INTRODUCTION

The long arm of chromosome 2 is unique in human autosomes, originating from the head-to-head fusion of two ancestral chromosomes at 2q13 with the ancestral centromere at 2q21. Terminal or interstitial deletion of the long arm of chromosome 2 is a rare copy number variations (CNVs), with approximately 100 cases reported in available literature. Furthermore, this deletion has been associated with epilepsy, intellectual disability, developmental delay, cardiovascular malformation, hypospadias and cryptorchidism, digital abnormalities, and other visceral organ anomalies. Clinical manifestations vary greatly based on the size and location of the deletion.

A deletion involving 2q24.3 has been previously reported, and the patient exhibited psychomotor retardation, low set ears, cranial sutural irregularities, and laryngomalacia. Microdeletion of 2q31.1 is deemed a clinically recognizable gene syndrome characterized by
short stature, moderate-to-severe developmental delay, microcephaly, hypotonia, specific craniofacial dysmorphisms, and upper/lower limb deformities associated with HOXD genes.\(^5\) Previously, chromosome deletions were discovered by Giemsa banding.\(^6\) Chromosome deletions spanning over 5 Mb are microscopically visualized on chromosome-banded karyotypes. Given the development of next-generation sequencing technology, CNV sequencing (CNV-seq) has been widely employed in recent years. Compared with conventional methodology, CNV-seq has advantages such as high throughput, high resolution, and relatively low cost.\(^7\) Moreover, CNV-seq can detect deletions above 100 Kb.

Herein, we describe a novel interstitial heterozygous deletion that encompasses the 2q24.3-q32.1 chromosomal region, as determined using CNV-seq and whole exome sequencing (WES). The deletion was found to affect 94 genes, of which 33 are associated with diseases, including HOXD13, MAP3K20, DLX1, DLX2, SCN2A, and SCN1A. We analyzed the clinical features and genes on the deletion region to further interpret the relationship between the deletion region and phenotype.\(^8\)

2 | METHODS

2.1 | Participants

The proband and parents were enrolled at The First Affiliated Hospital of Wenzhou Medical University. Written consent was obtained from the parents of the fetus prior to commencing the study. All study protocols were reviewed and approved by the ethics committees of The First Affiliated Hospital of Wenzhou Medical University. Relevant clinical records (symptoms, appearance and duration of symptoms, physical and ultrasound examination) were collected and examined.

2.2 | DNA extraction

According to the manufacturer’s standard instructions, genomic DNA was extracted from the fetal muscle and his parents’ peripheral blood samples conserved in EDTA using the Tissue Genome DNA Extraction Kit DP341 and Blood Genome DNA Extraction Kit DP329 (TianGen). DNA purity and concentration were determined using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). Genomic DNA was stored at −20°C until use.

2.3 | WES

Briefly, ultrasound was used to break genomic DNA into 250–300bp fragments. DNA libraries were constructed by end filling, adapter ligation, and polymerase chain reaction amplification.\(^9\) Then, the DNA libraries underwent hybridization capture and were enriched by the xGen Exome Research Panel v2.0 (IDT). High throughput sequencing was performed on the DNBSEQ-T7 platform (Beijing Genomics Institute). After filtration and quality control, clean reads were aligned to the University of California Santa Cruz (UCSC) human reference genome (hg19) using the Burrows-Wheeler mapping algorithm.\(^10\) Combined with OMIM, HGMD, SwissVar, Clinvar, and dbSNP, the genetic variation was analyzed, classified, and annotated with the American College of Medical Genetics (ACMG).

2.4 | CNV-seq

The DNA libraries were single-ended sequenced on the DNBSEQ-T7 platform (Beijing Genomics Institute), with a sequencing depth of 0.2x. Raw sequencing reads were processed according to the quality control standards and subsequently compared with the hg19 of the UCSC using Burrows-Wheeler Alignment.\(^10\) Using read counts, \(Z\)-scores, and log2Ratio, the in-house bioinformatics pipeline evaluated CNVs.\(^7\) The candidate CNVs were filtered with the Accurate Diagnosis of Genetic Diseases Cloud Platform (Quanpu). Subsequently, CNVs were annotated based on the publicly available databases, including Decipher, Clinvar, ISCA, OMIM, ClinGen and UCSC (http://genome.ucsc.edu).\(^11\) Finally, according to the ACMG guidelines, CNVs were divided into five categories: pathogenic, likely pathogenic, likely benign, uncertain clinical significance, and benign.\(^12\)

3 | RESULTS

3.1 | Clinical data

The male fetus (the proband) was the second child of young and non-consanguineous parents. The maternal pregnancy was uncomplicated. Family history included a spontaneous abortion (embryo arrest) at 8 weeks of gestation. No consanguinity was reported. Prenatal care showed no history of exposure to radiation and toxic agents. At the 23rd week of gestation, the fetus exhibited increased anterior nasal skin and nuchal fold (Figure 1A and B). The echocardiogram indicated ventricular septal defect and aortic dysplasia (Figure 1C–F). A routine prenatal ultrasound revealed abnormal fetal hand posture and an increased distance between fingers (Figure 1G–I). In addition, the gall bladder was unclear on the ultrasound image, along with the presence of polyhydramnios. The pregnancy was terminated, and the fetus was aborted owing to multiple malformations at 27 weeks of gestation, with a weight of 875 g (10th–25th percentile), length of 35 cm (25th–50th percentile), and a head circumference of 23.5 cm (10th–25th percentile). On physical examination, the fetus exhibited dysmorphic features, including proximally placed fourth finger and camptodactyly. As shown in the ultrasonic image, the distance between the thumb, index finger, and middle finger was increased, with splaying between the index and middle fingers (Figure 1). His feet were symmetrical with complete cutaneous syndactyly of the second and third digits (Figure 1K). The necropsy was refused.
3.2 | CNVs detection

WES revealed a heterozygous deletion at genomic position (chr2: 165125352-183581904) (Assembly hg19). CNV-seq confirmed the likely 18.46 Mb pathogenic CNVs on chromosome 2 (Figure 2A). This position corresponded to the 2q24.3 and 2q32.1 cytogenetic bands. The chromosomal constitution was as follows: 46,XY array2q24.3q32.1 (165125352-183581904)×1. The deletion affected 94 protein-coding genes, including HOXD13, MAP3K20, DLX1, DLX2, SCN2A, and SCN1A (Figure 2B). Both parents did not carry abnormal CNVs, which indicated that the deletion in the proband was de novo.
Herein, the proband presented camptodactyly, syndactyly, proximally placed fourth finger, ventricular septal defect, and aortic dysplasia. We identified a novel heterozygous interstitial deletion at chromosome 2q24.3–32.1 (chr2: 165125352–183581904), which could have markedly contributed to the fetal phenotype. The deletion involved 94 protein-coding genes, including 33 morbid genes related to recognizable clinical phenotypes. Among these, HOXD13, SCN2A, and SCN1A have exhibited haploinsufficiency in ClinGen. Table 1 summarizes the clinical features of patients with chromosome deletion from 2q24.3 to 32.1. In this study, the most prominent feature was deformity of the upper and lower limbs, including camptodactyly, syndactyly, and clinodactyly. All reported patients with 2q24.3–32.1 deletions appear to present limb abnormalities. Overall, 8/11 patients presented syndactyly, 4/11 patients exhibited camptodactyly, and 8/11 patients presented clinodactyly. As noted in mouse mutants, the HOXD cluster and surrounding regulatory sequences are considered the underlying cause of the limb phenotype in this region. Deletion, translocation, or disruption of this locus can reportedly cause camptodactyly, syndactyly, brachydactyly, ectrodactyly, and polydactyly. Considering the current case study, the deletion contained HOXD13, an essential gene for regulating and developing the genital tract and autopod that forms hands and feet. In addition, it has been suggested that the HOXD cluster can regulate the size and number of digits in a dose-dependent manner, indicating a negative relationship between the HOXD gene and digit number rather than qualitative functions. In the presence of multiple homozygous HOXD mutations, major limb defects are likely to occur. Heterozygous deletion of HOXD13 may lead to HOXD13 haploinsufficiency. The heterozygous loss-of-function variants reduce the production of functional protein binding to DNA, while sustaining the basic function of HOXD13 protein. Therefore, it exhibits a milder phenotype, similar to that observed in our proband and shows incomplete penetrance with some frequency. Meanwhile, it explains the limb phenotypes with different reported severity.

Spielmann et al. have found that Map3k20, a gene within the deletion region, is expressed in developing limbs. Furthermore, the authors summarized the clinical manifestations of Map3k20 mutations, including split-foot malformation with mesoaxial polydactyly, which is related to hearing loss and exhibits a possible clinical phenotype of cutaneous syndactyly. Herein, cutaneous syndactyly was an important malformation in the examined fetus. However, according to mouse experiments and reported pedigrees, the
| Phenotype                  | Lazier et al. | Tsai et al. | Svensson et al. | Pescucci et al. | Boles et al. | Dimitrov et al. [n = 5] | Our case |
|---------------------------|---------------|-------------|-----------------|-----------------|--------------|------------------------|----------|
| Start-end                 | 2q24.3-q31.1  | 2q31.1-31.2 | 2q31.1          | 2q24.3-q31.1    | 2q24.2-q31.1 | 2q24.3-q32.1           | 2q24.3-q32.1 |
| Size (Mb)                 | 10.4          | 3.4         | 2.518           | 10.4            | NS           | 2.74–16.9              | 18.46    |
| Gender                    | Female        | Female      | Female          | Female          | Male         | 1 M: 4 F               | Male     |
| Birth Height              | NS            | NS          | 10th–25th       | 25th–50th       | NS           | 2/5 [NBW] (1/5 NS)     | 25th–50th |
| Birth Weight              | 10th          | <3th        | 50th–75th       | 10th–25th       | 2890 g       | 3/5 [NBW] (1/5 NS)     | 10th–25th |
| Postnatal developmental retardation | +            | +           | +               | +               | +            | +                      | NA       |
| microcephaly              | +             | +           | +               | +               | 2/5          | −                      | −        |
| Cranial sutural irregularities | +           | −           | −               | −               | 2/5          | −                      | −        |
| ptosis/epicanthus         | +             | −           | −               | +               | 4/5          | −                      | −        |
| Low set/dysplastic ears   | −             | +           | +               | +               | 2/5          | −                      | −        |
| Bilateral limb deformity  | +             | +           | +               | NS              | 4/5 (1/5 NS) | +                      | +        |
| Syndactyly                | +             | −           | +               | +               | 3/5          | +                      | −        |
| Camptodactyly             | +             | −           | −               | +               | 1/5          | +                      | −        |
| Wide gap between digits   | +             | −           | −               | +               | 2/5          | +                      | −        |
| Clinodactyly              | +            | +           | +               | −               | 3/5          | +                      | −        |
| Tapering fingers          | −             | +           | −               | +               | 1/5          | −                      | −        |
| Wide halluces             | −             | +           | −               | +               | 1/5          | −                      | −        |
| Cardiac anomalies         | −             | −           | −               | +               | 2/5          | +                      | −        |
| Strabismus                | +             | −           | +               | −               | 1/5          | −                      | −        |

Note: NBW, normal birth weight; NS, not specified; NA, not applicable; +, present; −, absent.

*Extrapolated based on descriptive features.

Start-end: patient1, 2q24.3-q31; patient2, 2q31.1-q32.1; patient3, 2q31.1-q31.2; patient4, 2q24.3-q31.1; patient5, 2q31.1.
heterozygous deletion of Map3k20 did not induce abnormal morphological changes.

Severe limb deformities, including split hand and monodactyly, have also been reported, and DLX1 and DLX2 are speculated to be novel candidate genes. However, upper and lower limb malformations in the examined fetus did not confirm this possibility. Theisen et al. have reported individuals exhibiting deleted DLX1/DLX2 integrally, and no obvious limb phenotype was detected. In mutant mouse experiments, heterozygous/homozygous DLX1/DLX2 knockouts did not induce limb abnormalities, but could produce marked craniofacial and spinal abnormalities. Facial dysmorphism is a well-known feature in 2q31.1 microdeletion; however, no gene cluster has been defined. Interestingly, the examined fetus had no facial deformities, which could be attributed to the distinct expression of this gene in different species. Further experiments are warranted to determine whether DLX1/DLX2 deletion could explain craniofacial abnormalities.

Chromosomal deletion is frequently associated with congenital heart defects (CHD) of unknown pathogenesis. Examining the echocardiogram, our proband exhibited a ventricular septal defect and aortic dysplasia. The ascending aorta was significantly narrower than the pulmonary artery in the three-vessel and trachea view. Based on the echocardiogram, the examined fetus did not exhibit large ventricular septal defects and abnormal left ventricular development. Aortic dysplasia is primarily associated with chromosomal anomalies. Alison et al. have found that approximately 40% of patients with split hand and monodactyly mapped to chromosome 2 exhibited CHD, and DLX genes might affect the migration of neural crest cells to influence the formation of cardiovascular derivatives.

Ventricular septal defect is the most frequently detected CHD. Overall, 4/11 patients were found to exhibit a ventricular septal defect. TTN is located in the deleted region, encodes titin protein, and is overexpressed in the fetal heart and skeletal muscle. The large spectrum of observed cardiologic phenotypes suggests that titin-mediated defects (caused by TTN mutations) could underlie certain cardiac conditions with or without skeletal muscle involvement, such as ventricular septal defect. TTN mutations are also associated with dilated cardiomyopathy. In addition, ATF-2, one of the deleted genes, is critical for cardiomyocyte differentiation. ATF-2 has been shown to regulate the expression of five genes associated with left ventricular outflow tract obstruction. Moreover, it suggests that the heterozygous ATF-2 deletion could lead to heart defects.

SCN2A and SCN1A, two genes detected in the current fetus, are known to be associated with epilepsy. Haploinsufficiency of SCN2A and SCN1A is reportedly responsible for nervous system dysfunction. SCN1A has been associated with several epilepsy syndromes with distinct clinical severities, especially the Dravet syndrome (DS), a refractory childhood epilepsy characterized by intractable seizures, developmental disorders, and increased mortality. The heterozygous deletion of SCN2A mainly induces autism spectrum disorders and intellectual disability. However, given the death of our proband, several potential symptoms could not develop, and no neurological examinations, such as cerebral magnetic resonance and electroencephalogram, could be performed. Deletion of SCN2A and SCN1A genes did induce notable clinical effects in our proband.

In summary, we report a de novo interstitial deletion of 2q24.3-q32.1. This genomic segment involves 94 protein-coding genes, and 33 of these are related to recognizable clinical phenotypes. This case study further supports the role of HOXD13 haploinsufficiency in limb defects. Furthermore, we identified possible causative genes by analyzing gene function and phenotype. Certain defects may be due to the cumulative effect of genes in deleted fragments.

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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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