucoma. Renal tubular dysfunction includes tubular proteinuria, moderate-severe multiple sets of urinary amino acids (e.g., lysine and tyrosine), increased urinary phosphorus, low phosphorus, and high blood chloride renal tubular acidosis.[19] Case BY653 is a six-year-and-six-month-old female child with mental retardation, growth retardation, bilateral congenital cataracts, and tremor in the eyeballs. CMA revealed a 249-kb microdeletion in the Xq25q26.1 region that contains the *OCRL* gene (OMIM 300535). The patient is considered to have Lowe syndrome. This is the first report of haploinsufficiency of the *OCRL* gene being associated with Lowe syndrome.

We also found 47 (37.3%, 47/126) cases of de novo non-syndromic pathogenic CNVs. The size of the CNVs varied from 153 kb to 7.93 Mb. Additionally, the *ABAT*, *FTSJ1*, *DYNC1H1* and *SETBP1* genes were identified as DD/ID candidate genes. Case BY169 is a two-year-and-three-month-old female who has DD with epilepsy. CMA revealed a de novo 153-kb microduplication in the 16p13.2

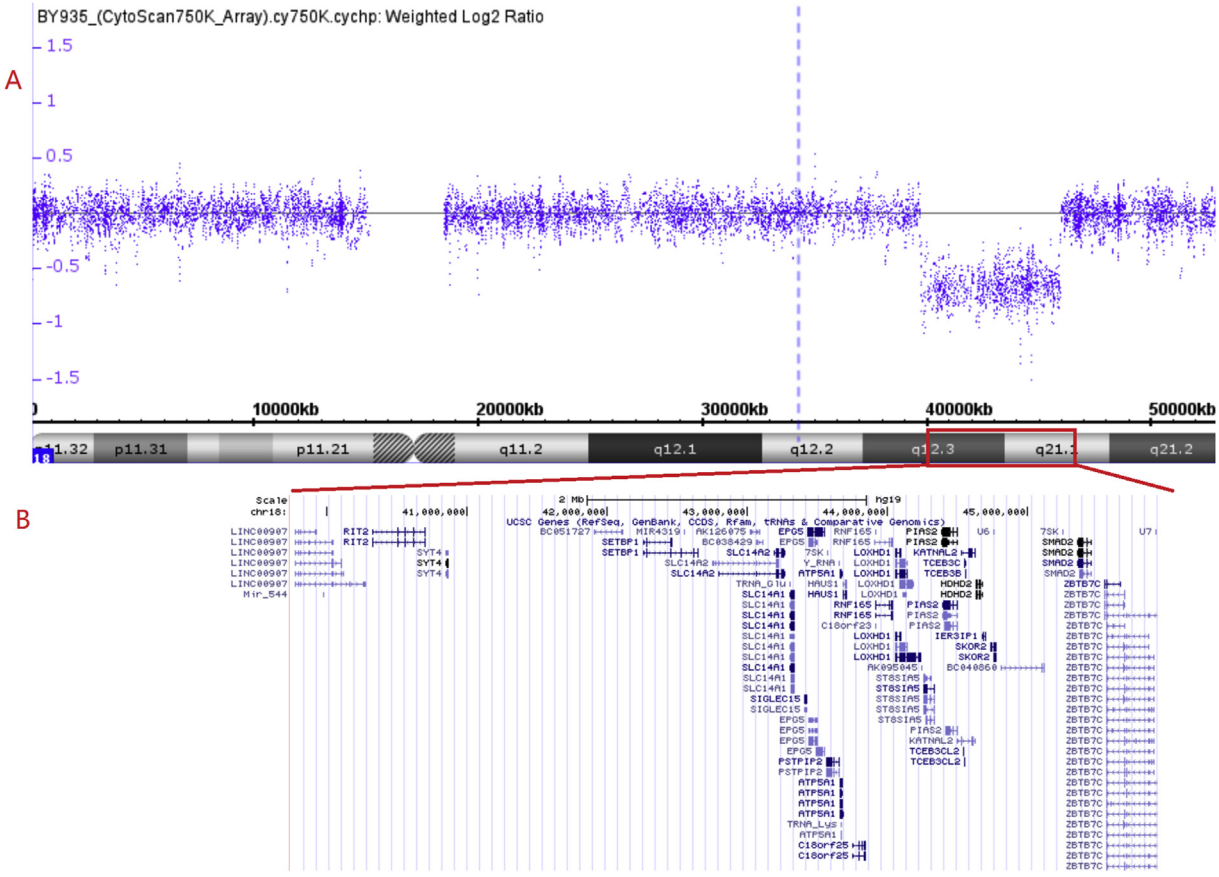

Figure 2 (A) CMA image showing a 6.21-Mb deletion at 18q12.3q21.1 (chr18: 39,747,201—45,955,757, hg19) from Case BY935. (B) Schematic from the UCSC genome browser (hg19) showing the position of the 18q12.3q21.1 deletion. The region overlaps with the *SETBP1 (611,060)*, *EPG5 (615,068)* and *KATNAL2 (614,697)* genes. The *SETBP1* gene may be a candidate gene of DD/ID.

region that contains the *ABAT* gene. The *ABAT* gene (OMIM 137150) encodes gamma-aminobutyrate transaminase, which catalyzes the conversion of gamma-aminobutyric acid (GABA), an important, mostly inhibitory neurotransmitter in the central nervous system, into succinic semialdehyde.[20] Mutations of the gene can cause GABA-transaminase deficiency with severe psychomotor retardation, seizures, hypotonia, and hyperreflexia.[21] Therefore, we speculate that *ABAT* is part of a novel class of genes that is associated with the development of the central nervous system. However, more effort should be taken to confirmed that the gene is dose sensitive and that functional haploinsufficiency will cause DD/ID. Case BY1403 is a two-year-and-3-month-old female with isolated DD. CMA revealed a de novo 4.49-Mb microduplication in the Xp11.23p11.22 region that contains the *FTSJ1* gene. Studies have shown that loss-of-function mutations of this gene are associated with mental retardation.[22,23] However, Nizon et al.[24] found that duplication of the gene also results in mental retardation. Moreover, our findings further confirmed that the *FTSJ1* gene is a dose-sensitive gene that is associated with DD/ID. Case BY1420 is a six-year-old female with ID and speech delay. CMA detected a de novo 3.85-Mb microdeletion in the 14q32.31q32.33 region that contains 36 OMIM pathogenic genes. Mutations in the *DYNC1H1* gene (OMIM 600112) can impair migrating embryonic nerve cells and lead to central neurological developmental defects.[25,26] Therefore, we conclude that the *DYNC1H1* gene is a central nervous system-related gene. Case BY935 is a six-year-and-one-month-old female with ID and facial dysmorphism. CMA detected a de novo 6.21-Mb microdeletion in the 18q12.3q21.1 region, which encompasses the *SETBP1* gene (OMIM 611060). Coe et al.[27] reported that haploinsufficiency of the *SETBP1* gene was associated with ID and loss of expressive language. Altogether, we speculated that the *SETBP1* gene is a dosage-sensitive gene that is associated with DD/ID. However, further investigation is required to explore the relationship between the *SETBP1* gene and the development of the central nervous system.

CMA also detects many CNVs of uncertain clinical significance, which can be difficult to interpret and can increase parental stress or anxiety. In our study, the detection rates of VOUSs were 15.3% (75/489) and 3.1% (15/489) before and after parental analysis, respectively. In particular, the interpretation of a VOUS can be assisted by information on whether it was inherited from a normal parent or de novo in the proband. In addition, parental studies can also help with the genetic counseling and evaluation of the recurrence risk of the genetic abnormalities in families following CMA in patients with DD/ID. We referred these patients to the clinical genetics team to help with the interpretation of VOUS. Other novel genetic diagnostic techniques are likely to be used to further increase the diagnostic yield in these patients. One of the most promising techniques is next

Table 4 Details of 15 patients with VOUS.

Case ID	Age	Gender	Clinical feature	CMA result	Size (Mb)	OMIM morbid gene (OMIM)
BY958	6y3m	F	Isolated ID	Dup1p31.1 (76,906,085—77,641,160)x 3	0.735	—
BY557	6y3m	F	ID, Epilepsy	Dup4q24 (102,343,698—103,114,497)x 3	0.771	—
BY1338	5y8m	M	ID, Epilepsy	Dup11q22.3 (108,425,169—108,691,250)x3	0.266	*EXPH5* (612878)
BY1436	4y8m	F	DD, Epilepsy	DupXq21.33 (96,421,808—97,073,270)x 3	0.651	*DIAPH2* (300108)
BY18	2m	F	DD, Ventriculomegaly, Megalencephaly	DupXp22.33 (201,704—356,244)x 3	0.155	—
BY245	2y1m	F	DD, Dandy-Walker malformation, Microcephaly	Dup 19p12 (20,912,176—21,364,322)x 3	0.452	—
BY280	4y1m	F	DD, Microcephaly	Dup 2p25.1 (10,620,166—10942,687)x 3	0.323	—
BY371	4y	M	DD, Microcephaly	Del 12p12.3 (16,440,575—16,672,265)x 1	0.232	—
BY448	1y2m	M	DD, Agenesis of the corpus callosum, Leukoencephalopathy	Dup 4q32.3 (165,907,055—166,146,754)x 3	0.240	—
BY967	1y5m	M	DD, Cerebral palsy	Dup 2q22.1 (140,678,236—141,296,109)x 3	0.618	—
BY982	11m	M	DD, Atrioventricular septal defect, Pulmonary stenosis	Dup 1q32.3 (213,088,380—213,588,624)x 3	0.5	—
BY450	4m	F	DD, Developmental Dysplasia of the Hip	Dup 5q14.1q14.3 (80,141,460—85,934,161)x 3	5.79	*MSH3* (600887), *RPS23* (603683), *XRCC4* (194363), *VCAN* (118661)
BY956	10m	M	DD, Shortening of the limbs	Dup 8p23.1 (6,388,462—7044,046)x 3	0.656	*MCPH1* (607117)
BY160	4y11m	M	DD, Cerebral palsy, Cleft palate, Ventricular septal defect	Del 10q21.2 (64,060,613—64,373,152)x1	0.313	*ZNF365* (607818)
BY944	8m	M	DD, Cleft lip and cleft palate, Clinodactyly	Dup 15q22.2q22.31 (61,240,544—63,887,162)x 3	2.65	*VPS13C* (608879), *TPM1* (191010), *CA12* (603263)

generation sequencing, a genome-wide method with even higher resolution than CMA.

In summary, our findings suggest the necessity of CMA as a routine diagnostic test for unexplained DD/ID in South China and determined a detection rate of 25.8%. We also showed that the SNP array-based analysis of DNA samples derived from the DD/ID patients is an efficient and productive method of identifying candidate genes.

Competing financial interests

The authors declare that they have no competing financial interests.

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