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## A de novo mutation in the COL1A1 gene leading to severe osteogenesis imperfecta: Case report and review of the literature

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**Case Description:** We report the case of a fetus with skeletal abnormalities and subcutaneous edema. Ultrasound imaging revealed suspected skeletal malformations, including hypoplastic long bones of all four limbs, poorly ossified calvarium, unrevealing nasal bones, and generalized subcutaneous edema. Whole-exome sequencing revealed a heterozygous mutation in COL1A1 [c.2174G>T/p.(G725V), NM\_000088.3]. According to the ACMG guidelines, it was determined to be a pathogenic variant and identified as a de novo variant(PS2+PP3\_Strong+PM2\_Supporting), which has not been reported in the HGMD, gnomAD, Clinvar, or other databases. This variation causes a glycine to valine substitution at position 725, which is located within the Gly Xaa-Yaa repeat in the helical domain of the collagen molecule.

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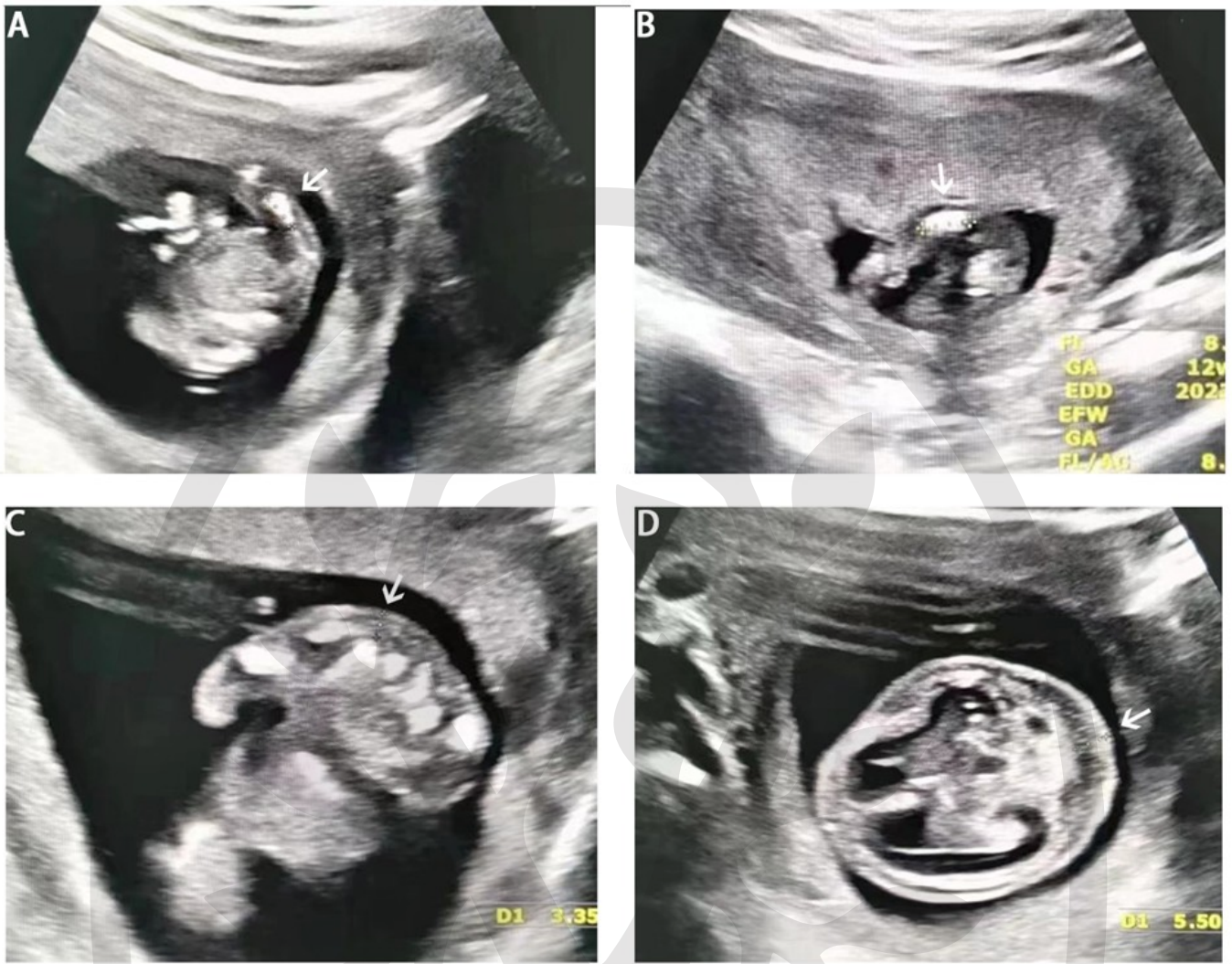
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**FIGURE 1** Ultrasound images of cases.(A) Humeral length: 7 mm. (B) Femur length: 8 mm. (C) The abdomen was approximately 3.5 mm thick. (D) The scalp was approximately 5.5 mm thick.



# A *de novo* mutation in the *COL1A1* gene leading to severe osteogenesis imperfecta: Case report and review of the literature

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## Abstract

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**Case Description:** We report the case of a fetus with skeletal abnormalities and subcutaneous edema. Ultrasound imaging revealed suspected skeletal malformations, including hypoplastic long bones of all four limbs, poorly ossified calvarium, unrevealing nasal bones, and generalized subcutaneous edema. Whole-exome sequencing revealed a heterozygous mutation in *COL1A1* [c.2174G>T/p.(G725V), NM\_000088.3]. According to the ACMG guidelines, it was determined to be a pathogenic variant and identified as a *de novo* variant(PS2+PP3\_Strong+PM2\_Supporting), which has not been reported in the HGMD, gnomAD, Clinvar, or other databases. This variation causes a glycine to valine substitution at position 725, which is located within the Gly Xaa-Yaa repeat in the helical domain of the collagen molecule.

**Conclusions:** The *COL1A1* mutation [c.2174G>T/p.(G725V), NM\_000088.3], is a novel pathogenic variant of severe OI. Our study expanded the OI *COL1A1* gene variation profiles in the Chinese population and provided a theoretical foundation for prenatal diagnosis, genetic counseling, and obstetric management.

**Keywords:** Osteogenesis imperfecta<sup>1</sup>, *COL1A1* gene<sup>2</sup>, Whole exome sequencing<sup>3</sup>, Prenatal diagnosis<sup>4</sup>, Hydrops fetalis<sup>5</sup>

## 1 Introduction



As one of the most common congenital disorders, fetal skeletal dysplasia is characterized by genetic and phenotypic heterogeneity, making it difficult to differentiate between these diseases on ultrasound[1]. Osteogenesis imperfecta (OI) is the most prevalent monogenic inherited skeletal dysplasia disease. It is predominantly autosomal dominant, but a few autosomal recessive cases have been reported, and it is rarely attributed to X-linked inheritance[2]. The incidence of OI ranges from approximately 1:15,000 to 1:20,000 births[3]. The main clinical manifestations of OI include short stature, skeletal anomalies, increased skeletal fragility, multiple fractures, other skeletal system pathologies, and extraskeletal manifestations such as dentinogenesis imperfecta, malocclusion, pulmonary dysfunction, and hearing impairment[4]. In 1979, Silence et al. initially categorized OI into four types, I-IV, based on patients' phenotypic characteristics and severity. In 2019, the Chinese guidelines for rare disease diagnosis and treatment further classified it into five types, I-V. Among them, type II OI is fatal, and the phenotype is the most serious, with patients usually succumbing in the perinatal period, and there is no efficacious treatment for live births[5]-[6]. For fetuses with potential OI suggested by ultrasound, genetic testing to ascertain the etiology and prenatal intervention are optimal prevention and management strategies, respectively. In this study, we conducted whole exome sequencing(WES) of the fetus, referring to prenatal ultrasound suggestive of OI, with normal copy number variation sequencing (CNV-seq) and classic karyotype. To validate the disease-associated candidate variant sites detected by WES, Sanger sequencing was used to elucidate the fetal genetic etiology and provide a basis for prenatal intervention, genetic counseling, and risk assessment of fertility in the family. In accordance with the CARE reporting checklist, we present the following case.

## 2 Case Presentation

A 24-year-old Chinese Han primigravida was found to carry a fetus with suspected skeletal dysplasia, which prompted our attention. No abnormalities were observed in the first trimester, and the couple did not have a history of radiation substance exposure or medication use, as claimed by the couple of primigravida. Additionally, the couple was previously healthy and did not have a family history of skeletal dysplasia or consanguineous marriage. During the routine check-up in the second trimester, other prenatal examinations did not reveal any abnormalities. However, ultrasound imaging at 18 weeks of gestation revealed suspected skeletal malformations, including hypoplastic long bones of all four limbs, poorly ossified calvarium, unrevealing nasal bones, and generalized subcutaneous edema (Figure 1). Details of the suspected fetuses are shown in Table 1. Subsequently, diagnostic screening was conducted by collecting amniotic fluid from fetal parents and peripheral blood samples.

After obtaining consent from the couple, we obtained a 20 ml sample of amniotic fluid via amniocentesis at 18 weeks of gestation. The amniotic fluid sample obtained was aliquoted into two tubes. One tube was utilized for chromosome karyotype analysis, and the other tube (QIAamp DNA Blood Mini Kit produced by German company Qiagen) was used to extract genomic DNA for CNV-seq and Trio-WES; 2 ml samples of each spouse's peripheral blood were collected to extract genomic DNA (Magnetic Blood Genomic DNA Kit from Tiangen Biochemical Technology Company) for Trio-WES.

Karyotype analysis revealed no abnormalities in the chromosome number or structure (Figure 2A). CNV-seq analysis did not reveal any chromosome aneuploidy or copy number variations (CNVs) in the genome, with a definite pathogenicity of more than 100 kb (Figure 2B). We identified a

heterozygous mutation of *COL1A1* [c.2174G>T/p.(G725V), NM\_000088.3], which were not present in either parent. Sanger sequencing corroborated the sequencing results for the whole exon group (Figure 2), and this variation was confirmed to be a *de novo* variation through sequencing of the parental samples.

The mutation was deemed pathogenic based on multiple *in silico* predictions of variant pathogenicity (Table 2), with a REVEL software prediction score of 0.997. This variation was absent from the ESP6500, EXAC, and 1000 human genome databases. Following the ACMG guidelines, the variation was determined to be pathogenic (PS2+PP3\_Strong+PM2\_Supporting). This mutation was not present in HGMD, gnomAD, Clinvar, or other databases, indicating that it was a novel mutation site.

Collagen type 1(Col1) is the principal connective tissue that forms the skeleton, sclere, skin, and teeth of the body. Collagen type 1 is a protein that is fundamentally composed of alpha 1 and alpha 2 chains. We used Uniport (<https://www.uniprot.org/>) to process protein conservation analysis at the mutation site 725, and the results showed that it is a conservation site in various species(Figure 2D).

### 3 Discussion

OI is a congenital skeletal dysplasia with a genetic classification of 22 types (OI type I to XXII) (<https://omim.org>). However, there is considerable overlap in the clinical phenotypes of these different types. As of November 11, 2022, the OI and Ehlers-Danlos Syndrome variant databases have included 20 genes associated with OI. Studies have demonstrated that pathogenic mutations cause approximately 85-90% of OI in the type I collagen ligand  $\alpha 1$  subunit/ $\alpha 2$  subunit (*COL1A1*/*COL1A2*). Despite the identification of thousands of pathogenic mutation types, a precise genotype-phenotype correlation has yet to be established[7].

In clinical practice, OI is classified into four types (I-IV) based on the clinical phenotype and severity. Type I OI is characterized by the mildest clinical phenotype, typically without skeletal malformations. The clinical phenotype of type II OI is the most grievous, with the most affected individuals dying in the perinatal period due to severe bone deformities. Some severe cases also present with fetal edema [8]. Type III OI is severe, presenting with progressive skeletal malformation and high-frequency fractures as well as abnormal teeth and hearing loss, and has a poor prognosis. Type IV OI is of moderate severity, between types I and III[5][6].

The *COL1A1* is located at 17q21.33, with a total length of 18 kb and 51 exons. Code-shifting, nonsense, and splicing mutations of *COL1A1* primarily lead to premature termination codons, producing hazardous and degradable truncated gene expression products. OI caused by such mutations typically presents mild clinical manifestations. Missense mutations in *COL1A1* are associated with the production of proteins with abnormal structures. The mutant subunits participate in and disrupt the triple-helix assembly of type I collagen, exacerbating the dominant-negative effect. These mutations typically result in severe phenotypes[9]. Studies in China, Italy, Poland, and other countries have demonstrated that missense mutations are the primary cause of OI with poor prognosis, the majority of which involve the substitution of glycine in the Gly-X-Y (X and Y are random amino acids) repeat sequences of the collagen molecular helix domain with other amino acids[10][11][12][13]. The substitution of glycine with branched nonpolar or charged amino acids likely results in a more severe form of OI[14]. Studies conducted in other contexts have corroborated

that *de novo* mutations, which have not been subjected to purifying selection, are associated with a more severe clinical presentation of OI than inherited *COL1A1* mutations[15]-[16].

This was the first pregnancy for couples who were not blood-related. No significant skeletal dysplasia or similar clinical manifestations were observed in the family history. Obstetric ultrasonography revealed severe limb shortening, poor skull ossification, absence of nasal bones, and generalized subcutaneous edema. According to research findings, fetuses afflicted with lethal skeletal dysplasia display considerable and premature reduction in the length of their long bones as well as disproportionate growth of the fetal abdomen and cranium. Notably, the ratio of femoral length to abdominal circumference serves as a reliable indicator of skeletal dysplasia lethality. Specifically, 92-96% of cases showed a ratio of femoral length to abdominal circumference below 0.16, which is a sign of fatal skeletal dysplasia[17]. The available sonographic parameters indicate the presence of a hypoplastic fetal skeletal system, which is indicative of severe OI. Studies have demonstrated that when a pregnant woman and her spouse have no apparent skeletal system malformations, prenatal ultrasound imaging reveals abnormalities of the fetal skeletal system in conjunction with other system malformations, which are likely caused by chromosomal abnormalities or copy number variations. When fetal malformations are mainly observed in the skeletal system, they are usually monogenic in origin[18]. In this study, karyotyping and CNV-seq did not reveal any abnormalities, suggesting that non-chromosomal numerical, structural, and copy number variations were associated with skeletal malformations and other ultrasonic manifestations. Trio-WES revealed that the fetus in this case carried the heterozygous c.2174G>T/p.(G725V) variant, which is categorized as a pathogenic variant based on ACMG guidelines. Neither of the parents had this mutation site, which was not present in the database or reported in the literature. Among the candidate genes, the missense variant in *COL1A1* appeared to be the most frequently observed pathogenic variant associated with OI. Therefore, the identified mutation appears to have arisen spontaneously during the early stages of human embryonic development. Based on this and the ultrasound-specific clinical presentation, the fetus was initially diagnosed with perinatal lethal osteogenesis imperfecta. The fetal *COL1A1* gene had a c.2174G>T/p.(G725V) variation, causing a glycine to valine substitution at position 725, which is located within the Gly Xaa-Yaa repeat in the helical domain of the collagen molecule and is a branched nonpolar amino acid, which might be the most responsible for the more severe fetal clinical phenotype. We have summarized the mutated genes of severe fetal OI caused by missense mutations in *COL1A1* and their associated clinical ultrasonic manifestations reported in the literature (Table 3). Ultrasound images exhibit high similarity, primarily displaying a shortened humerus, shortened femur, thoracic stenosis, and a poorly ossified calvarium. This finding was consistent with the ultrasound findings reported in the present study. Consequently, in the absence of a prenatal genetic diagnosis, ultrasound during pregnancy suggests that the aforementioned fetal manifestations could be utilized as the initial diagnostic basis. The patient chose to terminate their pregnancy after genetic confirmation. Regrettably, owing to the lack of aborted tissue, we were unable to definitively ascertain whether it was a perinatal lethal osteogenesis imperfecta.

OI is one of the primary causes of skeletal dysplasia in fetuses for which no adequate therapy exists. Currently, non-lethal patients are managed symptomatically, primarily through lifestyle modification, medications, surgery, and rehabilitative training[19][20]. Therefore, regular prenatal checkups, especially prenatal ultrasound examinations, are paramount for such diseases. Prompt prenatal diagnosis is necessary for fetuses with a family history or ultrasound findings suggestive of skeletal dysplasia, for early prenatal intervention and prevention of the birth of affected infants.

## 4 Conclusions

In conclusion, we present a case of OI in a fetus with skeletal dysplasia and generalized subcutaneous edema. The c.2174G>T/p.(G725V) *de novo* mutation of *COL1A1* in our patient was a novel pathogenic mutation of OI.

## 1 Conflict of Interest

The authors declare that they have no competing interests.

## 5 Funding Statement

This study was supported by projects funded by the National Key Research and Development Program of China (2018YFC1002900, 2018YFC1002903 and 2021YFC2701504).

## 6 Acknowledgments

We acknowledge all the participants in this study.

## 7 Ethics Approval Statement

The samples used in this study were collected with appropriate informed consent and approval from the Ethics Committee of Shandong Provincial Hospital, Affiliated with Shandong First Medical University(LCYJ: NO.2018-003).

## 8 Authors Contributions

**Yurong Lu** wrote the original draft. **Yijia Tian** wrote the original draft of this manuscript. **Jinxiu Liu**: Visualization. **Yifan Wang**: Visualization. **Xietong Wang**: Conceptualization; Methodology; Project administration, writing, review, and editing.

## 9 Patient Consent Statement

Images and information from individual participants were obtained from the individual's free prior informed consent.

## 12 Data Availability Statement

All the data involved in this study are available in the manuscript, tables, and figures.

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**FIGURE 1** Ultrasound images of cases.(A) Humeral length: 7 mm. (B) Femur length: 8 mm. (C) The abdomen was approximately 3.5 mm thick. (D) The scalp was approximately 5.5 mm thick.

**FIGURE 2** A. Karyotype analysis results of cases. B.CNV-seq results of fetal. C. (a)Pedigree information in this family. (b) Sanger validation peak plots for the proband (fetus) and family members. The fetal *COL1A1* gene has c.2174G>T / p.(G725V) heterozygous variation, which his parents do not carry. D. Protein conservation analysis in the mutation site 725 showed that it is a conservation site in various species.( <https://www.uniprot.org/>)

**TABLE 1** Ultrasonographic measurements of suspected fetal skeletal dysplasia.

Ultrasonographic	Measured value (mm)	Normal range in 18 weeks gestation (mm)
Biparietal Diameter(BDP)	42	34-45
Head circumference(HC)	59	128-161
Transverse diameter of the cerebellum	16	15.9-20.5
Abdominal Circumference (AC)	108	110-141

Femoral Length (FL)	8	21-29
Humeral Length (HL)	7	22.3-30.1
Fetal weight	118(g)	184-269(g)
Fetal skin thickness	Scalp thickness:5.5 Abdominal skin thickness:3.5	-
Other	The nasal bone of the fetus was not shown, the lip structure was not clearly displayed, the ossification of the skull ring was not obvious, the cerebellum was visible, the midline of the brain was in the middle. and the width of bilateral ventricles was about 4mm.	-

**TABLE 2** Effects of *COL1A1* mutations predicted with in silico tools.

Protein prediction algorithm	Score	Interpretation
Scale-Invariant Feature Transform (SIFT)	0	Damaging
Polymorphism Phenotyping version2_HDIV(Polyphen2 HDIV)	1	Probably-damaging
Polymorphism Phenotyping version2_HVAR (Polyphen2 HVAR)	0.999	Probably-damaging
Mutation Taster	1	Disease-causing
Protein Variation Effect Analyzer(PROVEAN)	-7.53	Damaging
Rare Exome Variant Ensemble Learner(REVEL)	0.997	Damaging
Combined Annotation-Dependent Deletion (CADD)	34	Damaging
phyloP	7.867	Conserved
phastCons	1	Conserved
SiPhy	18.429	Conserved

**TABLE 3** Gene and clinical manifestation of fetal severe OI caused by multiple missense mutations *COL1A1*.

References	Gene Nucleotide Change	Pathogenicity Level (Evidences)	Fetal Gestational Age	Radiographic Finding	Extraskel et al Manifestations in Ultrasound Examination	Fetal Sample for WES
Ji, Y.et al. [21]	Case 1: c.1634G>A (p.Gly545Asp)	-	Case 1: 17W	Case 1: The fetus is characterized by short humeri (10 mm) and short femora (10mm) Skull ossification is poor. with borderline personality disorder (BPD)(41 mm) and circumference (150 mm).	Case 1: A narrow chest and notch can be seen in the junction of the chest and abdomen, and the maximal pocket of amniotic fluid	Case 1: Amniotic fluid

Tanner, L. et al. [22]	Case 1: c.3290G>T(p.Gly1097Val)	-	Case 1: 21W4D	Case 1: Extremely short limbs corresponding roughly to the gestational age of 12 weeks. The thorax was so small; Computed tomography showed the fetus was abnormally short and poorly ossified long bones and absent ossification of the skull were evident; The Fetus was aborted at 22W	-	Case 1: Tissue after fetal abortion
Huang, H. et al. [23]	Case 1: c.1822G>A (p.Gly608Ser)	-	Case 1: 20W	Case 1: Short and incurved limbs, a small thorax, bowing long bones, and a reduced echogenic ring around the intracranial structures: Fetus was aborted at 17W	-	Case 1: Tissue after fetal abortion
Zhang, L. et al. [24]	Case 1: c.994G>A(p.Gly332Arg) Case 2: c.2362G>A(p.Gly788Ser) Case 3: c.2444G>C(p.Gly815Ala) Case 4: c.3505G>A(p.Gly169Ser) Case 5: c.3541G>A(p.Gly181Ser)	Case 1: P(PS2+PM1+PM2+P3+PP4) Case 2: LP(PM1+P2+PP3+PP4) Case 3: LP(PM1+P2+PP3+PP4) Case 4: LP(PS2+PM1+PM2+PP3+PP4) Case 5: LP(PS2+PM1+PM2+PP3+PP4)	Case 1: 24W6D Case 2: 31W3D Case 3: 30W1D Case 4: 26+W Case 5: 21+W	Case 1: Fetal whole body bone sonographic changes, clinical suspicion of osteogenesis imperfecta. Case 2: The fetal femur and humerus were about 4 W+ less than the corrected gestational age. and no obvious abnormality was found before 31 weeks. Case 3: The long bones of limbs are less than gestational age, bilateral femurs are slightly curved. Case 4: Fetal femurs are short and slightly curved on both sides. Case 5: The long bones of the fetus limbs are less than 4 standard deviations, the thoracic cavity is narrow; the nasal bone is absent. Case 1: The long bones of the fetus's limbs were short and curved. The Fetus aborted at 24W1D Case 2: The fetal femur, tibia, and fibula were initially found to be short and curved; Fetus was aborted at 23W5D. Case 3: Two affected pregnancies: (1) The right femur was initially found to be "telephone-like" at 22W; Both femurs were identified as short and curved at 24W; Fetus was aborted at 25W; (2) Both femurs were identified as short and curved at 20W6D: Fetus aborted at 32W; (3) Still pregnant before submission. Case 4: Limb long bones short and curved, abnormal ankle joint and foot posture at 13W4D; Fetus aborted at 17W.	Case 3: The diameter of the oval is larger, and the left ventricle has strong light spots Case 5: The eye spacing is widened; the scalp edema, the brain parenchyma is poor, the subdural effusion, and the abdominal fluid is effusion.	unknown
Yang, K. et al. [25]	Case 1: c.1678G>A(p.Gly560Ser) Case 2: c.2101G>A(p.Gly701Ser) Case 3: c.3557C>T(p.Pro186Leu) Case 4: c.2300G>T(p.Gly767Val)	Case 1: supporting +PS2+PP3) Case 2: P(P2+PM2+PM5 strong+PS2+PP3) Case 3: VUS(PP2+PM2+PM5) Case 4: LP(PP2+PM2+PM5+PP3)	Case 1: 23W4D Case 2: 22W6D Case 3: 22W;20W6D (two pregnancies) Case 4: 13W4D	Case 1: The long bones of the fetus's limbs were short and curved. The Fetus aborted at 24W1D Case 2: The fetal femur, tibia, and fibula were initially found to be short and curved; Fetus was aborted at 23W5D. Case 3: Two affected pregnancies: (1) The right femur was initially found to be "telephone-like" at 22W; Both femurs were identified as short and curved at 24W; Fetus was aborted at 25W; (2) Both femurs were identified as short and curved at 20W6D: Fetus aborted at 32W; (3) Still pregnant before submission. Case 4: Limb long bones short and curved, abnormal ankle joint and foot posture at 13W4D; Fetus aborted at 17W.	Case 4: NT (nuchal translucency) thickening (6.0 mm), anasarca	Case 1: Umbilical cord Case 2: Umbilical cord Case 3: Amniotic fluid; Umbilical cord Case 4: Amniotic fluid; Umbilical cord
Zhuang, J. et al. [26]	Case 1: c.1777G>A(p.Gly593Ser)	Case 1: PS2_very strong+PM5+PM2 supporting +PP3	Case 1: 19W	Case 1: Short and curved femurs and humerus in the fetus. (Specially, during her first pregnancy, a c.1777G>A mutation in the COL1A1 gene was detected in the fetus who exhibited skeletal dysplasia,	-	Case 1: Amniotic fluid

was 36 mm.



thus the family chose to terminate her pregnancy.)

Cao, J. et al. [27]	Case 1 : c.644G>A (p.Gly215Asp) Case 2 : c.2885G>A (p.Gly962Asp) Case 3 : c.994G>A (p.Gly332Arg)	Case 1: PVS1+PM2+PM5+P2+PP3 Case 2: PS2+PM2+PP3 Case 3: PS2+PM1+PM2+PP3+PP5	Case 1: 22W Case 2: 24W5D Case 3: 20W6D	Case 1: Mild bilateral femoral bowing Case 2: Bowing of the long bones, poor ossification of the skull and vertebrae, hypertelorism Case 3: Abnormality of calvarial morphology, short long bone, limb undergrowth, bowing of the long bones, fractures of the long bones	Case 1: Light spot in heart of fetus Case 1: hypertelorism	Case 1: Aborted fetuses and amniotic fluid samples Case 2: Aborted fetuses and amniotic fluid samples Case 3: Aborted fetuses and amniotic fluid samples
LI, R. et al. [28]	Case 1: c.644G>A (p.Gly215Asp) Case 2: c.2885G>A (p.Gly962Asp) Case 3: c.994G>A (p.Gly332Arg)	Case 1: PVS1+PM2+PM5+P2+PP3 Case 2: PS2+PM2+PP3 Case 3: PS2+PM1+PM2+PP3+PP5	Case 1: 22W Case 2: 24W5D Case 3: 20W6D	Case 1: Severe short extremities and severe curvature of the lower limbs Case 2: Severe short and curved extremities	Case 1: Facial dysmorphism (tall forehead, beaked nose, low-set ears, and open triangular-shaped mouth)	Case 1: Amniotic fluid Case 2: Amniotic fluid
Yang, Y. et al. [29]	Case 1: c.2605G>T (p.Gly869Cvs) Case 2: c.1804G>A (p.Gly602Arg)	-	Case 1: 24W Case 2: 25W	Case 1: Fetal extremities were short for gestational age. The femurs, humeri, and fibulas were bent some of which were telephone receiver-shaped. Case 2: Fetal nasal bones were not shown. The thorax was narrow and bell-shaped. Fetal limb bones were short for gestational age and some were abnormally bending. The fetus was aborted at 25W.	-	Case 1: Umbilical cord blood Case 2: Aborted fetuses and amniotic fluid samples