ANNALS OF MEDICINE
2025, VOL. 57, NO. 1, 2476038
https://doi.org/10.1080/07853890.2025.2476038

MEDICAL GENETICS & GENOMICS

OPEN ACCESS Check for updates

# Utility of whole exome sequencing in the evaluation of isolated fetal growth restriction in normal chromosomal microarray analysis

Xiaomei Shi[a], Yanling Huang[a], Hongke Ding[a], Lina Zhao[b], Wei He[a] and Jing Wu[a]

[a]Genetic Medical Center, Guangdong Women and Children Hospital. Xingnan Load, Guangzhou, China; [b]Department of Obstetrics, Guangdong Women and Children Hospital. Xingnan Load, Guangzhou, China

**ABSTRACT**

**Objective:** To investigate the application of whole exome sequencing (WES) in the prenatal diagnosis of isolated fetal growth restriction (FGR) with a normal result by chromosomal microarray analysis (CMA).

**Methods:** This retrospective study included singleton fetuses with isolated FGR in Guangdong Women and Children Hospital between July 2018 and August 2023. All fetuses were subjected to invasive prenatal testing with CMA and WES. Only cases with negative CMA results were included.

**Results:** r

A total of 135 fetuses were included. Ultrasonography identified short long bones in 39 fetuses and nonshort long bones in 96 cases. WES revealed pathogenic/likely pathogenic (P/LP) variants in 16(11.9%) fetuses and variants of uncertain significance (VUS) in 2 (1.5%) fetuses. Compared to the nonshort long bones group, the short long bones group had a significantly higher detection rate of P/LP variants (33.3% [13/39] vs. 3.1% [3/96], $p<0.001$, OR=15.5(4.1-58.5)). No significant differences were observed in the detection rates between severe FGR and nonsevere FGR (12.3% [13/106] vs. 10.3% [3/29], p=.000, OR=1.2(0.3-4.6)), or between the early-onset (12.9% [15/116]) and the late-onset group (5.3%[1/19],p=0.565, OR=2.7(0.3-21.5)).

**Conclusions:**

P/LP variants are more prevalent in fetuses with short long bones. WES is recommended for isolated FGR with short long bones, but further studies are needed to assess its utility in cases with nonshort long bones.

**KEY MESSAGES**

- Among fetuses diagnosed with isolated fetal growth restriction and a normal CMA result, WES identified clinically significant findings in 11.9% of fetuses.
- Compared to the nonshort long bones group, the short long bones group had a greater detection rate of P/LP variants.

**ARTICLE HISTORY**
Received 2 September 2024
Revised 20 February 2025
Accepted 23 February 2025

**KEYWORDS**
Whole exome sequencing; genetic abnormalities; prenatal diagnosis; FGR

## Introduction

Fetal growth restriction (FGR) refers to the inability of a fetus to maintain expected growth and is defined as an estimated fetal weight below the 10th percentile for gestational age [1]. It is a relatively common complication of pregnancy, affecting approximately 5-10% of newborns [2]. FGR is associated with high risks of perinatal morbidity and mortality and can lead to delayed postnatal growth and mental development as well as increased susceptibility to metabolic syndrome in adulthood [3,4]. Identifying pathological mechanisms is required for the optimal management of pregnancy. The aetiology of FGR is complex and includes infection, chemical substances, and abnormalities of the placenta or umbilical cord, among others [5–7].

Genetic abnormalities are important causes of FGR [8,9]. Conventional molecular diagnostic strategies include karyotyping and chromosomal microarray analysis (CMA). G-banding karyotyping and CMA have been the predominant strategies applied for detecting chromosomal abnormalities in FGR in clinical practice. Previous studies have shown that chromosomal abnormalities can explain 20% of FGR cases [10–14]. However, a diagnosis remains unattainable in many

**CONTACT** Jing Wu wujing020@126.com Genetic Medical Center, Guangdong Women and Children Hospital. Xingnan Load, 521, Guangzhou 511400, China

cases. Thus, additional diagnostic tools are necessary to further determine the genetic cause of FGR and to provide appropriate counselling. Recently, two large-scale prospective studies revealed that whole exome sequencing (WES) provides an additional diagnostic yield of 8-10% for fetuses with an ultrasound anomaly, a normal karyotype and normal CMA results [15,16]. The ACMG stated that WES may be considered for a fetus with any ultrasound anomaly when standard CMA and karyotype analysis have failed to yield a definitive diagnosis, but it did not metion whether WES should be performed for isolated FGR [17]. A recent review of 146 cases by Pauta M et al. found that WES analysis resulted in a 12% increase in the diagnosis of isolated growth restriction in fetuses with normal chromosomal analysis results [18]. However, comparisons of the severity and classification by gestational age of FGR were not analyzed in this review.

In the present study, WES was performed to identify genetic causes for 135 fetuses with isolated FGR and normal CMA results and to estimate the application value of WES in the prenatal diagnosis of isolated FGR.

## Materials and methods

### *Ethics approval*

The study was approved by the Medical Ethics Committee of Guangdong Women and Children Hospital [202201109]. All participants were informed of the purpose, experimental procedures and potential risks of the study and signed an informed consent form. All experiments were performed in accordance with the Declaration of Helsinki and National Regulations for Ethics of Biological Medical Sciences on Human Studies released by the Ministry of Health, China.

### *Subjects*

Fetuses who were diagnosed with FGR by prenatal ultrasonography at our centre between July 2018 and August 2023 were enrolled in this study. Fetal crown-rump length (CRL) was measured by ultrasound during the first trimester of pregnancy to assess gestation. The Hadlock formula is utilized to calculate the estimated fetal weight (EFW) from the biparietal diameter, abdominal circumference, and femur length [19]. FGR is defined as an estimated fetal weight <10th percentile for gestational age according to the Chinese population references [20]. Following prenatal detection of FGR, a systematic sonographic assessment for associated anomalies was performed. Fetus was considered isolated FGR if no other structural abnormalities were noted. The exclusion criteria were as follows: (1) multiple pregnancies and (2) FGR with structural anomalies.

After diagnosis, all parents were given comprehensive counselling. CMA and WES of the fetuses and parents (parent–fetus trios) were offered to all fetuses. Written informed consent was obtained from pregnant women before invasive prenatal diagnostic testing. Information on maternal demographic characteristics and clinical data on the current pregnancy was obtained. All fetuses underwent invasive prenatal diagnostic testing *via* the collection of amniotic fluid by amniocentesis or umbilical cord blood by cordocentesis. Only patients with normal CMA results were included.

All isolated FGR patients were divided into two groups according to the ultrasound features: (1) short long bones group, which refers to FGR related only to short long bones; and (2) nonshort long bones group, which indicates a proportionately small biparietal diameter, head circumference (HC), abdominal circumference (AC), and femoral length (FL), as demonstrated by fetal ultrasound. FGR that occurred before 32 gestational weeks was considered early onset FGR, and after 32 gestational weeks was considered late onset FGR. Cases with an EFW below the 3rd percentile were diagnosed with severe FGR and EFW between the 3rd to 10th percentile were diagnosed with non-severe FGR.

### *WES analysis*

The genomic DNA of fetus-parental trios was extracted using a Qiagen DNA Blood Midi/Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The DNA library was sequenced on an Illumina NovaSeq 5000 (Illumina, Inc., San Diego, CA, USA) for paired-end 150-bp reads, yielding an average coverage above 100×, with 95% of target bases covering at least 20×. The sequencing data for the human assembly GRCh37/hgl9 were analysed by NextGENe software, which is responsible for variation alignment, calling, and annotation. The annotation variants were screened according to a small allele frequency (MAF) < 0.01. For the filtered data, genotype driven variant analysis was conducted, combined with phenotype driven analysis, to find the pathogenic or likely pathogenic variants associated with the phenotype. The genomic variation database (http://dgv.tcag.ca/dgv/app/home), DECIPHER database (https://decipher.sanger.ac.uk/), and OMIM database (http://www.ncbi.nlm.nih.gov/omim) were employed. Variants were evaluated with the predictors included in the Varsome website (https://Varsome.com/),

VarSEAK website (https://varseak.bio/), and SpliceAI software [21]. All of the selected variants were then classified into five categories, including pathogenic (P), likely pathogenic (LP), benign, likely benign, and variants of uncertain significances (VUS), according to the American College of Medical Genetics and Genomics (ACMG) guidelines [22]. Variants suspected to be clinically significant were confirmed by Sanger sequencing. Before undergoing WES, a detailed explanation of the possible results, including the possibility and significance of VUS would offered by genetic counselors. A multidisciplinary team of clinical and laboratory geneticists, obstetricians and genetic counselors reviewed all the rare phenotype-related variants to identify the reportable variants.

### Pregnancy outcome

The maternal and fetal clinical characteristics, which included maternal age, gestational age at the suspicion of FGR, umbilical artery Doppler results, amniotic fluid volume, gestational disease, neonatal outcomes, and postpartum growth and development from all pregnancies, were ascertained from delivery room records or by phone enquiry if the pregnancy was not followed up and delivered in our centre.

### Statistical analysis

SPSS 20.0 software was used for data analysis. The chi-square test was used to test the differences between WES yield in relation to different group. Chi-square test for continuous calibration was used in cases where a table cell contained < 5 observations. $p < 0.05$ was considered statistically significant. Odds ratio (OR) is derived using the OR formula for a 2-dimensional table with a confidence interval of 95%.

## Results

### Clinical features

A total of 135 fetus-parental trios were successfully analysed by WES, including 96 cases with nonshort long boness and 39 cases with short long boness. Based on the gestational age at diagnosis,116 cases were considered early onset FGR, and 19 cases were late onset FGR. One hundred and six fetuses were severe FGR, and 29 fetuses were non-severe FGR. The mean maternal age was 29.6 ± 4.1 years. The mean gestational age at diagnosis was 28.1 ± 3.9 weeks. A total of 108 prenatal samples were obtained by amniocentesis, and 27 were obtained by cordocentesis. The clinical characteristics of the patients included in this study are summarized in Table 1.

**Table 1.** Maternal and fetal characteristics in this study.

| Characteristic | Mean ± SD/ or n (%) |
|---|---|
| Maternal features | |
| Maternal age | 29.6 ± 4.1 (year) |
| Gestational age at diagnosis | 28.1 ± 3.9 (weeks) |
| Fetal features | |
| Short long bones group | 39/135 (28.9%) |
| Non-short long bones group | 96/135 (71.1%) |
| EFW blow the 3rd percentile | 106/135 (78.5%) |
| EFW between the 3rd to 10th percentile | 29/135 (21.5%) |
| Diagnosed <32 GW | 116/135 (85.9%) |
| Diagnosed ≥32 GW | 19/135 (14.1%) |
| Methods of prenatal diagnosis | |
| Amniocentesis | 108/135 (80.0%) |
| Cordocentesis | 27/135 (20.0%) |
| Outcomes | |
| Live born | 85/135 (63.0%) |
| Termination of pregnancy | 42/135 (31.1%) |
| intrauterine fetal demises | 5/135 (3.7%) |
| Lost to follow up | 3/135 (2.2%) |

EFW: estimated fetal weight; GW: weeks of gestation.

### Analysis of WES results

WES revealed P/LP variants in 11.9% (16/135) of fetuses. The clinical characteristics of the 16 fetuses are shown in Table 2. Among the diagnostic results, de novo mutations were identified in 12 (75.0%) fetuses with dominant inheritance, and compound heterozygotes were identified in 4 (25.0%) fetuses with autosomal recessive inheritance.

In addition to these 16 fetuses, two fetuses had genetic variants that were not P/LP but merited further clinical and molecular investigations, and these were classified as VUS (Table 3). The total proportion of fetuses with VUS was 1.5% (2/135).

### Subgroup analysis of different types of FGR

In the nonshort long bones group ($n = 96$), there were 3 patients with P/LP variant, for a positivity rate of 3.1%. In the short long bones group ($n = 39$), there were 13 patients with P/LP variant, for a positivity rate of 33.3%. Compared to that in the nonshort long bones group, the percentage of patients who underwent WES was greater in the short long bones group (chi-square test for continuous calibration, $\chi^2 = 21.4$, $p < 0.001$, OR = 15.5(4.1-58.5)).

P/LP variants were found in 13 patients in the severe FGR group and 3 patients in the nonsevere FGR group. In total, 12.3% (13/106) of the patients in the severe FGR group and 10.3% (3/29) of those in the nonsevere FGR group had P/LP variants, which was not a significant difference (chi-square test for continuous calibration, $\chi 2 = 0$, $p = 1.000$, OR =

**Table 2.** Pathogenic and likely pathogenic variants identified by WES in fetuses with FGR.

| No | GA | Fetal biometric measurements (Z-scores) BPD | HC | AC | FL | EFW | Results of WES | Disease (OMIM ID) | Pregnancy outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 31+3 | 0.66 | 0.62 | 0.30 | −4.39 | −1.74 (4.1%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 2 | 30+4 | 1.29 | 1.46 | 0.18 | −5.50 | −2.20 (1.7%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 3 | 26+6 | 1.32 | 1.27 | −0.72 | −3.03 | −2.15 (1.6%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 4 | 32+5 | 0.99 | 0.33 | −1.80 | −6.23 | −4.11 (0%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 5 | 30 | 2.47 | 1.91 | −0.05 | −3.98 | −1.69 (4.5%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 6 | 30+5 | 0.08 | 0.48 | −0.12 | −4.66 | −2.33 (1%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 7 | 30+6 | 2.61 | 2.58 | −0.44 | −3.93 | −1.77 (3.8%) | FGFR3 c.1138G>A(p.Gly380Arg), het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 8 | 31 | 1.28 | 1.72 | 0.00 | −4.92 | −1.97 (2.4%) | FGFR3 c.1626C>A(p.N542K) het,de novo,P,AD | Achondroplasia (100800) | TOP |
| 9 | 20 | 1.93 | 1.60 | −0.91 | −6.49 | −5.0 (0%) | FGFG3 c.742C>T(p.Arg248Cys) het,de novo,P,AD | Achondroplasia/ Thanatophoric dysplasia type 1(100800/187600) | TOP |
| 10 | 29+3 | −0.28 | −1.40 | −1.34 | −2.71 | −3.0 (0.1%) | COL1A2 c.1801G>A(p.Gly601Ser) het,de novo,P,AD | Osteogenesis imperfecta (20301472) | TOP |
| 11 | 29+5 | 0.73 | −0.08 | −0.51 | −4.30 | −2.67 (0.4%) | CUL7 c.5011C>T(p.Q1671*), Het.pat,LP,AR CUL7 c.4585C>T(p.R1529*) Het.mat,LP,AR | Three M syndrome (273750) | TOP |
| 12 | 24+4 | −0.02 | 1.24 | −0.76 | −5.42 | −3.52 (0%) | CUL7 c.2416C>T(p.R806*) Het.pat,LP,AR c.3489delC(p.F1164Sfs*61) Het.mat,LP,AR | Three M syndrome (273750) | TOP |
| 13 | 28+4 | −0.83 | −1.21 | −1.07 | −3.74 | −3.25 (0.1%) | NAGLU c.1693C>T(p.Arg565Trp) Het.pat,LP,AR NAGLU c.1562C>T (p.Pro521Leu) Het.mat,LP,AR | Sanfilippo type IIIB (252920) | IUTD |
| 14 | 30+5 | −1.11 | −0.91 | −3.06 | −1.94 | −3.74 (0%) | NR2F1c.1117C>T (p.R373*) het,de novo,LP,AD | Bosch-Boonstra-Schaaf Optic atrophy syndrome (615722) | TOP |
| 15 | 23+3 | −2.16 | −2.49 | −2.48 | −3.17 | −4.97 (0%) | TCF20 c.5124_5125del (p.Cys1708Trpfs*11) het,de novo,LP,AD | Developmental delay with variable intellectual impairment and behavioral abnormalities(618430) | TOP |
| 16 | 26+5 | −3.22 | −2.34 | −3.61 | −0.41 | −4.3 (0%) | LIG4 c.833G>T (p.Arg278Leu) Het.pat,P,AR LIG4 c.1271_1275del (p.Lys424Argfs*20) Het.mat,P,AR | DNA ligase IV (LIG4) deficiency (606593) | TOP |

Z: Z score; P: pathogenic; LP: likely pathogenic; Pat: Parental inherited; Mat:Maternal inherited; AD: Autosomal dominant; AR: Autosomal recessive; TOP: termination of pregnancy;IUTD:intrauterine fetal demises.

**Table 3.** VUS Identified by WES in fetuses with FGR.

| No | GA | Fetal biometric measurements (Z-scores) BPD | HC | AC | FL | EFW | Results of WES | Disease (OMIM ID) | Pregnancy outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 19+1 | −2.29 | −1.27 | −2.59 | −3.75 | −5.94 (0%) | SBDS c.184A>T (p.Lys62*) Het.pat,P,AR SBDS c.218G>C(p.Ser73Thr) Het,mat,VUS,AR | Shwachman-Diamond syndrome (260400) | TOP |
| 2 | 32+3 | 0.28 | −0.70 | −0.79 | −1.72 | −1.90 (1.9%) | ZC4H2 c.635C>T (p.S212F) het,de novo,VUS,XLD | Wieacker-Wolff syndrome (314580) | Delivery at $36^{+2}$w female,2.1kg Normal growth and development |

Z: Z score; P: pathogenic; LP: likely pathogenic; Pat: Parental inherited; Mat: Maternal inherited; AD: Autosomal dominant; AR: Autosomal recessive; TOP: termination of pregnancy; IUTD: intrauterine fetal demises.

1.2(0.3-4.6)). The rate of P/LP variants was 12.9% in the early onset group (15/116), which was not significantly different from that in the late-onset group (5.3%, 1/19). (Chi-square test for continuous calibration, $\chi 2 = 0.3$, $p = 0.565$, OR = 2.7(0.3-21.5)). However, in the nonshort long bones group, all the P/LP variants were found to be associated with severe and early-onset FGR. Characteristics of these findings are listed in Table 4.

### *Pregnancy outcome*

Among the 16 P/LP variant patients, 15 pregnancies were terminated, and one pregnancy resulted in intrauterine fetal demise. Among the two VUS patients, one parent chose to continue the pregnancy, and one chose to terminate the pregnancy. Of the 119 remaining pregnancies, 4 ended in fetal intrauterine demise, 27 ended in termination, and 3 were lost to follow-up.

**Table 4.** Detection rates of P/LP variants in different subgroup.

| Group | Number of Cases | Number of P/LP Variants | Detection Rate (%) | *P*-value | Odds Ratio (95% CI) |
|---|---|---|---|---|---|
| Short Long Bones | 39 | 13 | 33.3 | <0.001 | 15.5 (4.1-58.5) |
| Non-Short Long Bones | 96 | 3 | 3.1 | | |
| Severe FGR | 106 | 13 | 12.3 | 1.000 | 1.2 (0.3-4.6) |
| Non-Severe FGR | 29 | 3 | 10.3 | | |
| Early-Onset FGR | 116 | 15 | 12.9 | 0.565 | 2.7 (0.3-21.5) |
| Late-Onset FGR | 19 | 1 | 5.3 | | |

Among the 85 deliveries, two infants died within 1 month, four infants were diagnosed with growth retardation, and 79 exhibited normal growth and development after birth.

## Discussion

Previous studies have demonstrated the clear advantages of WES in prenatal diagnosis in fetuses with structural anomalies. Petrovski et al. performed a prospective cohort study of 234 trios with an incremental yield of prenatal WES to CMA of 10% in fetal structural anomaly cases. Twenty-nine FGR patients with other structural anomalies were analysed. Ten percent (3/29) of these had diagnostic genetic variants [16]. It is clear that if additional structural anomalies are detected, WES should be offered to the parents in addition to CMA for nonisolated FGR. However, data on the yield of WES for isolated FGR are still rare.

Meler E et al. reviewed the genetic syndromes that can cause FGR without structural defects and showed that isolated FGR, particularly extreme FGR (EFW below the first centile), may also be a sign of a syndrome caused by a single-gene mutation, such as Cornelia de Lange syndrome, 3 M syndrome and floating-Harbour syndrome [23]. To estimate the performance of WES in patients with isolated FGR, Pauta M et al. reviewed the data of 146 fetuses with isolated FGR to determine the diagnostic yield of WES above that of CMA or karyotyping. The study revealed that a monogenic disorder was prenatally found in association with apparently isolated FGR in 12% of these fetuses [18]. In our study, we found that WES detected 11.9% of P/LP variants, which is consistent with a previous study.

In the present study, among the 16 fetuses with a genetic diagnosis, de novo mutations were identified in 75.0% of fetuses with dominant inheritance, and compound heterozygotes were identified in 25.0% of fetuses. Furthermore, 56.3% of fetuses contained variants in FGFR3, which is one of four distinct membrane-spanning tyrosine kinases that serve as high-affinity receptors for numerous fibroblast growth factors and plays essential roles in skeletal development. The variant c.1138G > A in fetuses 1-7 and the variant c.1626C > A in fetus 8 are associated with achondroplasia (OMIM:100800). The variant c.742C > T in fetus 9 results in acondroplasia or Thanatophoric dysplasia type 1 (OMIM:100800/187600) [24]. Fetus 10 had a de novo heterozygous variant c.1801G > A in COL1A2, which resulted in Osteogenesis imperfecta (OMIM:20301472) [25]. Fetus 14 had a de novo heterozygous variant of NR2F1 (c.1117C > T), which was consistent with Bosch-Boonstra-Schaaf optic atrophy syndrome (OMIM:615722) [26]. Fetus 15 had a de novo heterozygous variant (c.5124_5125del) in TCF20. Heterozygous mutation of this gene causes developmental delay with variable intellectual impairment and behavioural abnormalities (OMIM:618430) [27]. Four fetuses exhibited an autosomal recessive inheritance pattern. Fetuses 11 and 12 all carried compound heterozygosity variants in GUL7. The pathogenic variation in GUL7 results in 3 M syndrome (OMIM:273750) [28]. Fetus 13 had two variants of NGAL (c.1693C > T and c.1562C > T). The variants of NAGLU were parentally inherited, causing Sanfilippo type IIIB (OMIM:252920) [29]. Fetus 16 carried a compound heterozygosity mutation in LIG4 (c.833G > T(p. Arg278Leu) and c.1271_1275del (p.Lys424Argfs*20). Mutations in this gene cause autosomal recessive DNA ligase IV deficiency (OMIM:606593) [30].

As an important biometric measurement in prenatal ultrasound screening, short long bones may be ascribed to FGR, chromosomal abnormalities or skeletal dysplasia [31]. Recently, Mone F et al. reviewed the data of 452 cases to determine the incremental yield of prenatal exome sequencing in cases of isolated FGR associated with placental insufficiency ($n = 116$) or in cases of isolated short long bones ($n = 336$). The apparent incremental yields with prenatal sequencing were 4% in isolated FGR with evidence of placental insufficiency and 48% in isolated short long bones [32]. In our study, the diagnostic yield of WES for P/LP variants in the nonshort long bones and short long bones was 3.1% and 33.3%, respectively. This result was consistent with the study of Mone F. Regarding the high diagnostic yields in the short long bones group, the majority of these identifications were skeletal dysplasias, which typically exhibit additional characteristics in ultrasound features. For example, the prenatal ultrasound features for achondroplasia include reduced

femur length and humeral length, as well as increased biparietal diameter and head circumference. However, in our study, of the 8 cases diagnosed with achondroplasia, only 2 cases showed long bones shortening and macrocephaly. Therefore, we emphasize that further WES analysis for fetuses with short long bones should be conducted timely to spare the family from a delayed diagnosis or misdiagnosis. Early prenatal diagnosis of skeletal dysplasias provided tangible benefits for parents by allowing them to mentally prepare, make plans for termination of pregnancy or delivery and treatment. As the genetic causes of isolated FGR with short long bones become clearer, gene panels for common skeletal system genes, such as FGFR3, GUL7, and COL1A1, can be used to assess the genetic characteristics of fetuses with prenatal isolated FGR and short long bones when WES is not readily available. However, the diagnostic incremental yield in non-short long bones is low. Given the limitations of the current study, including its small sample size and single-center design, further investigation is warranted. We hope that further prospective investigation by multicenter and prospective research may be warranted to evaluate the utility of WES for FGR with non-short long bones. A precise diagnosis of FGR can assist parents in making informed clinical decisions. For fetuses with a poor prognosis, timely termination of pregnancy can mitigate the financial burden associated with postnatal care and reduce the overall burden on society and families. Consequently, during prenatal genetic counseling, counselors must discuss with parents the possibility of identifying P/LP variants through WES, along with the associated costs. Ultimately, the decision to undergo WES is at the discretion of the parents.

Peng et al. reviewed the data of 128 FGR fetuses with no additional anomalies who underwent genetic testing with karyotyping and CMA. They demonstrated an association between the chromosomal abnormality rate and FGR severity: the rate increased from 7.8% in fetuses with EFW below the 10th centile to 10% in those with EFW below the 5th centile and to 18% for fetuses with EFW below the 3rd centile [33]. Hang Z et al. found 8 P/LP variants in 51 singleton fetuses with isolated and severe FGR (EFW < 3rd percentile) and reported that WES can increase the diagnostic yield for isolated and severe FGR in fetuses with normal CMA results by 15.7% [34]. However, not study assessed the rate of positivity for the severity of isolated FGR by WES. In this study, the prevalence of the P/LP variant was 12.3% in the severe FGR group and 10.3% in the nonsevere FGR group, which was not a significant difference. However, in the nonshort long bones group, all the P/LP variants were found in the severe isolated FGR group. These findings suggest that WES is more valuable for fetuses with severe isolated FGR with non-short long boness. However, further studies should be performed to confirm this hypothesis.

Previous studies have also shown that the chromosomal abnormality rate in FGR decreases with advancing gestational age [35,36]. This study also demonstrated the detection rate of P/LP variants in fetuses with FGR diagnosed at different gestational ages. However, there was no significant difference between the early-onset group and late-onset group. Due to the limited number of patients with late-onset FGR, additional studies are needed to better define this discrepancy.

In addition to its diagnostic potential, the widespread application of WES has led to an increasing identification of VUS, posing significant challenges in clinical practice. In this study, WES revealed VUS in 2 (1.5%) fetuses, highlighting the complexity of interpreting these results in a clinical context. Given the uncertainty associated with VUS, it is recommended that clinicians enhance patient monitoring through regular examinations and ultrasounds. Timely interventions should be considered if necessary, based on emerging clinical findings. Moreover, intensive follow-up is essential after birth to track the clinical course and gather additional data that may clarify the significance of these variants.

There are several limitations in our study. The retrospective design may introduce selection bias, thereby potentially influencing the interpretation of our results. Moreover, the single-center nature may restrict the diversity of our patient population, which may compromise the external validity and generalizability of our findings. Additionally, the limited follow-up duration represents a significant limitation of this study, particularly in the context of long-term phenotypic outcomes. Given the relatively short duration of follow-up, our findings may not fully capture the long-term implications of the observed phenotypes or the potential evolution of these phenotypes over time. To address this limitation, future studies should prioritize extended follow-up periods to provide a more comprehensive understanding of long-term phenotypic outcomes. Furthermore, statistical comparison between other etiological categories such as gestational complications, intrauterine infection and confined placental mosaicism was not possible since these analysis was not performed for all cases. We hope that further prospective investigations involving multi centre and prospective research may be warranted to evaluate the application value of WES in the prenatal diagnosis of isolated FGR.

## Conclusion

Among fetuses diagnosed with isolated fetal growth restriction and a normal chromosomal microarray analysis, WES identified clinically significant findings in 11.9% of fetuses. Compared to the nonshort long bones group, the short long bones group had a greater detection rate of P/LP variants. In summary, our study proposes that WES should be offered for isolated FGR with short long boness. However, more studies are needed to investigate the utility of WES for isolated FGR with nonshort long boness.

## Acknowledgments

We are grateful to the patient and her family who participated in this study. We obtained consent for the publication of the patient's photograph and results. Xiaomei Shi: analysed and interpreted the data and wrote the final version of the article; Yanling Huang and Hongke Ding: performed the WES analysis; and Lina Zhao and Wei He: collected the data and edited the manuscript. Jing Wu: project development and manuscript writing. All the authors have read and approved the final manuscript.

## Author contributions

CRediT: **Xiaomei Shi**: Data curation, Formal analysis, Writing – original draft; **Yanling Huang**: Formal analysis, Software; **Hongke Ding**: Formal analysis, Software; **Lina Zhao**: Resources, Writing – review & editing; **Wei He**: Resources, Writing – review & editing; **Jing Wu**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

## Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Guangdong Women and Children Hosiptal. All pregnant women received genetic counseling and signed a written consent before the test. All procedures performed in this study were 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.

## Disclosure statement

The authors report no conflict of interest.

## Funding

This study was supported by the Traditional Chinese Medicine Bureau of Guangdong Province, China (20221045), and the Guangzhou Science and Technology Planning Project (grant 202103000047 to AY).

## ORCID

Yanling Huang http://orcid.org/0000-0001-8275-812X
Jing Wu http://orcid.org/0000-0003-4213-4106

## Data availability statement

All data generated during and/or analyzed during the current study are available upon request by contact the corresponding author.

## References

[1] McCowan LM, Figueras F, Anderson NH. Evidence-based national guidelines for the management of suspected fetal growth restriction:comparison, consensus, and controversy. Am J Obstet Gynecol. 2018;218(2S):S855–S868. doi: 10.1016/j.ajog.2017.12.004.
[2] American College of Obstetricians and Gynecologists' Committee on Practice Bulletins-Obstetrics and the Society for Maternal-Fetal Medicine. ACOG practice bulletin No. 204: fetal growth restriction. Obstet Gynecol. 2019;133:e97–109.
[3] Olga L, Sovio U, Wong H, et al. Association between antenatal diagnosis of late fetal growth restriction and educational outcomes in mid-childhood: a UK prospective cohort study with long-term data linkage study. PLoS Med. 2023;20(4):e1004225. doi: 10.1371/journal.pmed.1004225.
[4] Della Gatta AN, Aceti A, Spinedi SF, et al. Neurodevelopmental outcomes of very preterm infants born following early foetal growth restriction with absent end-diastolic umbilical flow. Eur J Pediatr. 2023;182(10):4467–4476. doi: 10.1007/s00431-023-05104-y.
[5] Figueras F, Gratacos E. An integrated approach to fetal growth restriction. Best Pract Res Clin Obstet Gynaecol. 2017;38:48–58. doi: 10.1016/j.bpobgyn.2016.10.006.
[6] Longo S, Borghesi A, Tzialla C, et al. IUGR and infections. Early Hum Dev. 2014;90(Suppl 1):S42–S44. doi: 10.1016/S0378-3782(14)70014-3.
[7] Giouleka S, Tsakiridis I, Mamopoulos A, et al. Fetal growth restriction: a comprehensive review of major guidelines. Obstet Gynecol Surv. 2023;78(11):690–708. doi: 10.1097/OGX.0000000000001203.
[8] Nowakowska BA, Pankiewicz K, Nowacka U, et al. Genetic background of fetal growth restriction. Int J Mol Sci. 2021;23(1):36. doi: 10.3390/ijms23010036.
[9] Borrell A, Grande M, Meler E, et al. Genomic microarray in fetuses with early growth restriction: a multicenter study. Fetal Diagn Ther. 2017;42(3):174–180. doi: 10.1159/000452217.
[10] Monier I, Receveur A, Houfflin-Debarge V, et al. Should prenatal chromosomal microarray analysis be offered for isolated fetal growth restriction? A French multicenter study. Obstet Gynecol Surv. 2022;77(6):336–338. doi: 10.1097/01.ogx.0000831036.52081.a6.
[11] Zhu H, Lin S, Huang L, et al. Application of chromosomal microarray analysis in prenatal diagnosis of fetal growth restriction. Prenat Diagn. 2016;36(7):686–692. doi: 10.1002/pd.4844.
[12] An G, Lin Y, Xu L, et al. Application of chromosomal microarray to investigate genetic causes of isolated fetal growth restriction. Mol Cytogenet. 2018;11(1):33. 0604 doi: 10.1186/s13039-018-0382-4.
[13] Chen Y, Xie Y, Jiang Y, et al. The genetic etiology diagnosis of fetal growth restriction using single-nucleotide polymorphism-based chromosomal microarray analysis. Front Pediatr. 2021;9:743639. doi: 10.3389/fped.2021.743639.

[14] Zhou H, Cheng K, Li Y, et al. The genetic and clinical outcomes in fetuses with isolated fetal growth restriction: a chinese single-center retrospective study. Front Genet. 2022;13:856522. doi: 10.3389/fgene.2022.856522.
[15] Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393(10173):747–757. doi: 10.1016/S0140-6736(18)31940-8.
[16] Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393(10173):758–767. doi: 10.1016/S0140-6736(18)32042-7.
[17] Monaghan KG, Leach NT, Pekarek D, et al. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22(4):675–680. doi: 10.1038/s41436-019-0731-7.
[18] Pauta M, Martinez-Portilla RJ, Meler E, et al. Diagnostic yield of exome sequencing in fetal growth restriction: systematic review and meta-analysis. Prenat Diagn. 2023;43(5):596–604. 03-04 doi: 10.1002/pd.6339.
[19] Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements-a prospective study. Am J Obstet Gynecol. 1985;151(3):333–337. doi: 10.1016/0002-9378(85)90298-4.
[20] Leung TN, Pang MW, Daljit SS, et al. Fetal biometry in ethnic Chinese: biparietal diameter, head circumference, abdominal circumference and femur length. Ultrasound Obstet Gynecol. 2008;31(3):321–327. doi: 10.1002/uog.5192.
[21] Jaganathan K, Kyriazopoulou PS, Mcrae JF, et al. Predicting splicing from primary sequence with deep learning. Cell. 2019;176(3):535–548.e24. doi: 10.1016/j.cell.2018.12.015.
[22] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–424. doi: 10.1038/gim.2015.30.
[23] Meler E, Sisterna S, Borrell A. Genetic syndromes associated with isolated fetal growth restriction. Prenat Diagn. 2020;40(4):432–446. doi: 10.1002/pd.5635.
[24] Mortier GR, Cohn DH, Cormier-Daire V, et al. Nosology and classification of genetic skeletal disorders: 2019 revision. Am J Med Genet A. 2019;179(12):2393–2419. doi: 10.1002/ajmg.a.61366.
[25] Zhang ZL, Zhang H, Ke YH, et al. The identification of novel mutations in COL1A1, COL1A2, and LEPRE1 genes in Chinese patients with osteogenesis imperfecta. J Bone Miner Metab. 2012;30(1):69–77. doi: 10.1007/s00774-011-0284-6.
[26] Kaiwar C, Zimmermann MT, Ferber MJ, et al. Novel NR2F1 variants likely disrupt DNA binding: molecular modeling in two cases, review of published cases, genotype-phenotype correlation, and phenotypic expansion of the Bosch-Boonstra-Schaaf optic atrophy syndrome. Cold Spring Harb Mol Case Stud. 2017;3(6):a002162. doi: 10.1101/mcs.a002162.
[27] Torti E, Keren B, Palmer EE, et al. Variants in TCF20 in neurodevelopmental disability: description of 27 new patients and review of literature. Genet Med. 2019;21(9):2036–2042. doi: 10.1038/s41436-019-0454-9.
[28] Hu L, Wang X, Jin T, et al. Identification of two CUL7 variants in two Chinese families with 3-M syndrome by whole-exome sequencing. J Clin Lab Anal. 2020;34(7):e23265. doi: 10.1002/jcla.23265.
[29] Elmas M, Gogus B, Kılıçarslan F, et al. Genotype to Phenotype: identification of Mucopolysaccharidosis Type IIIB (Sanfilippo's B) Case Using Whole Exome Sequencing. J Pediatr Genet. 2021;10(1):74–76. 0301 doi: 10.1055/s-0040-1708555.
[30] Jiang J, Tang W, An Y, et al. Molecular and immunological characterization of DNA ligase IV deficiency. Clin Immunol. 2016;163:75–83. doi: 10.1016/j.clim.2015.12.016.
[31] Kaijomaa M, Ulander VM, Ryynanen M, et al. Risk of adverse outcomes in euploid pregnancies with isolated short fetal femur and humerus on second-trimester sonography. J Ultrasound Med. 2016;35(12):2675–2680. doi: 10.7863/ultra.16.01086.
[32] Mone F, Mellis R, Gabriel H, et al. Should we offer prenatal exome sequencing for intrauterine growth restriction or short long boness? A systematic review and meta-analysis. Am J Obstet Gynecol. 2023;228(4):409–417.e4. doi: 10.1016/j.ajog.2022.09.045.
[33] Peng R, Yang J, Xie HN, et al. Chromosomal and subchromosomal anomalies associated to small for gestational age fetuses with no additional structural anomalies. Prenat Diagn. 2017;37(12):1219–1224. doi: 10.1002/pd.5169.
[34] Huang Y, Liu C, Ding H, et al. Exome sequencing in fetuses with short long boness detected by ultrasonography: a retrospective cohort study. Front Genet. 2023;14:1032346. doi: 10.3389/fgene.2023.1032346.
[35] Anandakumar C, Chew S, Wong YC, et al. Early asymmetric IUGR and aneuploidy. J Obstet Gynaecol Res. 1996;22(4):365–370. doi: 10.1111/j.1447-0756.1996.tb00990.x.
[36] Drummond CL, Gomes DM, Senat MV, et al. Fetal karyotyping after 28 weeks of gestation for late ultrasound findings in a low risk population. Prenat Diagn. 2003;23(13):1068–1072. doi: 10.1002/pd.715.