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ORIGINAL ARTICLE

Initial clinical and molecular investigation of 20q13.33 microdeletion with 17q25.3/14q32.31q32.33 microduplication in Chinese pediatric patients

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Abstract

Background: Limited research has been conducted regarding the elucidation of genotype–phenotype correlations within the 20q13.33 region. The genotype–phenotype association of 20q13.33 microdeletion remains inadequately understood. In the present study, two novel cases of 20q13.33 microdeletion were introduced, with the objective of enhancing understanding of the genotype–phenotype relationship.

Methods: Two unrelated patients with various abnormal clinical phenotypes from Fujian province Southeast China were enrolled in the present study. Karyotype analysis and chromosomal microarray analysis (CMA) were performed to investigate chromosomal abnormalities and copy number variants.

Results: The results of high-resolution G-banding karyotype analysis elicited a 46,XY,der(20)add(20)(q13.3) in Patient 1. This patient exhibited various clinical manifestations, such as global developmental delay, intellectual disability, seizures, and other congenital diseases. Subsequently, a 1.0-Mb deletion was identified in the 20q13.33 region alongside a 5.2-Mb duplication in the 14q32.31q32.33 region. In Patient 2, CMA results revealed a 1.8-Mb deletion in the 20q13.33 region with a 4.8-Mb duplication of 17q25.3. The patient exhibited additional abnormal clinical features, including micropenis, congenital heart disease, and a distinctive crying pattern characterized by a crooked mouth.

Conclusion: In the present study, for the first time, an investigation was conducted into two novel cases of 20q13.33 microdeletion with microduplications in the 17q25.3 and 14q32.31q32.33 regions in the Chinese population. The presence of micropenis may be attributed to the 20q13.33 microdeletion, potentially expanding the phenotypic spectrum associated with this deletion.

Jianlong Zhuang and Na Zhang contributed equally to this work.

KEYWORDS

14q32.31q32.33 microduplication, 17q25.3 microduplication, 20q13.33 microdeletion, chromosomal microarray analysis, copy number variants

1 | BACKGROUND

Conventional chromosome karyotype analysis techniques are limited to detecting chromosome structural variations larger than 5 Mb, resulting in a low detection rate for identifying the etiology of pediatric patients with congenital genetic disorders such as developmental delay and intellectual disability. In contrast, chromosomal microarray (CMA) technology can effectively detect copy number variants (CNVs), as well as most uniparental diploids and triploids. A previous large-scale study enrolled patients with developmental delay, intellectual disability, multiple malformations, and autism for CMA detection, demonstrating a 10% incremental yield of CMA over conventional karyotype analysis (Miller et al., 2010). At present, CMA is frequently recommended as the first-line detection method for children with complex and rare diseases (Manning, 2010; Miller et al., 2010; Xu et al., 2018).

The long-arm terminal deletion of 20q13.33 represents a rare structural variation, often associated with chromosome duplications, though primarily inherited rather than being de novo (Shabtai et al., 1993). To the present knowledge, less than 30 cases of 20q13.33 microdeletions are available in existing research. The *CHRNA4* (OMIM 118504) and *KCNQ2* (OMIM 602235) genes localized at the 20q13.33 region have been associated with autosomal dominant epilepsy. Mutations in *CHRNA4* would lead to autosomal dominant nocturnal frontal lobe epilepsy (*ENFL1*, OMIM 600513), while mutations in *KCNQ2* are associated with benign neonatal epilepsy (*EBN1*, OMIM 121200) (Singh et al., 1998; Steinlein et al., 1995; Weckhuysen et al., 2012). In prior research and databases, the variable expressivity of 20q13.33 microdeletion has been observed. Commonly, individuals with 20q13.33 microdeletions will exhibit abnormal clinical features including severe limb malformations, skeletal abnormalities, intellectual disability, developmental delay, hypotonia, strabismus, and seizures (Ardalan et al., 2005; Béna et al., 2007; Mefford et al., 2012; Traylor et al., 2010).

In the present study, two additional unrelated patients were reported with 20q13.33 microdeletions accompanied by chromosome duplications. These patients displayed abnormal clinical characteristics including language and motor developmental delay, intellectual disability, congenital heart disease (CHD), and seizures. Furthermore, Patient 2 exhibited additional features of micropenis and a distinctive crying pattern characterized by a crooked mouth.

2 | MATERIALS AND METHODS

2.1 | Subjects

Enrolled in the present study were two novel unrelated patients with language and motor developmental delay, intellectual disability, seizures, and other congenital diseases from Fujian province, South China. Both parents of these patients reported no family history of genetic diseases, and they denied having a consanguineous marriage. After obtaining signed informed consent, karyotype and chromosomal microarray analyses were performed on the enrolled subjects. Ethics Committee approval was obtained from the Institutional Ethics Committee of Quanzhou Women's and Children's Hospital prior to the commencement of the study (2020No.31).

2.2 | Conventional karyotype analysis

Approximately 2 mL of peripheral blood was collected from the patients for G-banding karyotype analysis, following a previously described protocol (Zhuang et al., 2019). The peripheral blood lymphocytes were cultured for 72 h and harvested using a SinochromeChromprepII automatic chromosome harvesting system (Lechen Biotechnology Co., Ltd., Shanghai, China) according to the standard protocols. 20 metaphases were counted, and 5 metaphases were analyzed for each case. The karyotype nomenclature and diagnosis were conducted according to ISCN 2020.

2.3 | High-resolution G-banding analysis

About 2 ~ 3 mL of peripheral blood was collected from Patient 1 for high-resolution G-banding analysis with 550 ~ 650 bands, and 1 mL of peripheral blood was inoculated into peripheral blood lymphocyte culture medium and cultured for 72 h. Subsequently, 5-fluorouracil and uracil nucleoside were added and the culture was continued for 16 h, followed by the addition of thymine and culture for an additional 4 h. Ethidium bromide was then added and cultured for 45 min. Finally, the cultured

cells were harvested using conventional manufacturing protocols.

2.4 | DNA extraction

Peripheral blood samples of approximately 3~5 mL were obtained from the enrolled subjects. Genomic DNA was extracted using the QIAamp DNA Blood Kit (QIAGEN, Germany) according to the manufacturer's protocol (www.qiagen.com).

2.5 | Chromosomal microarray analysis

CMA was conducted using Affymetrix Cytoscan 750 K chip (Life Technologies, American) according to a previously described protocol (Zhuang et al., 2021). The single-nucleotide polymorphism (SNP) and copy number variants (CNVs) were analyzed using Genotyping Console and Chromosome Analysis Suite software. The pathogenicity interpretation of CNVs was conducted according to the standards and guidelines set forth by the American College of Medical Genetics (ACMG) (Kearney et al., 2011). Reference resources such as the Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv), Online Mendelian Inheritance in Man (OMIM) (https://omim.org/), DECIPHER (https://decipher.sanger.ac.uk/), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), and other relevant databases were utilized for the analysis.

3 | RESULTS

3.1 | Subject information

3.1.1 | Patient 1

The patient was a male infant born at full term with a birth weight of 2.9 kg. He was the only child in the family, and his parents, both in their early 30s, denied any history of inherited diseases. The infant's height and weight showed gradual increases after birth, and he exhibited normal feeding habits without convulsions. However, epileptic symptoms were observed at 14 days after birth, prompting the initiation of anti-epileptic drug therapy, which effectively controlled the symptoms. The patient exhibited delayed developmental milestones; he achieved independent walking at 1 year and 2 months but was unable to crawl and could not pronounce "Baba" or "Mama" at 2 years and 5 months of age. Subsequent physical examination revealed normal head circumference (49 cm) and muscular tension, along with moderate malnutrition. Additionally, the patient presented with orbital hypertelorism, strabismus, low-set nose and ears, and widened interocular distance, with no other apparent abnormalities. The brain magnetic resonance imaging (MRI) of the patient revealed mild bilateral ventricular enlargement, slightly widened sulci and cisterns, and abnormal signals in the left internal capsule. At the age of 5, the patient exhibited severe language developmental delay and intellectual disability, as he could only speak a few words. However, with the standardized administration of anti-epileptic medication (oxcarbazepine oral liquid), epileptic symptoms did not occur.

3.1.2 | Patient 2

Patient 2 was a 4-year-old boy born at full term with a birth weight of 2.7 kg and a normal head circumference of 34 cm. Following birth, several notable abnormalities were observed, including crooked mouth crying, feeding difficulty, a single palmar crease on both hands, and micropenis. His parents denied consanguinity and any family history of genetic diseases. Cardiac ultrasound showed the existence of CHD with patent ductus arteriosus, persistent left superior vena cava and pulmonary hypertension. At 10 days of age, the boy experienced seizures characterized by apnea and unnatural curling of the limbs, which spontaneously resolved within several seconds. The brain MRI imaging did not show any notable findings, but electroencephalography (EEG) revealed a significant number of multifocal sharp waves and a partial seizure during sleep, potentially originating from the left frontal lobe. Chest x-ray at Day 16 after birth elicited enlargement of the heart, which was consistent with a diagnosis of CHD. The patient exhibited normal muscular tension and no notable facial deformities upon physical examination. However, developmental milestones were significantly delayed; at the age of 4 years, the patient was unable to walk independently and could only speak sporadically. As such, a diagnosis of language/motor developmental delay and intellectual disability was established. The parents voluntarily discontinued the use of anti-epileptic medications for approximately 1 year, during which no seizures occurred.

3.1.3 | Karyotype analysis results

As shown in Figure 1a, high-resolution karyotype analysis was performed to analyze the chromosomal abnormality in Patient 1. An additional fragment was observed in the 20q13.3 region, and his karyotype was then described as 46,XY,der(20)add(20)(q13.3). In Patient 2, conventional karyotype analysis was performed, revealing no significant chromosomal abnormalities. The karyotype was described as 46,XY, indicating a normal male chromosomal composition (Figure 1b).

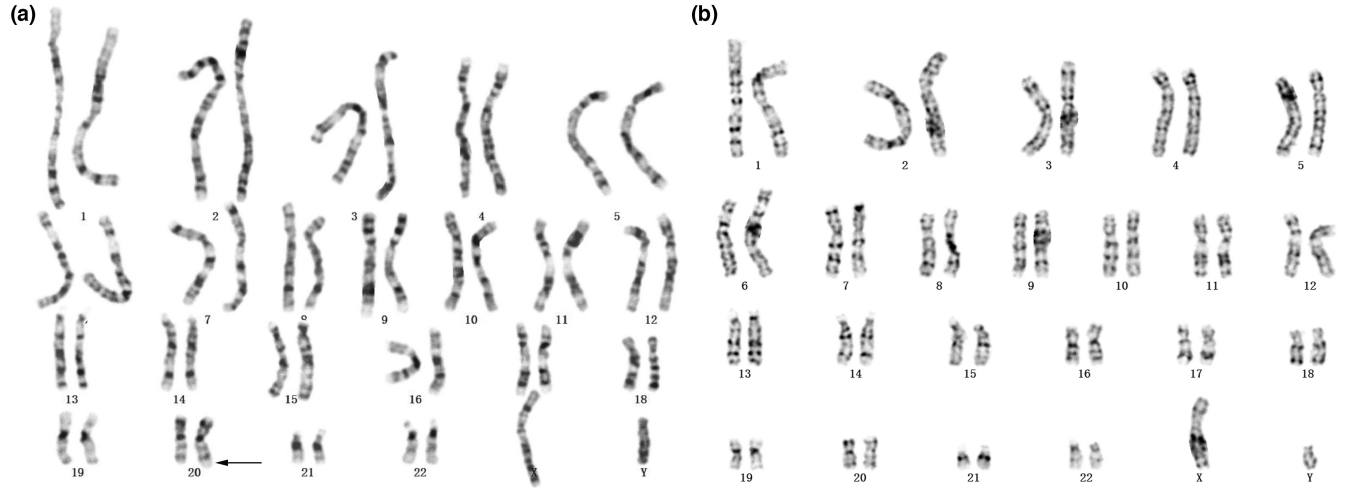


FIGURE 1 Karyotype analysis results of the enrolled patients. (a) Patient 1 was subjected to high-resolution karyotype analysis and the results demonstrated an additional fragment in the 20q13.3 region. The arrow indicates the chromosomal abnormality. (b) Patient 2 was subjected to conventional karyotype analysis, which showed no significant abnormality, with the karyotype described as 46,XY.

3.1.4 | Chromosomal microarray analysis results

The CMA detection results demonstrated a 1.0-Mb deletion in the 20q13.33 region [arr[GRCh37]20q13.33(61854236-62915555)x1] in Patient 1, which contained 26 OMIM genes including *KCNQ2*, *ARFGAP1*, *EEF1A2*, and *CHRNA4*. In addition, a 5.2-Mb duplication in the 14q32.31q32.33 region [arr[GRCh37]14q32.31q32.33(102042054-107285437)x3] was also detected, which covered 39 OMIM genes (Figure 2a,b). Further CMA detection results showed a 1.8-Mb deletion in the 20q13.33 region [arr[GRCh37]20q13.33(61041280-62915555)x1] in Patient 2, containing 42 OMIM genes (*KCNQ2*, *ARFGAP1*, *EEF1A2*, *GATA5*, and *CHRNA4*). A 4.8-Mb duplication in the 17q25.3 region [arr[GRCh37]17q25.3(76203763-81041938)x3] was also detected, containing 75 OMIM genes (Figure 2c,d). Parental genetic studies were not available for either case. Following the ACMG guidelines, the 20q13.33 microdeletions, 14q32.31q32.33, and 17q25.3 microduplications were interpreted as pathogenic CNVs.

3.1.5 | Relevant database information of the detected CNVs and literature review

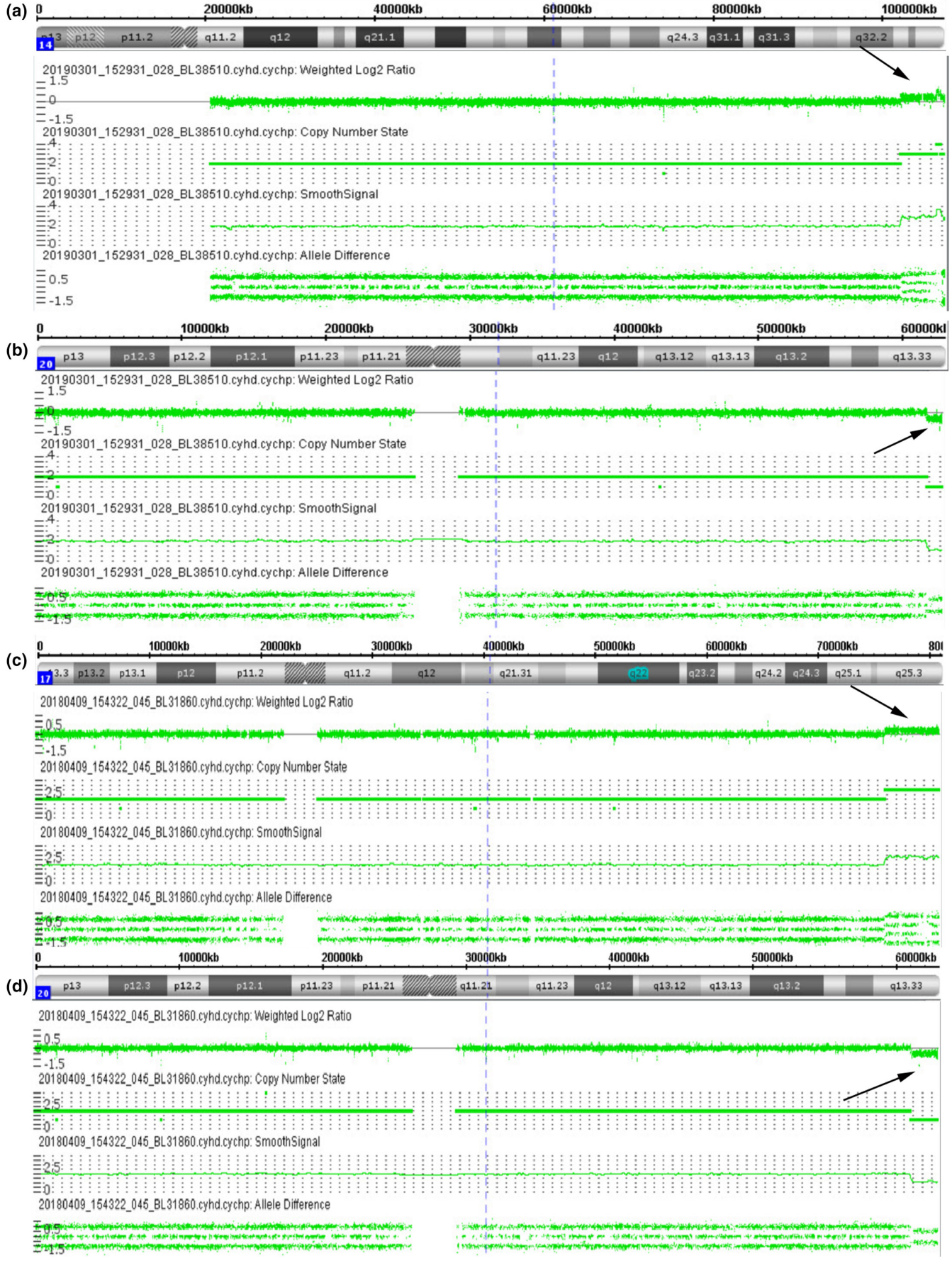
At present, limited reports regarding 20q13.33 microdeletions are available in the existing literature, with some cases listed in Table 1. Notably, no reports exist concerning patients harboring a 20q13.33 deletion along with a 14q32.31q32.33 duplication. In the DECIPHER database, several cases with duplication fragments less than 5 Mb in the 14q32.31q32.33 region from the present report were interpreted as likely pathogenic variants (Table 2). These individuals commonly exhibited clinical features such as global developmental delay, intellectual disability, hypotonia, abnormalities of higher mental function, behavioral abnormalities, and other congenital anomalies. Additionally, as outlined in Table 3, occurrences of 20q13.33 deletion and 17q25.3 duplication were rarely reported, with only two studies available.

4 | DISCUSSION

The distal long-arm microdeletion of 20q13.33 represents a rare structural variation, often associated with a diverse range of clinical phenotypes. These phenotypes commonly include intellectual disability, developmental delay, seizures, and other congenital anomalies. In the present study, two unrelated patients with 20q13.33 microdeletion and 14q32.31q32.33 microduplication or 17q25.3 microduplication were found to exhibit congenital genetic disorders using CMA technology.

The genotype–phenotype correlations of 20q13.33 microdeletions have not been fully elucidated, and there do not appear to be clinically recognizable dysmorphic

FIGURE 2 Chromosomal microarray analysis (CMA) results of the enrolled patients. The arrows indicate the abnormal copy number variants regions. (a, b) The CMA results elicited a 5.2-Mb duplication in the 14q32.31q32.33 region and a 1.0-Mb deletion in the 20q13.33 region in Patient 1. (c, d) The CMA results demonstrated a duplication of 4.8 Mb in the 17q25.3 region and a 1.8-Mb deletion in the 20q13.33 region in Patient 2.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20190301_152931_028_BL38510.cyhd.cychp: Weighted Log2 Ratio
20190301_152931_028_BL38510.cyhd.cychp: Copy Number State
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TABLE 1 Comparison of clinical findings in patients with 20q13.33 microdeletion in existing literature.

	Traylor et al. (2010)					Béna et al. (2007)	Mefford et al. (2012)	Lewis et al. (2018)
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5			
Deletion size	1.1-Mb	1.61-Mb	1.08-Mb	560-kb	1.0-Mb	1.1 ~ 1.6-Mb	1.5-Mb	NM
Sex/Age	M/9 years	F/4 years	M/3 years	M/9 years	M/6 years	F/4 years	M//7 years	F/7 years
Global developmental delay	+	+	+	+	+	+	+	+
Intellectual disability	+	NM	NM	NM	NM	Learning disability	NM	NM
Seizures	+	+	−	+	+	−	+	+
Dysmorphism features	−	+	+	+	−	−	−	−
Other	−	−	−	−	−	Convergent strabismus	−	Failure to thrive

	Mosca-Boidron et al. (2013)	(Pascual et al., 2013)			The present study	
		Patient 1	Patient 2	Patient 3	Patient 1	Patient 2
Deletion size	556-kb	521-kb	520.7-kb	1.4-Mb	1.0-Mb	1.8-Mb
Sex/Age	M/9 years	M/10 months	F/1.5 years	F/3.7 years	M/4.3 years	F//3.7 years
Global developmental delay	NM	+	+	+	+	+
Intellectual disability	NM	NM	NM	NM	+	+
Seizures	−	+	+	+	+	+
Dysmorphism features	−	−	−	−	+	−
Other	Autism spectrum disorders	−	−	Hearing loss, short stature, idiopathic macrocytosis, severe eczema, etc.	Strabismus	Crooked mouth crying, CHD, micropenis

Abbreviations: −, absent; +, present; CHD, congenital heart disease; F, female; M, male; NM, not mentioned.

TABLE 2 14q32.31q32.33 microduplications less than 5.0-Mb with likely pathogenic/pathogenic classification in the DECIPHER database.

DECIPHER patients	Location (GRCh38)	Inheritance	Pathogenicity	Phenotypes
274,922	14:102082020–102307753, 225.73 kb	Maternal	LP	Abnormality of higher mental function, behavioral abnormality
402,149	14:105470441–105785009, 314.57 kb	De novo	LP	Abnormal respiratory system physiology, abnormality of the vasculature, asymmetry of the thorax, blepharophimosis, cerebral calcification, feeding difficulties in infancy, hearing impairment, hypotonia, intellectual disability, etc.
501,113	14:102949431–103274928, 325.50 kb	Unknown	LP	Intellectual disability, seizures
303,812	14:104816988–105187460, 370.47 kb	Unknown	LP	Global developmental delay
402,225	14:105612056–106,649,016, 1.04 Mb	De novo	LP	Aplasia/hypoplasia of the cerebral white matter, autistic behavior, cerebral atrophy, delayed speech and language development, EEG abnormality, hypotonia, intellectual disability, etc.
276,341	14:102540896–103679744, 1.14 Mb	De novo	LP	2–3 toe syndactyly, aggressive behavior, global developmental delay, sensorineural hearing impairment, short fourth metatarsal, strabismus, thick lower lip vermilion
454,366	14:105606325–106873725, 1.27 Mb	Parental balanced rearrangement	LP	Abnormal iris pigmentation, asplenia, delayed speech and language development, duodenal atresia, fetal pyelectasis, global developmental delay, hydronephrosis, intestinal malrotation, microcephaly, renal hypoplasia
Our case	14:101575717–106877229, 5.2 Mb	Unknown	P	Global developmental delay, intellectual disability, seizures, moderate malnutrition, strabismus, low-set nose and ears, eye distance widening

Abbreviations: LP, likely pathogenic; P, pathogenic.

TABLE 3 Clinical findings in patients with 20q13.33 microdeletion associated with 17q25.3 microduplication.

		Ürel-Demir et al. (2020)			
	Marques et al. (2015)	**Patient 1**	**Patient 2**	**Patient 3**	**The present study**
Deletion size	2.1-Mb	1.594-Mb	1.635-Mb	1.594-Mb	1.8-Mb
Duplication size	1.4-Mb	4.357-Mb	4.429-Mb	4.357-Mb	4.8-Mb
Origin	NA	Maternal *t*(17;20)	Maternal *t*(17;20)	Maternal *t*(17;20)	NA
Sex/Age	M/6 years 2 months	M/12 years	M/15 years	M/9 years	M/4 years
Developmental delay	+	+	+	+	+
Intellectual disability	+	+	+	+	+
Seizures	+	+	+	+	+
Microcephaly	+	+	+	−	−
Hypotonia	+	−	−		+
Facial dysmorphism	+	+	−	−	+
CHD	+	+	+	−	+
Urogenital Abnormalities	−	Cryptorchidism	Cryptorchidism	−	Micropenis
Single palmar crease	−	+	−	−	+
Skeletal abnormalities	+	+	+	+	−
Other abnormalities	Craniosynostosis	Stereotypic movements	Strabismus	Unilateral pelviectasis	Crooked mouth crying

Abbreviations: −, absent; +, present; CHD, congenital heart disease; M, male; NA, not available; NM, not mentioned.

features in the patients (Traylor et al., 2010). In a previous study (Traylor et al., 2010), six individuals with 20q13.33 microdeletions ranging in size from 561 kb to 6.8 Mb were identified. The individuals exhibited abnormal clinical features including intellectual disability, developmental delay, speech and language deficits, seizures, and dysmorphic features. Additionally, in a previous report, a de novo 1.1 ~ 1.6-Mb deletion in the 20q13.33 region was identified in a patient with learning disability affecting particularly expressive speech, without seizures, and obvious dysmorphic features (Béna et al., 2007). Moreover, neonatal epilepsy and developmental disorder were identified to be associated with a 1.5-Mb deletion in the 20q13.33 region, which also manifested pyridoxine-dependent epilepsy. However, the researchers considered this association to be a coincidental event (Mefford et al., 2012). Furthermore, a study also reported a 20q13.33 microdeletion in a girl with epilepsy, global developmental delay, and failure to thrive (Lewis et al., 2018). One report detailed three unrelated individuals with deletions in the 20q13.33 region, all presenting with neonatal seizures necessitating anti-epileptic drugs, alongside developmental delay (Pascual et al., 2013). However, several studies have presented patients with 20q13.33 microdeletion who do not exhibit seizure disorders (Béri-Deixheimer et al., 2007; Mosca-Boidron et al., 2013; Roberts et al., 2004). Several cases (not all cases) presented seizures, suggesting incomplete penetrance. In the present study, the patients with 20q13.33 microdeletion displayed seizures, developmental delay, and intellectual disability, which may be partially attributed to the 20q13.33 microdeletion.

In the present study, an extremely rare 14q32.31q32.33 microduplication was also detected in Patient 1. Few reports of 14q32.31q32.33 microdeletion/microduplication are available in the existing literature. A previous study presented a case of a 14q32.31q32.33 microduplication compound with 14q32.33 microdeletion in a 29-month-old patient with spasticity and periventricular white matter changes on MRI (Ramaswamy et al., 2011). However, the authors are more inclined to consider that the microdeletion of 14q32.33 is responsible for the abnormal white matter observed on MRI. Despite the identification of a 14q32.11q32.33 microduplication covering *BCL11B*, *CCNK*, *YY1*, *DYNC1H1*, and *PACS2* candidate genes in a patient with developmental delay, intellectual disability, and facial dysmorphism (Han & Park, 2021), in the present report, CNVs were found to contain *DYNC1H1* and *PACS2* genes as well. Pathogenic mutations in *PACS2* are associated with developmental and epileptic encephalopathy 66 (Olson et al., 2018). In addition, pathogenic *DYNC1H1* mutations have been linked to mental retardation, specifically autosomal dominant 13 (Vissers et al., 2010). As delineated in Table 2, several cases with 14q microduplications smaller than those in our report were interpreted as likely pathogenic variants. Most of these cases exhibited global developmental delay and intellectual disability. Thus, it is plausible that the 14q32.31q32.33 microduplication in Patient 1 may also contribute to the abnormal clinical phenotypes of global developmental delay and intellectual disability.

In the present study, a 4.8-Mb 17q25.3 microduplication was also identified in Patient 2. The distal duplication of 17q is a rare CNV with only several reported cases exhibiting variable phenotypes. Notably, approximately 85% of these cases are inherited from parents with balanced translocations (Upadia et al., 2018). Patients with 17q distal duplication commonly manifest developmental delay, intellectual disability, short stature, microcephaly, facial dysmorphism, cleft palate, skeletal anomalies, and other congenital anomalies (Lukusa & Fryns, 2010). A recent study (Wang et al., 2020) identified a pure 17q25.3 duplication in a 4-year-old Chinese pediatric patient with global developmental delay, intellectual disability, short stature, facial dysmorphism, attention deficit hyperactivity disorder, microcephaly, skeletal anomalies, and multiple congenital anomalies. In addition, a previous study conducted by Probst et al. (Probst et al., 2015) suggested a correlation between 17q25.3 duplication and abnormal cardiac development. Thus, it is possible that the 17q25.3 microduplication may also contribute to developmental delay, intellectual disability, and CHD in Patient 2, as observed in the present study. At present, only two cases of 20q13.33 microdeletion with 17q25.3 microduplication have been reported in the existing literature (Table 3). A patient presenting with both CNVs exhibited a range of clinical features including intellectual disability, developmental delay, microcephaly, short neck, syndactyly, cardiac defects, craniosynostosis, and other abnormalities. This case was first reported by Marques et al. (Marques et al., 2015). Subsequently, a more recent study (Ürel-Demir et al., 2020) identified three patients with both CNVs who presented similar clinical features but without craniosynostosis. Thus, the belief of the present authors is that both the 20q13.33 microdeletion, and the 17q25.3 microduplication may contribute to the abnormal features observed in the patient. In the present report, additional clinical features of micropenis and crooked mouth crying were also observed in the patient. As shown in the DECIPHER database, a case with de novo 869.7-kb deletion in the 20q13.33 region exhibited intellectual disability, EEG abnormality and micropenis [DECIPHER ID: 401250, likely pathogenic]. Based on the described findings, the belief of the present authors is that 20q13.33 microdeletion may be the main reason for the additional clinical feature of micropenis. Nevertheless, it

is challenging to ascertain whether the additional clinical feature of crooked mouth crying is attributable to the 20q13.33 deletion or the 17q25.3 duplication.

Despite the present study presenting two novel cases of 20q13.33 microdeletion and further emphasizing the genotype–phenotype correlations with language and motor developmental delay, intellectual disability, and seizures, several limitations were also present in this study. One limitation was the inability to check parental chromosomes by FISH for a balanced translocation, as both families refused this testing. In addition, more research is needed to further confirm the relationship between the additional features (micropenis, congenital heart disease, and crooked mouth crying) and 20q13.33 microdeletion.

In conclusion, two novel cases of 20q13.33 microdeletion with microduplications in the 17q25.3 and 14q32.31q32.33 regions in the Chinese population were investigated in the present study. The findings suggest that the new feature of micropenis may be associated with 20q13.33 microdeletion, thereby broadening the phenotype spectrum of this condition. Additionally, the present study further underscores the utility of chromosomal microarray analysis in the etiological diagnosis of children with congenital complex diseases.

AUTHOR CONTRIBUTIONS

JZ and NZ wrote the article. SZ and JW performed the karyotype analysis. HZ and YJ recruited the participants and analyzed the data. NZ, CC, and JZ revised and polished the paper. All authors approved the final article.

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FUNDING INFORMATION

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics Committee approval was obtained from the Institutional Ethics Committee of Quanzhou Women's and Children's Hospital for the commencement of this study (2020No.31). All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT STATEMENT

We received informed consent from the study participants' guardians, and they agreed to the publication of a report on the study.

CONSENT FOR PUBLICATION

We confirm that written informed consent was signed by the patient's parents for publishing their own and their children's genetic data and relevant information, and the written informed consent is available on request.

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