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ORIGINAL ARTICLE

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Intellectual disability in two Chinese sisters caused by a 3p26.3p25.3 microdeletion and a 14q32.13q32.33 microduplication inherited from the mother with 46, XX, t (3, 14) (p25; q32)

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Abstract

Background: Genetic factors associated with intellectual disability (ID) include chromosomal aberrations, copy number variations (CNVs), and pathogenic variants. Identifying the genetic etiologies is beneficial for patient classification, therapy, management, and prognostic evaluation. Emerging genetic tests are helpful in identifying these genetic causes.

Methods: We enrolled two girl siblings with ID. Trio whole-exome sequencing (WES) and Copy number variation sequencing (CNV-Seq) were performed for genetic molecular analysis in these probands and their parents. The parents also accepted high-resolution G-banded karyotype studies.

Results: No significant homozygous or heterozygous variants were identified through WES. By CNV-seq, we identified an abnormal 3p26.3p25.3 microdeletion and 14q32.13q32.33 microduplication in the two girl siblings but not in their parents. A balanced translocation 46, XX, t (3, 14) (p25; q32) was found in their mother.

Conclusion: The affected siblings have similar phenotype, including ID, short stature, and microcephaly. Their mother had a history of seven first-trimester miscarriages and one elective termination because of multiple malformations. This abnormal karyotype was also thought to be responsible for the mother's recurrent miscarriage. WES in combination with CNV-seq analysis is very helpful for identification of the genetic causes of ID without positive karyotype findings.

KEYWORDS

balanced translocation, copy number variations, intellectual disability, miscarriage, whole-exome sequencing

1 | INTRODUCTION

Intellectual disability (ID) is characterized by significant deficits in both intellectual (reasoning, learning, problem solving) and adaptive functioning in the conceptual, social, and practical domains, originating before the age of 18 years. Between 1% and 3% of children worldwide are affected by this type of developmental disorder (Ilyas, Mir, Efthymiou, & Houlden, 2020; Leonard & Wen, 2002; Maulik, Mascarenhas, Mathers, Dua, & Saxena, 2011). The etiology of ID can be divided into

two groups: (a) exogenous factors, such as maternal alcohol abuse during pregnancy, delivery complications, uncontrolled maternal medical condition, postnatal trauma, and exposure to toxic/infectious agents; (b) genetic factors (Lee, Cascella, & Marwaha, 2020). In addition to diagnosis of ID based on clinical phenotype, identification of the etiologies is beneficial for the classification of patients, as well as selection of appropriate therapy, management, and prognostic evaluation. However, due to the high heterogeneity of etiology and presentation, a genetic diagnosis for most cases of ID is still lacking. Advances in sequencing technologies have made it possible to identify the genetic etiologies of many forms of ID (Harripaul, Noor, Ayub, & Vincent, 2017; Vissers, Gilissen, & Veltman, 2016). These genetic factors include chromosomal aberrations, copy number variations (CNVs), and pathogenic variants of over 700 genes (Harripaul et al., 2017; Vissers et al., 2016).

Here, we present the cases of two Chinese sisters with ID accompanied by short stature. Abnormal CNVs (3p26.3p25.3 microdeletion and 14q32.13q32.33 microduplication) were identified in the children but not found in their parents. Furthermore, through high-resolution G-banded karyotype studies, a balanced translocation, 46, XX, t (3, 14) (p25; q32) was identified in the children's mother.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

Ethical approval for this study was obtained from the Institutional Review Board at the Children's Hospital of Chongqing Medical University, Chongqing, China (Ethical approval no. 2018-64). Informed consent was obtained from the patient's parents.

2.2 | Patients and pedigree

After obtaining written informed consent from their parents, the family members were enrolled. In November 2019, the two patients attended the Pediatric Department of Qianjiang Central Hospital of Chongqing as outpatients. They were then transferred and followed-up at the Department of Neurology of the Children's Hospital of Chongqing Medical University. Peripheral blood was collected from the family members after signing informed consent. All of the available clinical characteristics of the patient, along with the aforementioned auxiliary examination results are summarized in this report.

2.3 | Whole-exome sequencing and copy number variation sequencing analysis

Based on human genome reference sequence hg19/GRCh37, whole-exome sequencing (WES) and copy number variation sequencing (CNV-Seq) analysis were performed on the siblings and their parents at MyGenostics Inc. The Genome Aggregation Database (gnomAD), DGV, dbVar, DECIPHER, and several other online databases were used as references. The mutations were predicted using in silico software, including SIFT, SIFT_Predict, PolyPhen_2, PolyPhen_2_Predict, SPIDEX, REVEL_score, MutationTaster, MutationTaster_Predict, MCAP_score, MCAP_pred, GERP++, and GERP++_Predict.

2.4 | G-banded karyotype studies

High-resolution G-banding karyotype analyses were performed using a standard method at the Center for Clinical Molecular Medicine of the Children's Hospital of Chongqing Medical University.

3 | RESULTS

3.1 | Clinical characterization

The two female siblings (Patient 1, aged 13 years and 6 months; Patient 2, 10 years and 6 months) were born to a nonconsanguineous Chinese family (Tu Jia nationality). Patient 1 (III 8, Figure 1) was delivered at 38 weeks and 4 days of gestation, gravida 5 para 1 (Figure 1). The girl was born by normal delivery with a birth weight of 2,200 g. Patient 2 (III 10, Figure 1) was born through normal delivery, G7P2 (Figure 1), with a birth weight of 2,600 g. The length and head circumference of both siblings at birth are not known.

The children were presented at the outpatient department of our hospital by their parents who reported a global developmental delay (GDD) since early childhood. Both of siblings began to walk independently at 2 and half years old and spoke their first words at 3 years of age. Despite slight improvements, the girls were still able to speak only a very few words. They exhibited obvious developmental delay in terms of intelligence and social/personal and activities of daily life. To date, the siblings were unable to attend mainstream schools, and received specialist education.

Examination showed that the children had no dysmorphic facial features. The physical characteristics of Patient 1 were as follows: weight 28 kg; height 123 cm (z-score = −5.09 *SD*); BMI 18.5 (z-score = −0.28 *SD*); and head circumference 48 cm. The physical characteristics of Patient 2 were as follows: weight 19 kg; height 110 cm (z-score = −4.87 *SD*); BMI 15.7 (z-score = −0.63 *SD*); and head circumference 47 cm. These data highlighted the short stature and microcephaly of both patients, but no other characteristic dysmorphic features were observed. Both siblings exhibited severe intellectual delay, with an IQ < 30 using the Chinese Wechsler Intelligence Scale for Children.

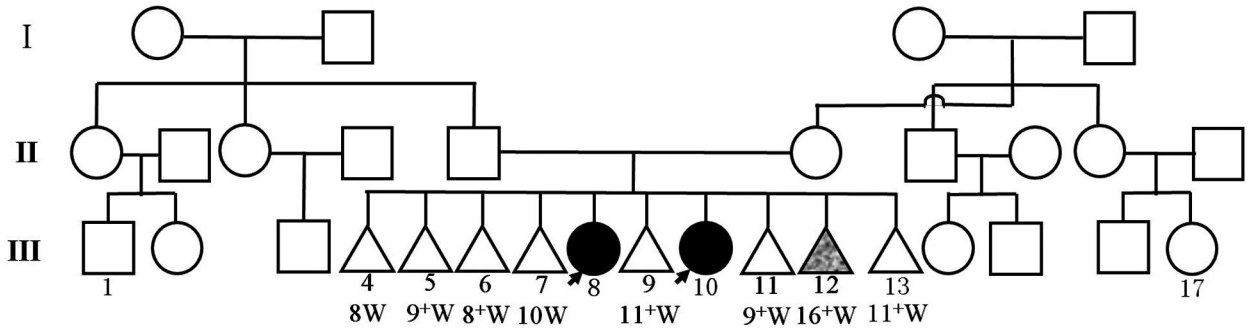

FIGURE 1 The two probands (black arrow) had similar phenotypes of intellectual disability (III 8, 10), their mother had a history of seven first-trimester miscarriages (III 4, 5, 6, 7, 9, 11, 13) without any identified causes and one elective termination due to multiple malformations (III 12)

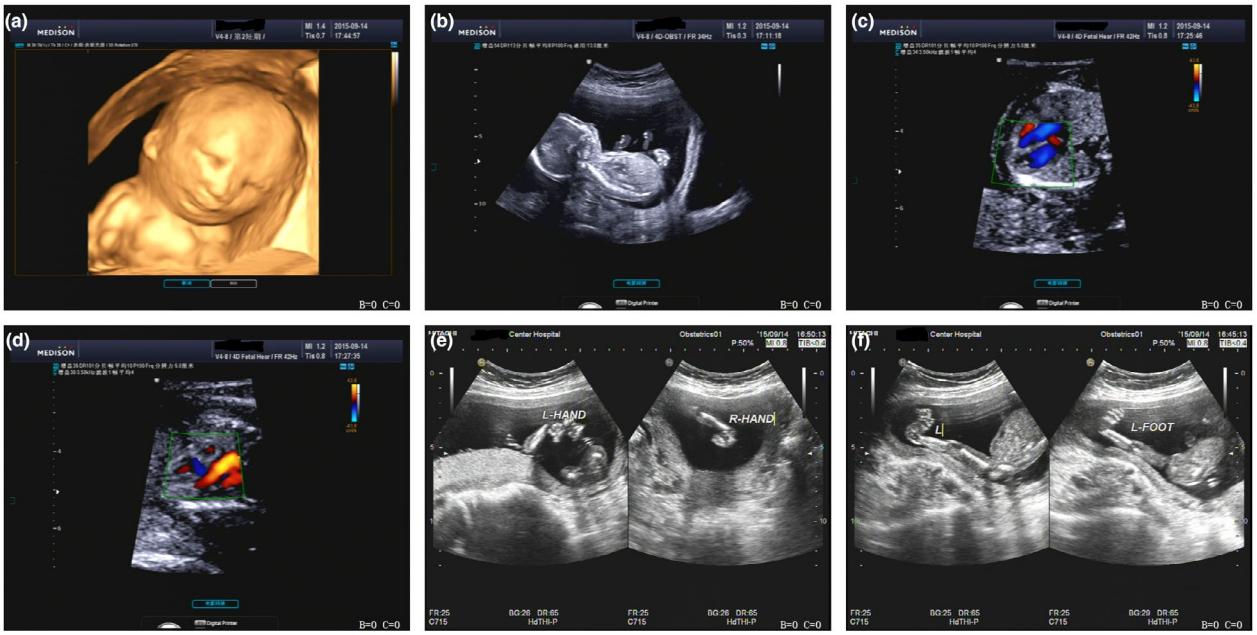

FIGURE 2 Fetal abnormalities (17 weeks and 3 days of gestation) were observed by color Doppler ultrasonography. (a) Widened bilateral eye space; (a and b) micrognathia; (c and d) Atrioventricular septal defect; (e) Abnormal bilateral wrist joint; (f) Left foot varus

For both girls, the blood biochemical parameters and thyroid and growth hormone levels were normal. Blood and urinary metabolic screening indicated normal results. In color Doppler ultrasonography, no abnormalities were found in their heart and abdominal organs. Their electroencephalography results were also normal. At the age of 8, brain magnetic resonance imaging (MRI) and peripheral blood karyotyping were performed for Patient 1 and her results were normal, although these tests were not completed for Patient 2. Computer assisted assessment indicated bone ages of 13 years for Patient 1 (13 year 11 month old) and 9 years and 6 months for Patient 2 (10 year 6 month old).

The mother had a history of seven first-trimester miscarriages (III 4, 5, 6, 7, 9, 11, 13) without any identified causes (Figure 1). The mother's ninth pregnancy (III 12, Figure 1) was artificially aborted at 17 weeks and 3 days of gestation because of multiple malformations (widened bilateral eye space, micrognathia, atrioventricular septal defect, abnormal bilateral wrist joint, and left foot varus) revealed by color Doppler ultrasonography (Figure 2); karyotyping of this fetus was not completed. Approximately 5 years previously, the parents underwent comprehensive laboratory examinations due to recurrent spontaneous miscarriages. The result of peripheral blood karyotyping and blood biochemical analysis were normal. Thyroid function was also normal, no pathogens were detected in serum (rubella virus, cytomegalovirus, herpes virus, toxoplasma, HIV, syphilis), or the genital tract (*Mycoplasma genitalium*, chlamydia, gonococcus). The father's semen analysis was normal. The mother's reproductive tract was also normal according to color Doppler ultrasonography and hysteroscopy.

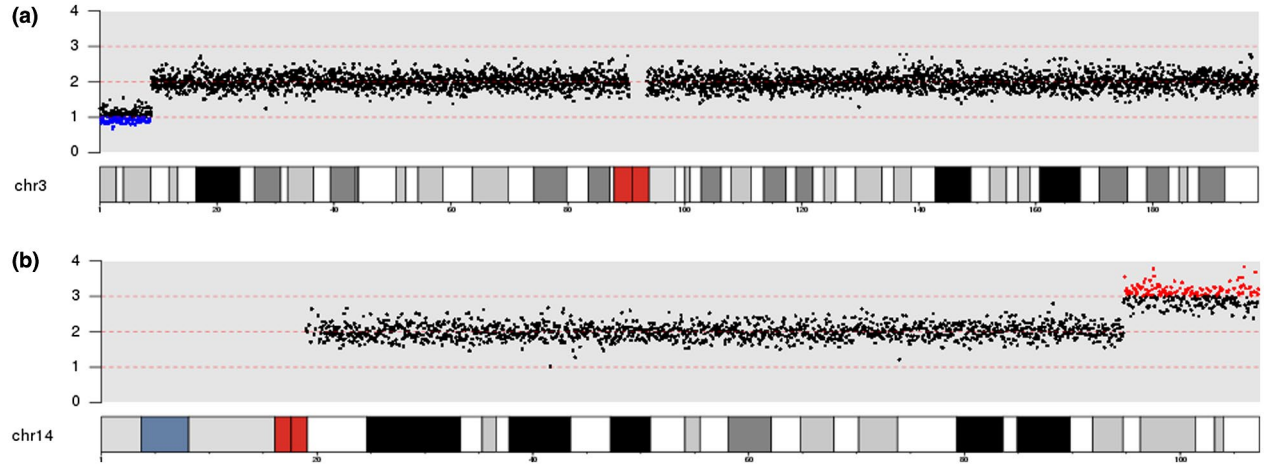

FIGURE 3 CNV-Seq analysis indicated. (a) a 3p26.3p25.3 microdeletion ((chr3:239325–8794930)*1); (b) a 14q32.13q32.33 microduplication ((chr14:94953639–105996131)*3). CNV-Seq, copy number variation sequencing

3.2 | Molecular genetics

After filtering the WES variant data according to the standards and guidelines of the American College of Medical Genetics and Genomics (Richards et al., 2015) and combined with the clinical features of the siblings, no significant homozygous or heterozygous variants were identified. CNV-seq analysis performed on the two siblings revealed a 3p26.3p25.3 microdeletion ((chr3:239325–8794930)*1) and a 14q32.13q32.33 microduplication (chr14:94953639–105996131)*3) (Figure 3). However, these abnormal CNVs were not identified in their parents. High-resolution G-banded karyotype studies revealed a balanced translocation 46, XX, t (3, 14) (p25; q32) in the mother, but not in the father (Figure 4).

4 | DISCUSSION

The genetic alterations of ID can be detected by karyotyping, chromosomal microarray analysis (CMA), and next-generation sequencing (NGS). Karyotyping and CMA have long been first-line approaches to the diagnosis of unexplained GDD/ID, but detect only large deletions, insertions and CNVs. Remarkable progress in NGS-based genetic testing, including panel-based NGS, WES, and whole-genome sequencing (WGS), has improved the discovery of genetic variants in over 7,000 rare diseases (Deciphering Developmental Disorders Study, 2015; Li, Anderson, Ginns, & Devlin, 2018; Lindstrand et al., 2019; Srivastava et al., 2019). It has been reported that the diagnostic yield of WES in 3,350 individuals with neurodevelopmental disorders was 36% (Srivastava et al., 2019). A recent study showed a diagnostic yield of 55.7% by clinical exome sequencing in severe GDD/ID (Stojanovic et al., 2020). In a study of 1,051 children with ID/GDD conducted in China, gene structure variation detected by karyotype analysis combined with CNV detection and WES increased the proportion of definitive etiological confirmed cases from 56.2% to 78.9%, respectively (Liao et al., 2019). Because of its potential for high throughput and cost effectiveness, WES is becoming widely used as a first-tier test, allowing detection of SNVs, INDELs, and CNVs covering multiple exons. WGS may become another powerful tool for genomic research due to the marked reduction in sequencing costs (Li et al., 2018; Lindstrand et al., 2019; Michelson et al., 2011; Srivastava et al., 2019).

In the present study, both children had GDD since early childhood, including deficits in walking, verbal skills, and learning. When we first encountered these siblings, we initially investigated any exogenous factors that may have caused their ID. However, there were no birth complications, trauma, toxin/infectious agent exposure or malnutrition. No abnormality was observed at the older sister's MRI; therefore, we considered the influence of genetic factors since both siblings exhibited highly similar phenotypes and their mother had experienced several spontaneous abortions without identified causes. Patient 1 and her parents had previously undergone chromosomal analysis but no abnormalities were identified; therefore, we decided to perform WES and CNV-seq analysis for the two siblings and their parents.

No significant homozygous/heterozygous variants were identified in the siblings by WES, while CNV-seq analysis revealed a 3p26.3p25.3 microdeletion and a 14q32.13q32.33 microduplication. Neither of these abnormal CNVs were found in the DGV and DECIPHER databases. However, in the dbVar database, a highly similar copy number loss (nssv13646324, 3p26.3–26.1(chr3:61891–8600971)*1) was reported to be responsible for developmental delay and/or other significant developmental or morphological phenotypes; hence, it was considered that the 3p26.3p25.3

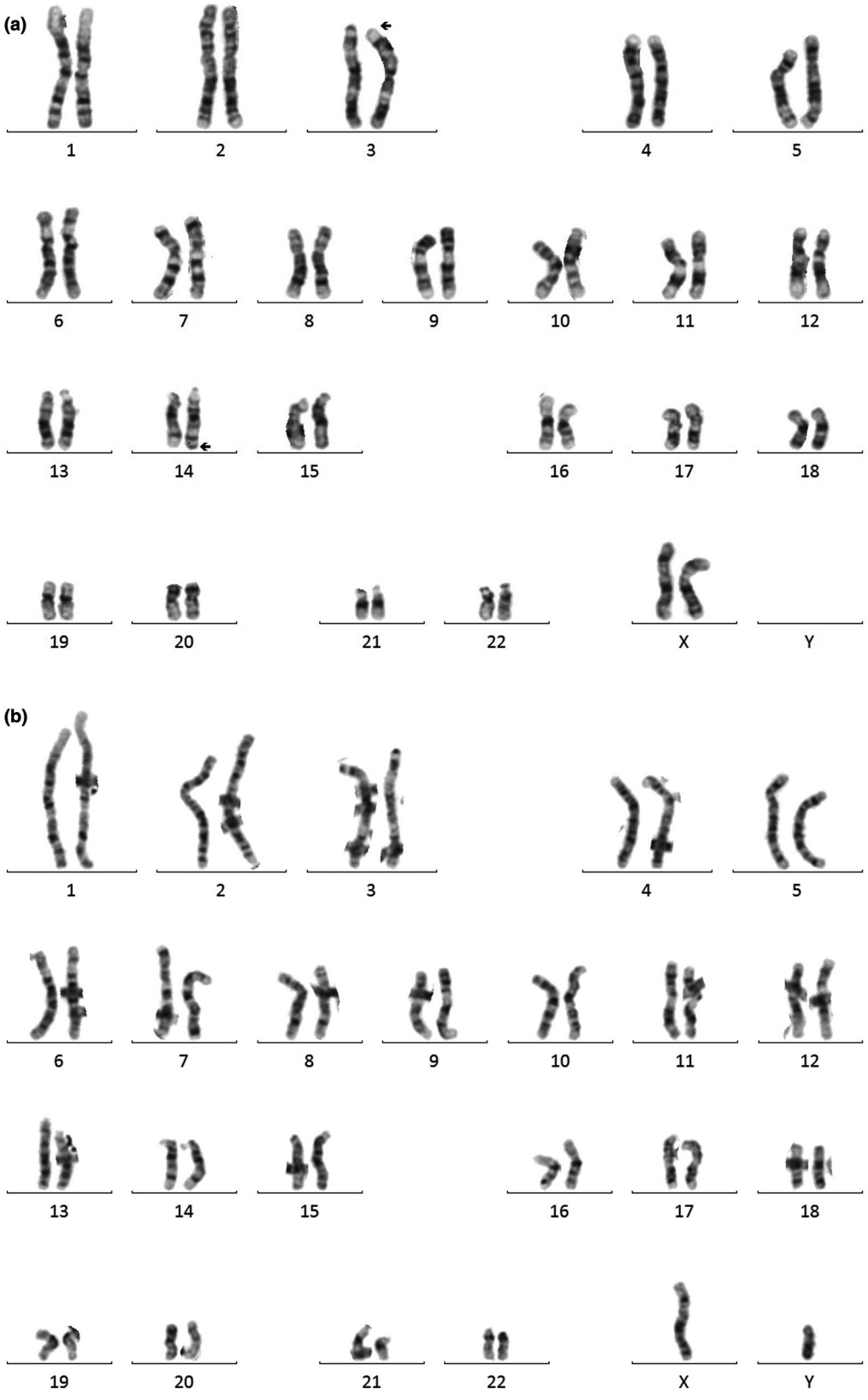

FIGURE 4 G-banded karyotype of the parents. (a) A balanced translocation 46, XX, t (3, 14) (p25; q32) was found in the mother; (b) The karyotype of the father was normal

microdeletion identified in these siblings was pathogenic. Also in dbVar, another copy number gain (nssv13652284, 14q32.12–32.33 (chr14:92432614–106358753)*3) was found to be associated with developmental disabilities or

congenital anomalies; hence, the 14q32.13q32.33 microduplication was deemed to be pathogenic. Subsequently, we aimed to confirm that these abnormal CNVs were the causes of the ID in these two patients.

The absence of these abnormal CNVs in the sibling's parents was a source of confusion. Considering the seven miscarriages experienced by their mother in addition to the elective termination of a fetus with multiple malformations, we speculated that these pathogenic CNVs may have arisen in the parents, and the possible existence of a balanced translocation. Therefore, we repeated the G-banded karyotype studies and detected a balanced translocation 46, XX, t (3, 14) (p25; q32) in the mother, although the father's karyotype was normal. Despite the mother's balanced translocation, she was clinically healthy, although her offspring inherited the abnormal karyotype through a chromatin rearrangement and loss during conception. The mother's abnormal karyotype may explain her recurrent spontaneous miscarriages and fetal abnormalities, as well as the two siblings' ID and it is regrettable that this balanced translocation was not detected in the earlier karyotype study. It can be speculated that duplicate detection in combination with other cytogenetics/cytogenomics diagnostic tests will be helpful in diagnosing this condition.

In summary, we present the cases of two female sibling with ID. The genetic causes of this condition had remained undetected for many years until our sequencing revealed pathogenic abnormalities of a 3p26.3p25.3 microdeletion and a 14q32.13q32.33 microduplication. These abnormal CNVs were found to be inherited from their mother who had the balanced translocation 46, XX, t (3, 14) (p25; q32). The mother's abnormal karyotype was also thought to be responsible for the recurrent miscarriages and fetal abnormalities. Therefore, WES in combination with CNV-seq analysis are very helpful for identification of genetic causes of ID, especially in cases without positive karyotype findings.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Zhong Min designed and supervised the study, and wrote the manuscript. Zhong Min and Wei Yongjuan carried out the diagnosis and follow-up of the patients. Dai Ying and Guo Hui collected the data, and performed the literature review. Chen Yuanyuan was responsible for WES, CNV-seq, and karyotype. All the authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data are not available for public access because of patient privacy concerns, but are available from the corresponding author (Zhong Min) on reasonable request.

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