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The Genetics of 241 Fetuses With Talipes Equinovarus: A 8-Year Monocentric Retrospective Study

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ABSTRACT

Objective: This study aims to investigate the utility of chromosomal microarray analysis (CMA) and whole exome sequencing (WES) in fetuses diagnosed with talipes equinovarus (TE), as well as to explore the genetic factors contributing to TE.

Methods: The study reviewed a total of 241 fetuses with TE between January 2015 and December 2023, categorizing them into two groups based on the absence or presence of additional ultrasound anomalies: 163 cases (67.6%) in the isolated TE group and 78 cases (32.4%) in the syndromic TE group. Karyotyping and CMA were performed for all cases, with WES being performed for 18 cases that had normal karyotype and CMA results.

Results: The results indicated a total detection rate of 16.2% (39/241) using karyotyping and CMA. Furthermore, the detection rates of karyotyping and CMA in the isolated TE group and syndromic TE group were 10.4% (17/163) and 28.2% (22/78) respectively, showing a statistically significant difference ($p < 0.05$). WES was conducted on 18 fetuses with normal karyotyping and CMA results. A total of six cases, consisting of five cases with pathogenic single nucleotide variant (SNV) and one case of variants of uncertain significance (VUS), were identified, resulting in a detection rate of 33.3% (6/18). The identified SNVs was associated with the *RIT1, GNPNAT1, PEX1, RYR1, ASCC1, and GDAP1* genes. The detection rates of WES in the isolated TE group and syndromic TE group were 25% (1/4) and 35.7% (5/14) respectively, with no statistically significant difference ($p > 0.05$). The overall diagnostic yield of genetic testing was 18.7% (45/241) in fetuses with TE.

Conclusion: When prenatal ultrasound identifies fetal TE, chromosome karyotyping and CMA should be considered as the first-line diagnostic tests. Unlike previous studies, this study recommended WES in cases of normal CMA results for both isolated and syndromic fetal TE.

1 | Introduction

Fetal talipes equinovarus (TE) is the most common congenital foot deformity, with the global incidence of about 0.15% (Ansar et al. 2018), the incidence in Chinese population is slightly lower than about 0.03% (Dietz 2002). On ultrasound examination of the fetus, TE is diagnosed by the presence of plantar and long axial sections, as well as an unchanged posture despite movement

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of the lower limbs of the fetus. Various factors contribute to the development of TE, such as chromosomal abnormalities, copy number variants (CNVs), neuromuscular disorders, and environmental influences (Lochmiller et al. 1998; Tredwell et al. 2001; Basit and Khoshhal 2017).

Current common tests for prenatal diagnosis, including karyotyping and CMA, are commonly utilized for fetal structural abnormalities. However, due to the complexity of the etiology of TE and the variability in genotype–phenotype correlations, prenatal genetic diagnosis remains challenging. In recent years, whole exome sequencing (WES) has been increasingly utilized for the evaluation of fetal structural abnormalities, particularly in cases involving skeletal deformities and increased nuchal translucency (NT), due to its ability to analyze single nucleotide variants (SNV) and insertion and deletion (indel) mutations (Diderich et al. 2020; Mustafa et al. 2023; Qiao et al. 2021). However, the routine use of WES in the fetal TE remains a subject of debate. This study retrospectively examines the characteristics of TE cases at our institution in order to assess the utility of chromosomal microarray analysis (CMA) and WES in prenatal genetic diagnosis, with the aim of providing recommendations for genetic counseling.

2 | Methods

2.1 | Study Subjects

Between January 2015 and December 2023, a total of 241 fetuses diagnosed with TE via ultrasound at the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region were included in the study. Inclusion criteria required singleton pregnancies, fetuses with TE, and parental consent for invasive prenatal diagnosis.

All pregnant women and their family members consented to participate in this study, which was approved by the Medical Ethics Committee of the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, China.

2.2 | TE Diagnosis Criteria

Ultrasonography revealed the presence of plantar and long axial sections, as well as a consistent posture during movement of the lower limbs of the fetus (Viaris de le Segno et al. 2015). Fetuses diagnosed with isolated TE were categorized into the isolated TE group, whereas fetuses displaying TE along with other abnormal morphological features were classified into the syndromic TE group.

Each pregnant woman underwent a level II or level III fetal anatomic scan by sonographers specialized in high-risk cases.

2.3 | Invasive Prenatal Diagnosis

Ultrasound-guided transabdominal chorionic villus sampling (CVS) was performed at 11–14 weeks of gestation. Amniocentesis was performed at 16–25 + 6 weeks of gestation. Cordocentesis was performed at more than 26 weeks of gestation.

Ultrasound-guided transabdominal chorionic villi sampling (CVS) was conducted between 11 and 14 weeks of gestation (4 cases), amniocentesis between 16 and 25 + 6 weeks of gestation (146 cases), and cordocentesis after 26 weeks of gestation (89 cases).

2.4 | Karyotype Analysis, CMA, and WES

Karyotype analysis was carried out on G-banded metaphases with a resolution of 400 bands following standard cytogenetic protocols.

Genome-wide single nucleotide polymorphism (SNP) array analysis for CMA was performed using the Illumina 300 K Human CytoSNP-12 BeadChip, containing over 300,000 SNPs of the human genome. Data analysis was conducted using Illumina Genome Studio and KaryoStudio software. Subsequently, all identified copy number variations (CNVs) were further scrutinized by consulting various public CNV databases, such as the UCSC Genome Browser, ClinGen Dosage Sensitivity Map, ClinVar, DECIPHER, OMIM, and Database of Genomic Variants.

Sequencing libraries were prepared with the Agilent SureSelect Human All Exon v6 kit (Agilent, CA) and subjected to exome sequencing on a HiSeq2500 platform (Illumina, CA). Following the removal of duplicate reads, the sequencing data was aligned to the human reference sequence hg19 using BWA and the Genome Analysis Toolkit (GATK HaplotypeCaller). The analysis of CNV was conducted utilizing the read depth method through a in-house pipeline, with subsequent visual inspection of CNVs of notable interest using the Integrative Genomics Viewer. SNVs and indels were annotated and prioritized using TGex software (LifeMap Sciences, Alameda, CA). Following that, the variants were filtered based on: (i) variation within the exon-flanking region of a 10-bp gene; (ii) less than 0.1% frequency in public databases like the 1000 Genomes Project, Exome Sequencing Project, and ExAC, as well as our in-house databases; (iii) Utilizing prediction tools such as MutationTaster, CADD, SIFT, or PolyPhen2 to identify loss-of-function alleles or damaging missense variants. The identified variants were assessed and categorized following the guidelines established by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (Richards et al. 2015).

2.5 | Statistical Analysis Method

Statistics were analyzed using SPSS 19.0 and Pearson 2 tests. A p-value of less than 0.05 was considered statistically significant.

3 | Results

3.1 | Basic Information

The study involved 241 pregnant women with an average age of 29.56 ± 5.53 years. All pregnant women were from the Han and Zhuang Chinese populations. Prenatal diagnosis was conducted between 12 + 5 and 34 + 3 weeks gestation. Among the cases, 98 were identified as unilateral TE (40.7%) and 143 as bilateral TE (59.3%). There were 174 male fetuses (72.2%) and 67 female

fetuses (27.8%). Chromosome karyotype and SNP array analysis were performed on all 241 cases, with 18 cases also undergoing WES among the negative results.

The study found that the detection rate of karyotyping and/or CMA was 16.2% (39 out of 241 cases), with 28 cases (11.6%) showing chromosomal anomalies and 11 cases (4.6%) displaying likely pathogenic (LP) or pathogenic (P) CNVs. Specifically, in the isolated TE group, the detection rate of karyotyping and/or CMA was 10.4% (17 out of 163 cases), while in the syndromic TE group, the detection rate was 28.2% (22 out of 78 cases), showing a statistically significant difference with a p-value of less than 0.05.

WES was conducted on 18 fetuses with normal karyotyping and CMA results. A total of six cases, consisting of five cases with pathogenic SNV and one case of variants of uncertain significance (VUS), were identified, resulting in a detection rate of 33.3% (6/18). The detection rates of WES in the isolated TE group and syndromic TE group were 25% (1/4) and 35.7% (5/14) respectively, with no statistically significant difference ($p > 0.05$).

The overall diagnostic yield of genetic testing was 18.7% (45/241) in fetuses with TE.

3.2 | Karyotyping Results

The karyotyping anomalies in this study included sixteen cases of trisomy 18 (6.7%), two cases of trisomy 21, one case of 47, XXY, one case of 47, XYY, one case of 48, XXY,+18, one case of triploidy. There were six cases of chromosomal structural abnormalities, which were detailed in Table 1.

The detection rates of karyotyping in the isolated TE group and the syndromic TE group were 6.1% (10/163) and 23.1% (18/78) respectively, with statistically significant difference ($p = 0.000$). The most frequently combined malformations in the syndromic TE group were musculoskeletal system, followed by the cardiovascular and central nervous system.

3.3 | CMA Results

The additional detection rate of P/LP CNVs was 4.6% (11/241), and the most common CNV was 22q11.2 microdeletion ($n = 2$) involving DiGeorge syndrome (DGS). The 10 cases of pCNVs are listed in Table 2. In addition, nine CNVs of VUS (3.7%, 9/241) were detected (Table 4). The detection rate of CMA in the isolated TE group was 4.3% (7/163) and the syndromic TE group was 5.1% (4/78), with no significant difference ($p > 0.05$).

3.4 | WES Results

The detection rate of WES was 33.3% (6/18). Specifically, the detection rate in the isolated TE group was 25% (1/4), whereas in the syndromic TE group it was 35.7% (5/14). Statistical analysis revealed no significant difference between these groups ($p > 0.05$). For further details, refer to Table 3.

A comparison of detection rates for karyotyping, CMA, and WES is shown in Table 5.

3.5 | Pregnancy Outcome

Among the 10 cases of pCNVs identified via SNP array, one case involved both TE and FGR, with 19p13.2p13.11 deletion (Table 2, case 7). Following genetic counseling, the couple opted to continue the pregnancy, resulting in the delivery of a full-term female infant with a birth weight of 1400 g. Unfortunately, the infant succumbed at 11 months of age due to developmental delays and recurrent infections.

Among the nine cases of VUS CNV, six resulted in live births. Notably, one case involved a fetus with a loss of heterozygosity (LOH) region across multiple chromosomes (Table 4, Case 4), and the newborn subsequently died postnatally due to dysphagia. Neither of these cases underwent WES.

In contrast, out of 187 cases with negative genetic test results, 116 (62%) opted to continue the pregnancy to term. This group included 94 cases of isolated TE (87.0%, 94/108) and 22 cases of syndromic TE (33.8%, 22/65). A statistically significant difference in delivery rates was observed between the two groups ($p = 0.000, \chi^2 = 51.967$).

4 | Discussion

4.1 | Application of Karyotyping and CMA in Fetal TE

Currently, there is ongoing research into the pathogenesis of TE. It is widely accepted that TE is a result of the interaction between genetic and environmental factors, with genetic factors playing a significant role in the onset and progression of TE. Chromosome karyotyping and CMA are commonly used methods for detecting genetic abnormalities, with CMA being the preferred diagnostic test for fetuses with structural abnormalities (Yong et al. 2016; Chen and Liu 2019; Sadler et al. 2019; Prenatal Screening and Diagnosis Group, Birth Defect Prevention and Control Professional Committee, Chinese Preventive Medical Association. Prenatal Diagnosis Group, Society of Medical Genetics, Chinese Medical Association 2023).

The prevalence of aneuploidy in fetuses with TE varied between 1.6% and 9.2% across different research studies, underscoring the significance of aneuploidy as a key genetic factor in TE (Lochmiller et al. 1998; Basit and Khoshhal 2017; Viaris de le Segno et al. 2015; Luo et al. 2022; Xie et al. 2023, 2020; Guo et al. 2016; Leyne et al. 2023). Research on the utilization of CMA technology in fetal TE remains limited and heterogeneous in its findings, the overall detection rates ranged from 5.9% to 10.4% (Singer et al. 2020; Huang et al. 2022a). In our own study, the overall CMA detection rate was 15.9%, surpassing that of traditional karyotyping (11.7%), aligning with findings by Xie et al. (2023). The 22q11.2 microdeletion is identified as the most prevalent CNV in fetuses with TE, exhibiting a detection rate ranging from 1.1% to 13.3%. This genetic anomaly is commonly associated with congenital

TABLE 1 | Cases with chromosome structural abnormalities.

Case	Ultrasonography abnormalities	Karyotyping	CNVs	Phenotype/disease	Size (Mb)	Pathogenicity	Source
1	Isolated TE	46,X,der(Y) t(Y;5) (q11.22;p11)	arr[GRCh37]Yq11.221q12(17447525_28980045)×0; 5p15.33p11(38139_46228333)×3	Oligoasthenozoospermia, azoospermia, developmental delay, mental retardation	11.5, 46.2	P	de novo
2	Isolated TE	46,XY,?del(7) (p21)	arr[GRCh37]7p21.3p21.1(8112014_18300541)×1	Contain TMEM106B gene, which was associated with hypomyelination	10.2	VUS	de novo
3	Isolated TE	46,XY,del(4) (q35)	arr[GRCh37]4q34.3q35.2(183132543_190880409)×1	Developmental delay, mental retardation, and multiple malformations (OMIM:600361)	7.7	P	de novo
4	TE, single umbilical artery, dilated lateral ventricles	46,XY,der(5) t(5;7)(p15;q21)	arr[GRCh37]5p15.33p15.2(38139_12392815)×1,7q21.11q36.3 (83599335_159119486)×3	Cri-du-chat syndrome (OMIM:123450)	12.4, 75.5	P	de novo
5	Isolated TE	46,XY,del(2) (q31.3q32.3)	arr[GRCh37]2q31.3q23.3(180823590_192561207)×1	Developmental delay, abnormal facial features, non-progressive encephalopathy, short stature	11.7	P	de novo
6	TE,pyelic separation	46,XN,del(11) (q14.1q22.3)	arr[GRCh37]11q14.1q22.3 (82108925_104532836)×1	Developmental delay, mental retardation, abnormal facial features	22.4	P	de novo

Abbreviations: P. pathogenic variants; VUS, variants of uncertain significance.

TABLE 2 | Abnormal CNVs results with normal chromosome karyotype.

Case	Ultrasonography abnormalities	CNVs	Phenotype/disease	Size (Mb)	Pathogenicity
1	TE, aberrant right subclavicular artery	arr[GRCh37]22q11.21(18896081_21928916)×1	DiGeorge syndrome (OMIM:188400)	3.0	P
2	Isolated TE	arr[GRCh37]22q11.21(18844632_21462353)×1	DiGeorge syndrome (OMIM:188400)	2.6	P
3	TE, micrognathia, hand deformity	arr[GRCh37]1q21.2q21.3(149815079_150416913)×1	NT/NF thickening, micrognathia, short femur	0.6	P
4	Isolated TE	arr[GRCh37]7q11.23(72401192_74282048)×1	Williams-Beuren syndrome (OMIM:613729)	1.9	P
5	Isolated TE	arr[GRCh37]15q13.2q13.3(30955149_1560532)×1	15q13.3 microdeletion syndrome (OMIM:612001)	1.6	P
6	Isolated TE	arr[GRCh37]2q37.3(240279945_243029573)×1	Developmental delay, with or without major morphological developmental abnormalities	2.8	P
7	TE, FGR	arr[GRCh37]19p13.2p13.11(13692602_16611978)×1	Abnormal facial features, FGR, muscle hypotonia, polyhydramnios	2.9	P
8	TE; mild tricuspid and mitral regurgitation	arr[GRCh37]15q11.2q13.1(23656946_28973396)×3	The 15q11-q13 duplication syndrome (OMIM:608636)	5.3	P
9	Isolated TE	arr[GRCh37]16p11.2(29634212_30199805)×3	16p11.2 duplication syndrome (OMIM:614671)	0.6	P
10	TE, bowing of the femurs	arr[GRCh37]17p12(14040338_15551871)×1	Neuropathy, Hereditary, with liability to pressure palsies (HNPP) syndrome (OMIM:601079)	1.5	P
11	Isolated TE	arr[GRCh37]9q34.3(138695235_141044489)×1	Kleefstra syndrome (OMIM:610253)	2.3	P

Abbreviation: P, pathogenic variants.

TABLE 3 | Cases with abnormal WES results.

Case	Ultrasonography abnormalities	WES	Phenotype/disease	Pathogenicity	Mode of inheritance	The source of the mutation
1	TE, fetal edema, angulation of right femur	*RIT1* gene c.270G>C/p.Met90Ile	Noonan syndrome type 8 (OMIM:615355)	Pathogenic (reported)	AD	de novo
2	TE, NF thickening, short limbs, narrow thorax	*GNPNAT1* gene c.305C>T/p.Thr102I1e, c.506G>T/p.Gly169Va1	Ain-Naz type limb-root-type skeletal dysplasia (OMIM:619598)	VUS (unreported/reported)	AR	Parents
3	TE, FGR	*PEX1* gene c.782-783delAA/p.Gln261fs*7, c.300-309delGGTATCTTGT/p.val101fs*27	Zellweger Spectrum disorders (OMIM:602136)	Pathogenic(unreported/ reported)	AR	Parents
4	TE, fetal edema, cystic hygroma	*RYR1* gene c.4080_4104delCCCGCAGGCGGGGGGAGAGGCGCAG/p. Gln362fs*28, c.11653C>T/p. Arg3885*	King-Denborough syndrome (OMIM:619542)	Likely pathogenic (reported)	AR	Parents
5	TE, flexion of right knee, postural fixation of both hands, Lateral ventricular dilatation	*ASCC1* gene c.923C>G/p. Ser311Ter, deletion of exon 5	Spinal muscular dystrophy, associated with congenital fracture type 2 (OMIM:616867)	Pathogenicity (unreported)	AR	Parents
6	Isolated TE	*GDAP1* gene c.485-1G>A homozygous mutation	Charcot–Marie–Tooth disease (CMT) (OMIM:608340)	Likely pathogenic (unreported)	AR	Parents

Note: In all cases that the source of AR mutations was parental origin: the first mutation was inherited from the father, while the second mutation was inherited from the mother. Parental consanguinity was denied in all cases.
Abbreviations: AD, autosomal dominant; AR, autosomal recessive; FGR, fetal growth restriction; NF, nuchal fold; VUS, variants of uncertain significance.

TABLE 4 | VUS CNVs in fetuses with talipes equinovarus.

Case	CNVs	Size (Mb)	Source	Outcome
Isolated TE group				
1	arr[GRCh37] 15q25.3(86819861_87070690)×1	0.25	Unknown	LB
2	arr[GRCh37] 16p13.3(105320_110560)×1	0.01	Unknown	LB
3	arr[GRCh37] 7p21.3p21.1(8112014_18300541)×1	10.2	de novo	TOP
4	arr[GRCh37] 1p36.21p35.3(14150709_29694741)×2 hmz, arr[GRCh37] 3p12.3q11.2(108619666_115279547)×2 hmz, arr[GRCh37] 3q13.13q13.31(78629833_97455908)×2 hmz, arr[GRCh37] 4q25q28.2(113972856_129254598)×2 hmz, arr[GRCh37] 7p22.1p21.3(31827180_93055650)×2 hmz, arr[GRCh37] 7p14.3q21.3(5257573_10177323)×2 hmz, arr[GRCh37] 9p22.1p21.2(18910581_27629693)×2 hmz, arr[GRCh37] 11q14.1qq14.2(78739822_86629236)×2 hmz, arr[GRCh37] 17p12q22(14678286_54902055)×2 hmz, arr[GRCh37] 18p11.32p11.31(139767_4609374)×2 hmz, arr[GRCh37] 18q21.31q22.3(56150114_71505511)×2 hmz	15.5 6.6 18.8 15.3 61.2 4.9 8.7 7.9 40.2 4.5 15.4	Unknown	LB
5	arr [GRCh37] 6p24.3(8803042_9959207)×3	1.15	Unknown	LB
6	arr[GRCh37] 11p15.4(5487596_5690691)×1	0.2	Unknown	TOP
7	arr[GRCh37] 4q31.22q32.2(147993702_164118272)×2 hmz, 16p12.2(21951415_22431170)×1	16.1 0.48	Unknown	LB
8	arr[GRCh37] 4q32.1q32.2(161363563_162109687)×1	0.75	Unknown	LB
	Complex TE group		Unknown	
9	arr[GRCh37] 14q24.3q31.3(77590183_89499467)×2 hmz	11.9	Unknown	TOP
10	arr[GRCh37] 2p25.3p25.1(192636199_238111471)×2 hmz, arr[GRCh37] 2p22.3p21(36508889_47058526)×2 hmz, arr[GRCh37] 2q11.2q21.2(79003_11293336)×2 hmz, arr[GRCh37] 2q32.3q37.3(97585936_133363129)×2 hmz	45.5 10.5 11.2 35.8	Unknown	TOP

Note: Parental consanguinity was denied in all cases.
Abbreviations: LB, live birth; TOP, termination of pregnancy.

heart defects, craniofacial abnormalities, and developmental delays (Luo et al. 2022; Xie et al. 2023; Cai et al. 2023; Homans et al. 2018, 2017). Homans JF et al. observed a notably elevated prevalence of TE in patients with DGS (OMIM:188400) at 3.3% (48 out of 1466 individuals), in contrast to the general population prevalence of approximately 0.1%, indicating a strong correlation between DGS and TE (Homans et al. 2018). In addition to DGS, our study identified multiple microdeletion/microduplication syndromes, including 5p15 deletion (Cri-du-chat syndrome) (OMIM:123450), 15q13.3 microdeletion syndrome (OMIM:612001), 16p11.2 duplication syndrome (OMIM:614671), Williams-Beuren syndrome (OMIM:613729). These syndromes have been linked to neurodevelopmental and growth abnormalities, consistent with findings from previous research (Cai et al. 2023; Homans et al. 2018). Furthermore, the detection rate of CMA in the syndromic TE group was significantly higher compared to the isolated TE group (28.6% vs. 9.9%), consistent with previous research by Huang et al. (27% vs. 5.4%) (Huang et al. 2022a). Our results suggest that the use of CMA in TE fetuses with ultrasound abnormalities may lead to a higher detection rate of genetic disorders.

TABLE 5 | Comparison of karyotyping, CMA, and WES.

Methods	Number of cases	Detection rate		*p*
		Isolated TE	Syndromic TE	
Karyotyping	241	6.1%	23.1%	0.000
CMA	241	4.3%	5.1%	> 0.05
WES	18	25%	35.7%	> 0.05

4.2 | WES In Fetal TE

The etiology of TE is multifaceted, and traditional genetic testing methods such as karyotyping and CMA may not fully elucidate the genetic factors contributing to the condition. In response to the growing need for precision medicine in prenatal diagnosis, the emergence of next-generation sequencing technology has significantly advanced the detection capabilities for TE in fetuses, with WES proving to be increasingly effective

(Corsten-Janssen et al. 2020). Presently, the involvement of *PITX1, TBX4*, and *HOXC11* genes in the pathogenesis of TE has been established. *PITX1* variants have been identified as a crucial gene for the development of TE in both humans and mice, while the *TBX4* transcription factor plays a role in the formation of limb muscle and tendon. Additionally, SNVs in *TBX4* and *HOXC11* have been linked to TE (Basit and Khoshhal 2017; Hordyjewska-Kowalczyk et al. 2022; Dobbs and Gurnett 2017). A recent study utilizing WES with a substantial sample size revealed 11 clinically significant SNVs in 12% of fetuses with TE, affecting 11 TE-related genes including *KLHL40, COL2A1, COL1A1, MAGEL2, TNNI2, TNNT3, TGM6, SOX9*, and *BRPF1*. Notably, six of these variants were novel and had not been previously reported (Huang et al. 2022b).

In the other two studies, the detection rate of WES in fetuses with TE ranged from 31.6% to 43.2%. Furthermore, the detection rate of WES in non-isolated TE fetuses was notably greater than that in isolated TE fetuses (Wang et al. 2023, 2024), underscoring the diagnostic utility of WES in TE fetuses, broadening the genetic landscape of TE-associated genes, and implying a strong correlation between syndromic TE and monogenic diseases.

In this study, WES detected five pathogenic/LP variants and one VUS in TE fetuses, with a detection rate of 33.3%, which was similar to previous studies (Huang et al. 2022b; Wang et al. 2023, 2024). The SNVs of the *RIT1, PEX1, ASCC1, GNPNAT1, RYR1*, and *GDAP1* genes detected in this study were all associated with the skeletal, muscular, and neurodevelopmental abnormalities, which may lead to TE in the fetuses. In contrast to prior research, the present study did not observe a statistically significant variance in the detection rate of WES between the isolated TE group and the syndromic TE group.

In WES case no. 6, the prenatal ultrasound finding was isolated TE, WES detected *GDAP1* gene c.485-1G>A homozygous mutation. Additionally, there were two siblings in this family were diagnosed with CMT4A due to progressive charcot–marie-tooth atrophy, TE, with *GDAP1* gene c.485-1G>A homozygous mutation. Ganglioside induced differentiation associated protein 1 (GDAP1) is involved in the division of peroxisomes, and genetic mutations can cause biological disorders of peroxisomes, whose phenotypes include TE (Sadler et al. 2019). It is related to Charcot–Marie–Tooth disease, type 4A (CMT4A, OMIM 214400), which is characterized by early onset (infantile to early childhood) severe, rapidly progressing demyelinating, axonal, or intermediate senior-motor neuropathy, usually affecting the more severe distal lower limbs first, then the proximal muscles and upper limbs. Nerve conduction velocities range from very slow to normal. In addition to the typical CMT phenotypes (distal muscle weakness and atrophy, sensory loss, high-arched feet), CMT4A patients also commonly experience motor retardation, vocal cord paralysis, mild sensory loss, loss of deep tendon reflexes, and skeletal malformations. Prenatal ultrasound is difficult to detect fetal muscle weakness, so the prenatal findings of fetuses with CMT4A could be isolated TE.

Case no. 3, which presented with prenatal ultrasound findings of TE and fetal growth restriction (FGR) without additional anomalies, was identified to harbor compound heterozygous mutations in the *PEX1* gene through WES analysis. Mutations in the *PEX1* gene lead to impaired peroxisome biogenesis and loss of peroxidase function in tissues and cells, resulting in autosomal recessive Zellweger spectrum disorder (OMIM:602136). Clinical manifestations of this disorder include craniofacial deformities, hypotonia, varying degrees of neurodevelopmental delay, epilepsy, liver and brain abnormalities, skeletal malformations, and cardiac defects. The literature reports the identification of compound heterozygous mutations in the *PEX1* gene in a 2-month-old boy presenting with hypotonia, feeding difficulties, and dyspnea (Alamatsaz et al. 2021). The mutations were inherited from parents. In our case, prenatal ultrasound initially revealed isolated TE during the first and early second trimester, followed by the development of features of FGR in the late second trimester.

Prior research has indicated that syndromic TE is predominantly linked to monogenic diseases. However, our study, along with two cases, demonstrates that isolated TE can also be associated with monogenic diseases, particularly those involving peroxisome disorders.

In the No. 2 WES case, ultrasound findings revealed TE, nuchal fold (NF) thickening, short limbs, and a narrow thorax, indicative of skeletal dysplasia. WES identified compound heterozygous mutations in the *GNPNAT1* gene, inherited from the parents, which, according to the ACMG guidelines, were classified as VUS. The pregnant woman previously underwent termination of pregnancy due to fetal short limb abnormalities, which were found to be associated with compound heterozygous mutations in the *GNPNAT1* gene identified by WES. Mutations in the *GNPNAT1* gene have been linked to Rhizomelic dysplasia, Ain-Naz type (OMIM 616510), a condition characterized by shortened limbs, particularly the proximal humerus and femur. Literature reports describe additional features of the disease including disproportionate short stature, wide metaphyseal ends of the humerus, absence of the femoral head, mild varus calf, high forehead, wide eye distance, and funnel chest (Ain et al. 2021). The phenotype observed in our case aligns with previously documented findings in the literature. Consequently, we posited that the mutations in the *GNPNAT1* gene was the underlying cause of this case, leading us to recommend preimplantation genetic testing for monogenic defects (PGT-M) to couples as a preventative measure against the possibility of recurrence in future pregnancies.

4.3 | Limitations of This Study

This study represents a single-center retrospective analysis. Given that our hospital serves as a regional referral center for many surrounding cities, there is a potential for selection bias. The sample size of cases subjected to WES was limited, indicating the need for additional research on the utility of WES in various forms of fetal TE.

5 | Conclusion

In summary, when prenatal ultrasound identifies fetal TE, chromosome karyotyping and CMA should be considered as the first-line diagnostic tests, with WES recommended in cases of

negative results for both isolated and syndromic fetal TE. In this study, we found two unreported genetic variation in syndromic TE fetuses through WES, expanded the disease spectrum of TE-related genes, and provided a new theoretical basis for the study of genetic etiology and pathogenic mechanism of fetal TE.

Author Contributions

Pingshan Pan: writing – original draft, investigation. **Dongbing Huang:** writing – original draft, investigation. **Jiangxuan Wei:** methodology. **Wei He:** methodology. **Peng Huang:** methodology. **Sheng Yi:** methodology. **Jing Huang:** data curation. **Dahua Meng:** data curation. **Shuyin Tan:** data curation. **Xinyan Li:** methodology. **Hongwei Wei:** supervision. **Linlin Wang:** supervision, writing.

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Ethics Statement

Ethics approval was obtained from the Guangxi Zhuang Autonomous Region Women and Children Care Hospital Ethics Committee.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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