Evaluation of Diagnostic Strategies for Fetal Skeletal Dysplasia Using Ultrasound Scan and Gene Testing: A Preliminary Study in Wu Han, China

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Research Article

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Abstract

Objective: The aim of this study was to deliver prenatal diagnosis through sonographic examination and gene variation testing, and to evaluate the outcome of applied strategies in prenatal diagnosis

Methods: From September 2015 to April 2021, the study investigated 24 cases with suspected short long bones, which were obtained from the prenatal diagnosis center of Tongji Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology. The likely pathogenic gene variants were analyzed by multiple approaches (including karyotype analysis, copy number variations and whole exome sequencing) and further determined with the accuracy of the prenatal diagnosis for fetal skeletal dysplasia through one year follow-up survey.

Results: We found fetal skeletal dysplasia or malformation in 8 cases (account for 33.3%) before 24 weeks of gestation and in the rest cases after 30 weeks of gestation. Out of 24 cases, likely pathogenic gene variants in \textit{FGFR3}, \textit{FBN2}, \textit{COL1A2}, \textit{CUL7} and \textit{DYNC2H1} were detected for 6 cases; genetic variants in \textit{FGFR3}, \textit{IMPAD1} and \textit{GORAB} as possibly lethal mutations were identified in other 6 cases; and gene variants in \textit{WNT1}, \textit{FBN1}, \textit{OBSL1}, \textit{COL1A1}, \textit{DYNC2H1} and \textit{NEK1}, known as Variant of Undetermined Significance (VUS), were found in 4 cases. The rest 8 cases showed undetectable mutation in the whole exome sequencing (WES) analysis. A genetic diagnosis determined 12 different skeletal dysplasia genotypes in 14/24 (58.3%) cases. The other 10 cases with wild type gene (41.7%) were normal and well developed in one-year follow-up survey after study.

Conclusion: Features of fatal skeletal dysplasia can be identified in utero using fetal ultrasound and gene testing. Sonographic examination combining with genetic diagnosis showed advance in prenatal diagnosis in the preliminary study and the applied strategy could be used to help with improving the accuracy of prenatal diagnosis for fetal skeletal dysplasia.

1. Introduction

Fetal skeletal dysplasia (FSD) is one of the most common fetal malformations, of which the incidence rate is approximately 2.4-4.5 out of 10,000 births (Liu et al., 2019; Krakow and Rimoin, 2010). It is defined as a group of bone and cartilage disorders with diverse clinical and genetic heterogeneity. The pathogenicity is closely associated with mutations of the genes encoding collagen (e.g. type I, II, IX, XI collagen) (Franzone et al., 2019). Current scientific study reported many pathogenic gene variations in the proband with skeletal dysplasia and variants occurred in autosomal genes of \textit{COL1A1}, \textit{COL1A2}, \textit{WNT1}, \textit{OBSL1}, \textit{FGFR3}, \textit{IMPAD1}, \textit{FBN2}, and \textit{GORAB} (Xu et al., 2020; Zheng et al., 2019; Cao et al., 2019). With the guideline of the Nosology & Classification of Genetic Skeletal Disorders (2015 Revision), FSD consists of 436 diseases which are classified into 42 groups according to syndromes, genetic information, and nosologic autonomy, while 364 different genes associated with the disorder have been reported (Bonafe et al., 2015). Therefore, the determination of FSD is challenging due to the diversity of FSD phenotypes, particularly in the diagnosis for prenatal diagnosis.

So far, ultrasound is the first-line screening method in the prenatal diagnosis of FSD (Yang K et al., 2019). However, the efficiency on prenatal diagnosis for FSD was limited by other factors, such as the absence of family history information or difficulty of determining specific symptoms for fetus in utero. Recently, with the advance in next-generation sequencing technology, high-throughput sequencing has been considered as an effective method for genetic diagnosis. E.g. whole exome sequencing (WES), is advantageous in identification of De novo and compound heterozygous variants (Fu et al., 2018; Chandler et al., 2018). Suggested by the American College of Medical Genetics and Genomics (ACMG), next-generation sequencing can be considered to increase the sensitivity of diagnosis when the tradition gene testing, such as chromosomal microarray analysis, failed to yield a definitive result for the diagnosis.

By analyzing the clinical symptoms and gene variation of 24 cases with suspected fetal skeletal dysplasia, this study successfully obtained some solid evidence for prenatal diagnosis of FSD combining the sonographic screening with gene sequencing test in the first or second trimester of gestation, and the outcomes provide scientific supports for genetic diagnosis consultation and clinical intervention of FSD.

2. Materials And Methods

2.1 Editorial policies and ethical considerations

Our study was approved by the Research Ethics Committee of Tongji Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology. Legal consents were obtained from all the participants in the study.

2.2 Patients information
Twenty-four cases were collected by the study since all cases were detected with suspected short limbs from primary ultrasound scanning in the prenatal diagnosis center (one of prenatal diagnosis referral centers in China) of Tongji Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology between September 2015 and April 2021. Out of 24 cases, the pregnant women in case 9 and 10 had osteogenesis imperfecta before pregnancy, while the clinical phenotypes of the pregnant women and their spouses of the rest cases (22 cases) were all normal. In addition, case 6 had induced labor previously due to the suspected short limbs of the fetus determined only by ultrasound examination. There was no significant abnormality in non-invasive prenatal test (NIPT) during pregnancy for the pregnant women in all 24 cases.

2.3 Ultrasound scanning

The biometric data of the fetal skeleton (including biparietal diameter (BPD), head circumference (HC), measurements of all long bones (including humerus length (HL), femur length (FL)), assessment of skeletal mineralization), and any other abnormalities were collected and analyzed in comparison with normal values. Out of 24 cases, only cases 6 and 22 were found by ultrasound screening that the values of NT (nuchal translucency) were higher than the normal (NT of case 6: 0.55cm; and NT of case 22: 0.58cm), while the rest of 22 cases were all detected as normal.

2.4 Sample collection

Amniotic fluid and blood samples were retrieved from umbilical cord by amniocentesis and cordocentesis individually under ultrasound at second trimester of gestation. For the cases of which the blood or amniotic fluid samples could not be collected, two pieces of fetal muscle tissue (2 ×2 cm, containing the skin) or umbilical cord (about 3 centimeters) were sampled after the termination of pregnancy.

2.5 Fetal karyotype analysis and copy number variations (CNV)

Amniotic fluid and umbilical cord blood samples were used for chromosome G band karyotype analysis and CNV test.

2.6 WES and familial validation

Fetal genomic DNA was extracted from amniotic fluid samples or the tissues samples of fetuses using a DNA Extraction Kit (TianGen, Beijing, China) according to the manufacturer's instructions and stored at ~20°C for further analysis. The genomic DNA of the couples in all cases was extracted from whole blood sample using the same protocol/Kit. The WES was performed for all the DNA samples. The sequencing data was used for analyzing likely pathogenic gene variation by the Polyphen and SIFT software. Once the pathogenic gene variations were detectable in the fetal samples, the genetic variants were further examined for parents by Sanger sequencing.

3 Results

3.1 Clinical information and ultrasound findings

Ultrasound imaging revealed normal amniotic fluid volumes and normal appearance of the brain, heart, liver, or kidneys in all cases, whereas the suspected skeletal malformations were detected. The lengths of humerus and femur in 24 cases were found to be either significantly lower or significantly higher than the normal value (mean±SD) at the same gestation period. The values of fetal biparietal diameter and head circumference in all cases were normal. The results of prenatal ultrasound were summarized in Table 1. In addition, the fetal skeletal dysplasia or malformation was detected for 8 cases before 24 weeks of gestation in sonogram screening but not seen in the rest of cases. The following sonogram results showed the fetal skeletal dysplasia or malformation was developed in the rest of cases after 30 weeks of gestation.

3.2 Abnormalities by chromosome G band karyotype analysis and copy number variations (CNV)

The chromosome G band karyotype revealed negative in all 24 cases, while CNV was undetected in 22 cases except of case15 and 23. In the case15, we found a 0.2MB duplication in the chromosome 7 q11.21, considered benign according to the available evidence; In the case 23, a 0.4MB duplication in the chromosome 18p11.31q11.23 was detected and known as VUS.

3.3 Abnormalities Detected by Whole exome sequencing (WES)

Out of 24 cases, 6 cases were identified with carrying the likely pathogenic variants in gene FGFR3, FBN2, COL1A2, CUL7 and DYNC2H1; 6 other cases were considered to carry likely lethal gene variants in gene FGFR3, IMPAD1 and GORAB; 4 cases were detected with variants in gene WNT1, FBN1, OBSL1, COL1A1, DYNC2H1 and NEK1, known as VUS; The rest 8 cases showed negative in WES (Table 2), which was further confirmed by Sanger sequencing.
For the fetus of case 4 carried the c.7842T>A (p. Ala2614Ala) variant in FBN1 gene and the deletion of c.2135-3_2135-2delCA in OBSL1 gene. The Sanger sequencing analysis further revealed that the gene variant was carried by the father and the deletion was carried by the mother (Fig. 1).

For the fetus of case 6, we detected the variant of c.700G>T (p.E234*) and the deletion of CDS4-5 in IMPAD1 gene. Both Sanger sequencing and qPCR determined that the heterozygous deletion of CDS4-5 and the homozygous mutation in gene IMPAD1 were inherited from their parents, resulting to a composite heterozygous mutation (Fig. 2).

In the case 9 and 10, the two women were diagnosed with osteogenesis imperfecta before pregnancy. Our analysis detected the variant of c.1118G>C (p. Gly373Ala) in COL1A2 gene for the fetus in case 9, and variant of c.178C>T (p. Arg60*) in GORAB gene for the fetus in case 10. The two variants originated from osteogenesis imperfecta and inherited from their mother (Fig. 3, 4).

4. Discussion

The prenatal diagnosis of FSD is important at the second trimester of gestation, however, it is still challenging due to diverse clinical and genetic heterogeneity of the disorder. For decades, ultrasound is widely used in the noninvasive detection of FSD. Pajkrt and Chitty (2019) found that FL and HL of the fetuses with skeletal dysplasia were 5% shorter than the normal value. Previous reports in China demonstrated that the diagnosis accuracy of continuous sequential follow-up ultrasound was over 80% and 87.2% in the second trimester of gestation (Wang et al., 2017). In this study, by ultrasound scanning, we found the HL and FL of the fetuses in the 24 cases was either lower or higher than the normal value except of case 10 (the woman with OI (osteogenesis imperfecta) before pregnancy). The BPD and HC of the fetus were normal in all the 24 cases. In one-year follow-up survey, we found the 8 infants (account for 33.3%) were normal in skeletal development after delivery when the HL and FL of these fetuses was 2SD-3SD lower than the mean value. Differently, when the HL and FL of the studied fetuses was 3SD less or 3SD more, only two newborns (8.33%) were normal. The results of this ultrasound-only dependent approach are inadequate for diagnosing the disorder and impossible to differentiate the complex types of FSD.

In recent years, NGS (WES and WGS, Whole exome sequencing and Whole gene sequencing) has been applied in the area of disease diagnosis (Han et al., 2020). The mutation detection tool needs to optimize since the detection rates of WES could be variable depending on many factors, such as the sample size, the analysis criteria, proband-only or trio WES, and so on (Chandler et al., 2018). More importantly, the complex of genetic variants was found to be associated with the diverse pathogenicity of FSD.

In case 4, The fetus carried the c.7842T>A (p. Ala2614Ala) variant in the FBN1 gene and the deletion of c.2135-3_2135-2delCA in OBSL1 gene. The mutation is synonymous mutation and the deletion is in the intron. The FBN1 gene variation (Newell et al., 2017) is reported to be associated with Weill Marchesani syndrome (clinical manifestations are short limb deformity, secondary glaucoma, short stature, etc.). The OBSL1 gene variation (Isik et al., 2021) is related to 3-M syndrome (clinical manifestations include severe intrauterine growth retardation, short stature, recessive spina bifida, compression deformation of long metaphysis). The inheritance patterns of Weill Marchesani syndrome and 3-M syndrome are both AR (autosomal recessive inheritance). The Sanger analysis indicated that the mutation was carried by the father and the deletion was carried by the mother (Fig. 1). The mother of case 4 had natural delivery by vaginal at 39+6 weeks of gestation, and the newborn did not show any abnormality of bone development (up to 18-monthes old) at the end of the following-up survey.

In case 6, we detected the variant of c.700G>T (p.E234*) and the deletion of CDS4-5 in IMPAD1 gene for the fetus. The heterozygous deletion of CDS4-5 and the homozygous variant in gene IMPAD1 were inherited from their parents, as a compound heterozygote mutation (Fig. 2). IMPAD1-related chondrodysplasia is an autosomal recessive disease (Rosario et al., 2011). The pregnant women had cesarean section at 38 weeks of gestation in this case, and the newborn had typical short limb deformity and died within one month after delivery.

In the study, there were two women (case 9 and 10) diagnosed with osteogenesis imperfecta before pregnancy. We detected the variant of c.1118G>C (p. Gly373Ala) in COL1A2 gene for the fetus in case 9, and found the variant of c.178C>T (p. Arg60*) in GORAB gene for the fetus in case 10 (Fig. 3, 4). The two variants related to osteogenesis imperfecta and inherited from their mother, but one genetic type is autosomal dominant (AD) (Auguscik-Duma et al., 2018) and the other is autosomal recessive (AR) (Yang et al., 2017). The fetus of case 9 died before delivery at 35 weeks of gestation, while the newborn of case 10 had no significant abnormality in bone development up to 2-years old in the following-up survey.

In the case 2, 5 and 8, we found the WNT1for COL1A1-related VUS in osteogenesis imperfecta, and DYNC2H1 and NEK1-related asphyxiative hypoplasia of thorax. Notably, the fetuses in these cases all had short lower limbs, which was determined after abortion, while the proteins encoded by the gene variants were predicted to be deleterious using the SIFT and Polyphen analysis. Our assumption includes: (1) the
limited data depth which failed to achieve 100% coverage of the exon sequences, resulting in the related pathogenic gene variants was dismissed; (2) the unknown gene variation outside the exons, including non-coding region and intron mutation, possibly responsible for the pathogenicity; (3) other environmental factors that have not been found. Furthermore, we had negative findings by WES, and the 8 infants were normal in skeletal development after delivery in the rest 8 cases.

5. Conclusions

In sum, skeletal dysplasia is mostly hereditary. Our study obtained 14/24 (58.3%) cases carrying 12 different skeletal dysplasia genotypes. The gene variants are commonly found in the IMPAD1, COL1A1, WNT1, FGFR3 and FBN1 genes. The one-year followed-up survey found that the newborns were normal and well developed in the rest of 10 cases (41.7%). This is an initial study that analyzed the clinical and genetic features of fatal skeletal dysplasia using fetal ultrasound and multiple sequence-based gene tests (including karyotype analysis, copy number variations and whole exome sequencing). The findings could be important foundation for further developing the diagnosis strategies of FSD. Sonographic examination combining with genetic diagnosis showed advance in prenatal diagnosis in the preliminary study and the applied strategy could be used to help with improving the accuracy of prenatal diagnosis for FSD in the future.

Abbreviations

FSD: fetal skeletal dysplasia

FGFR3: fibroblast growth factor receptor3

FBN1: Fibrillin-1

FBN2: Fibrillin-2

COL1A1: collagen type I alpha 1 Chain

COL1A2: collagen type I alpha 2 Chain

CUL7: cullin-7

DYN2H1: Dynein Cytoplasmic 2 Heavy Chain 1

IMPAD1: Inositol Monophosphatase Domain containing 1

WNT1: Wnt Family Member 1

GORAB: Golgi-associated Rab-binding protein

OBSSL1: Obscurin Like Cytoskeletal Adaptor 1

NEK1: NIMA (Never-in-mitosis A)-related kinase 1

VUS: variant of undetermined significance

WES: whole exome sequencing

WGS: whole gene sequencing

NIPT: non-invasive prenatal test

BPD: biparietal diameter

HC: head circumference

HL: humerus length

FL: femur length

NT: nuchal translucency
**Declarations**

**Availability of supporting data**

All data analyzed in our study are available upon reasonable request.

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**Ethics declarations**

**Ethical approval and Consent to participate**

The study was approved by the Research Ethics Committee of Tongji Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology. All parents of the fetuses provided a signed informed consent prior to intrauterine diagnosis and sample collection in our country.

**Consent for publication**

Yes

**Competing interests**

The authors declare that they have no conflict of interest.

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Tables

TABLE1 ultrasound findings in this study
| No. | Age | Gestation / Parity (G/P) | Gestation (weeks) | HL (cm) Value M±SD | FL (cm) Value M±SD | BPD (cm) | HC (cm) | Skeletal malformation |
|-----|-----|--------------------------|------------------|---------------------|---------------------|----------|--------|----------------------|
| 1   | 39  | G1P0                     | 30+2             | 4.3 5.2-4SD         | 4.5 5.8-5SD         | 7.7      | 27.4   | Unseen               |
| 2   | 24  | G1P0                     | 17+5             | 1.0 2.4-8SD         | 1.1 2.4-5SD         | 3.9      | 14.7   | Unseen               |
| 3   | 21  | G1P0                     | 32               | 4.6 5.5-5SD         | 4.9 6.2-5SD         | 8.3      | 29.3   | Unseen               |
| 4   | 36  | G1P0                     | 33+4             | 5.0 5.5-3SD         | 5.6 6.2-2SD         | 8.2      | 28.2   | Unseen               |
| 5   | 31  | G1P0                     | 23+2             | 3.2 3.9-3SD         | 2.9 4.1-6SD         | 5.5      | 21.3   | Angle bending of ribs, left lower limb bone and humerus |
| 6   | 29  | G2P0                     | 32               | 3.7 5.5-10SD        | 4.5 6.2-7SD         | 8.8      | 29.9   | Unseen               |
| 7   | 23  | G1P0                     | 24+5             | 2.9 4.1-6SD         | 3.7 4.4-3SD         | 5.1      | 21     | Scoliosis, bipedal varus, bipedal toes continuously hooked, bilateral humerus, tibia, fibula bent |
| 8   | 29  | G1P0                     | 30               | 4.6 5.1-3SD         | 4.8 5.8-3SD         | 8.1      | 28.4   | Bilateral femoral curvature |
| 9   | 35  | G1P0                     | 22+5             | 3.6 3-3SD           | 3.0 3.9-3SD         | 5.5      | 19.8   | Right femoral curvature |
| 10  | 25  | G1P0                     | 21               | 3.5 3.6            | 3.4 3.7            | 5.0      | 18.6   | Unseen               |
| 11  | 27  | G1P0                     | 22               | 2.6 3-6            | 2.7 3.9-5SD         | 5.3      | 19.6   | Unseen               |
| 12  | 30  | G1P0                     | 32+5             | 4.3 5.5-6SD         | 4.3 6.2-7SD         | 8.9      | 28.4   | Small thorax         |
| 13  | 32  | G1P0                     | 32               | 5.0 5.5-3SD         | 5.5 6.2-3SD         | 8.4      | 29.3   | Unseen               |
| 14  | 29  | G1P0                     | 25               | 3.6 4.2-3SD         | 3.9 4.6-2SD         | 6.4      | 23.4   | Unseen               |
| 15  | 29  | G2P0                     | 33               | 4.1 5.5-7SD         | 4.4 6.2-7SD         | 8.3      | 29.9   | Unseen               |
| 16  | 30  | G2P0                     | 38               | 5.6 6.1-2SD         | 6.4 7.1-3SD         | 9.3      | 32.6   | Unseen               |
| 17  | 30  | G1P0                     | 40+2             | 5.0 6.1-5SD         | 5.1 7.1-8SD         | 7.3      | 26.3   | Unseen               |
| 18  | 33  | G3P0                     | 25+4             | 3.2 4.2-5SD         | 3.5 4.6-4SD         | 5.23     | 18.62  | Scoliosis             |
| 19  | 33  | G3P0                     | 34+5             | 5.4 5.6-2SD         | 6.1 6.5-2SD         | 8.8      | 30.9   | Unseen               |
| 20  | 30  | G1P0                     | 15+6             | 1.0 1.8-9SD         | 1.1 1.8-5SD         | 3.3      | 11.7   | Small thorax, equinus |
| 21  | 35  | G4P1                     | 36               | 5.6 5.9-2SD         | 6.4 6.8-2SD         | 9.0      | 32.1   | Unseen               |
| 22  | 29  | G3P1                     | 16               | 1.3 2.1-5SD         | 1.3 2.1-4SD         | 3.7      | 13.5   | Bilateral temporal bone depression |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 23 | 32 | G1P0 | 22 | 2.2 | 3.6-7SD | 2.3 |
|   |   |   |   |   | 3.9-6SD | 5.7 |
| 24 | 30 | G1P0 | 30+5 | 4.7 | 5.1-2SD | 5.0 |
|   |   |   |   |   | 5.8-3SD | 7.7 |

TABLE 2 Variants of the fetuses identified in the study
| NO. | Bone gene encoded | Nucleotide mutation | Amino-acid change | heterogeneity | Mutation type | Inheritance type | Inheritance type | Pregnancy Outcomes |
|-----|------------------|---------------------|-------------------|--------------|--------------|-----------------|-----------------|-------------------|
| 1   | FGFR3            | c.1015C>T          | p. Arg339Ter      | het          | Likely pathogenic | De novo | AD            | Induced labor    |
| 2   | WNT1             | c.1027G>C          | p. Glu343Gln      | het          | VUS          | Paternal | AR            | Induced labor    |
| 3   | FGFR3            | c.1144G>A          | p. Gly282Arg      | het          | pathogenic   | De novo | AD            | Induced labor    |
| 4   | FBN1             | c.7842T>A          | p. Ala2614Ala     | het          | VUS          | Biparental | AR            | Vaginal delivery |
| 5   | OBSL1            | c.2135-3_2135-2delCA| p.2135-3_2135-2delCA | heterozygous | pathogenic   | Biparental | AR            | Cesarean section |
| 6   | COL1A1           | c.824G>A           | p. Gly275Asp      | het          | VUS          | De novo | AD            | Induced labor    |
| 7   | IMPAD1           | c.700G>T           | p.E234*           | homozygous   | pathogenic   | Biparental | AR            | Cesarean section |
| 8   | DYNC2H1          | c.2641G>T          | p. Asp881Tyr      | het          | VUS          | Biparental | AR            | Induced labor    |
| 9   | NEK1             | c.859C>G           | p. Pro287Ala      | het          | VUS          | Biparental | AR            | Induced labor    |
| 10  | COL1A2           | c.1118G>C          | p. Gly373Ala      | het          | Likely pathogenic | Maternal | AD            | Induced labor    |
| 11  | GORAB            | c.178C>T           | p. Arg60*         | het          | pathogenic   | Maternal | AR            | Cesarean section |
| 12  | N                | N                   | N                 | N            | N            | N                | N                | Cesarean section |
| 13  | FGFR3            | c.1138G>A          | p. Gly380Arg      | het          | pathogenic   | De novo | AD            | Induced labor    |
| 14  | N                | N                   | N                 | N            | N            | N                | N                | Vaginal delivery |
| 15  | CUL7             | c.3355+5G>A        | p. V1252Gfs*23    | het          | Likely pathogenic | Biparental | AR            | Induced labor    |
| 16  | N                | N                   | N                 | N            | N            | N                | N                | Vaginal delivery |
| 17  | N                | N                   | N                 | N            | N            | N                | N                | Vaginal delivery |
| 18  | DYNC2H1          | c.4072C>T          | p. Arg1358Cys     | het          | Likely pathogenic | De novo | AR            | Induced labor    |
| 19  | N                | N                   | N                 | N            | N            | N                | N                | Cesarean section |
| 20  | FGFR3            | c.2420G>C          | p.*807Sext*101    | het          | Likely pathogenic | De novo | AD            | Induced labor    |
| 21  | N                | N                   | N                 | N            | N            | N                | N                | Cesarean section |
| 22  | FGFR3            | c.1948A>G          | p. Lys650Glu      | het          | pathogenic   | De novo | AD            | Induced labor    |
| 23  | FGFR3            | c.742G>T           | p. Arg248Cys      | het          | pathogenic   | De novo | AD            | Induced labor    |
| 24  | N                | N                   | N                 | N            | N            | N                | N                | N                 |
Fig. 1 case 4: A The fetus carried the c.7842T>A (p. Ala2614Ala) mutation in the FBN1 gene. B The deletion of c.2135-3_2135-2delCA in the OBSL1 gene was detected. The Sanger verification revealed that the mutation was carried by the father while the deletion was carried by the mother.

Fig. 2 case 6: A The fetus carried the c.700G>T (p.E234*) mutation in the IMPAD1 gene and inherited from mother by Sanger sequencing verification. B The deletion of CDS4-5 in IMPAD1 gene was detected in the fetus and inherited from father by qPCR verification.
**Fig. 3 case 9:** The variant of c.1118G>C (p. Gly373Ala) in *COL1A2* gene of the fetus and the mother.

**Figure 3**

See image above for figure legend

**Fig. 4. case 10:** A The variant of c.178C>T (p. Arg60*) in *GORAB* gene of the mother. B The heterozygous mutation in GORAB gene of the fetus.

**Figure 4**

See image above for figure legend