Molecular Diagnosis and Prenatal Counseling of a Chinese Family with Spondyloepiphyseal Dysplasia congenital

Lihong Fan
Huzhou Maternity and Child Care Hospital
Yuqing Xu
Women's Hospital, School of Medicine, Zhejiang University
Guosong Shen
Huzhou Maternity and Child Care Hospital
Xueping Shen
Huzhou Maternity and Child Care Hospital
Kefeng Tang
Huzhou Maternity and Child Care Hospital
Juan Yao
Huzhou Maternity and Child Care Hospital
Longfei Ji (jilongfei882006@126.com)
The First People's Hospital of Huzhou, https://orcid.org/0000-0002-1230-9051

Research

Keywords: spondyloepiphyseal dysplasia congenital, targeted next-generation sequencing, missense mutation, COL2A1, prenatal diagnosis.

Posted Date: May 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-497606/v1

Molecular diagnosis and prenatal counseling of a Chinese family with spondyloepiphyseal dysplasia congenital

Lihong Fan[1], Yuqing Xu[2], Guosong Shen[1], Xueping Shen[1], Kefeng Tang[1], Juan Yao[1], Longfei Ji[3*]

[1]Center of Prenatal Diagnosis, Huzhou Maternity & Child Health Care Hospital, Huzhou, 313000, Zhejiang, China.

[2] Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, 310006, Zhejiang, China.

[3]Clinical Laboratory, The First People's Hospital of Huzhou, Huzhou, 313000, Zhejiang, China

*Correspondence: Longfei Ji, Clinical Laboratory, The First People's Hospital of Huzhou, Huzhou, 313000, Zhejiang, China. Email: jilongfei882006@126.com.

Aim: To identify the gene mutation and complete prenatal counseling in a Chinese family with spondyloepiphyseal dysplasia congenital (SEDC).

Materials and Methods: A Chinese family with SEDC was enrolled. Their detailed clinical features, skeletal radiographic features, and laboratory results were obtained. The peripheral blood samples of the family members were used for the targeted next-generation sequencing (NGS), and Sanger sequencing confirmation. Bioinformatics analysis and genotype-phenotype correlation analysis were used to identify the gene mutation.

Amniocentesis, fetal chromosome analysis and Sanger sequencing were completed for prenatal diagnosis.

Results: A missense mutation c.3392G>T (p. Gly1131Val) of *COL2A1* was found in the proband and the fetus. The mutation was confirmed to be likely pathogenic and would damage the structure of stable triple-helical type II collagen.

Conclusion: A novel pathogenic c.3392G>T (p. Gly1131Val) mutation in *COL2A1* leading to SEDC was identified, which expanded the genotypic spectrum and phenotypic spectrum of SEDC. In addition, we wish to emphasize that prenatal diagnosis and genetic counseling should be carried out in a family with SEDC for better procreative management.

Keywords: spondyloepiphyseal dysplasia congenital, targeted next-generation sequencing, missense mutation, *COL2A1,* prenatal diagnosis.

Introduction

Spondyloepiphyseal dysplasia congenital (SEDC; OMIM #183900) is a rare autosomal dominant inherited chondrodysplasia. It is characterized by common skeletal deformities concluding short trunk, dwarfism, cervical spine subluxation, scoliosis, coxa

vara, kyphosis, pes planus, and metaphyseal changes (Xiong et al., 2018). This disorder is mainly caused by mutations in the *COL2A1* gene (Anderson et al.,1990; Nishimura et al., 2005).

The *COL2A1* gene (MIM #108300) is located on 12q13.11-q13.2 and encodes type II procollagen alpha-1 chain which is a major component of type II collagen expressed in chondrocytes and vitreous humor (Anderson et al., 1990; Barat-Houari et al., 2016). The most common type of mutation in *COL2A1* is a substitution that changes codons for obligatory glycine residues in the Gly-X-Y triplet repeats (characteristic for the triple-helical domain) to codons for other amino acids, such as valine, arginine, and so on. More importantly, there is no particular mutation hotspot (Terhal et al., 2012).

Previous reports have indicated that SEDC significantly reduces the quality of life of the patients and their families. Even worse, the disease results in huge psychological and economic pressure on them(Miyoshi et al., 2004; Terhal et al., 2012). Thus, it is very important to carry out prenatal diagnosis and counseling for prevention.

Here, we aim to identify the pathogenic mutation on a Chinese pedigree with SEDC by NGS. We believe that our study would put insight into the molecular diagnosis of the rare disease, as well as

contribute to its prenatal diagnosis and pregnancy management.

Material and methods

Subjects

A Chinese family affected with SEDC was enrolled and the pedigree was shown in Fig.1A. The proband (III_1) was 36 years old with a height of 145cm. He had an abnormal gait when he was one year old and then X-ray films showed hip dislocation (data not shown). He has felt pain in the hip since he was 25 years old, especially after long-distance walking. X-ray check revealed skeletal anomalies (Fig.1B). His pregnant wife was non-consanguineous and healthy. They came to our hospital for molecular diagnosis and prenatal counseling. This study was approved by the institutional review board of Huzhou Maternity & Child Health Care Hospital for clinical research. All the family members gave their informed consent.

Genetic testing and data analysis

2740 genes associated with skeletal system disease were carried out to identify genomic variants by targeted NGS. The peripheral blood samples of the proband and his family members were used to extract DNA using Lab-Aid 820 DNA blood Mini Kit

(Zeesan, China). Based on the high-throughput sequencing technology, the samples were detected by using the Sure Select Human All Exon V6 (Agilent, American). The sequencing was performed on Illumina HiSeq2000 sequencer. The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), Leiden Open Variation Database 3.0 (LOVD 3.0, http://www.lovd.nl/3.0/home) and literature of Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/) were used to confirm the missense mutation. Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (http://provean.jcvi.org/index.php) and ClinPred (https://sites.google.com/site/clinpred/) were used to predict whether the mutation affects protein function. Online clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to analyze the evolutionarily conserved sequences among species (human, cattle, dog, horse, chimpanzee, rat, chicken and rabbit). Then Sanger sequencing was performed to confirm the gene mutation. The c.3392G>T mutation was amplified using the following primers: Forward (5′-GTCCTGGCTTTTCTCTGACG-3′) and Reverse (5′-AGCGAGAGGCTGATTCATGT-3′). The Sanger sequencing

was performed by an ABI 3130 DNA analyzer.

Prenatal diagnosis

Thirty milliliters of amniotic fluid were obtained by amniocentesis under ultrasound guidance. Fifteen milliliters of amniotic fluid were used for chromosomal karyotyping and the other fifteen milliliters were used for Sanger sequencing.

Results

The sequencing showed a novel missense mutation c.3392G>T (p. Gly1131Val) in exon 48 of *COL2A1* (Fig. 2A), while Sanger sequencing revealed that except for the fetus, none of his family members carried the mutation (Fig. 2B). The mutation has not been reported in the HGMD, LOVD 3.0, or the Clinvar database. SIFT (PROVEAN v1.1.3), ClinPred, and PolyPhen-2 (version 2.2) predicted the gene mutation as 'deleterious', 'probably damaging', and 'probably damaging', respectively. According to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015), the missense mutation was identified to be "likely pathogenic". Besides, this mutation site is highly conserved among different species (Fig. 2C), indicating its functional

importance throughout evolution. chromosomal karyotyping showed that the fetal karyotyping was 46, XX (Fig. 3A). Ultrasound findings of the fetus indicated short head circumference, short abdomen circumference, short humerus length (HL), and short femur length (FL) at 22^{+3} weeks of gestation, which were all at least lower than -2SD (<-2SD) (Fig.$3B_1$, $3B_2$, $3B_3$). In addition, the fetus showed scoliosis (Fig.$3B_4$). Considering the gene mutation and abnormal structures of the fetus, the couple decided to terminate the pregnancy.

Discussion

In summary, we report here a novel missense mutation, c.3392G>T (p. Gly1131Val), in the *COL2A1* gene in a Chinese family. It is diagnosed as SEDC by clinical features and genetic test. Moreover, prenatal diagnosis and genetic counseling were completed through amniocentesis and Sanger sequencing. The new finding points to the new gene mutation in the genotypic spectrum of SEDC and the significance of prenatal genetic counseling for such a family.

SEDC is a rare genetic disease caused by *COL2A1* mutations(Li et al., 2014). Mature type II collagen fibers consist of three α-1 chains encoded by *COL2A1*. These chains twist

together and present a triple helix domain. N- and C-telopeptide regions at both ends allow the initiation of the triple-helical configuration (Fig.2A). Amino acid substitution of the triple-helical domain can slow down the triple-helical conformation folding and damage the collagen transport in cells, which leads to the over modification of the protein, the destruction of cartilage homeostasis, and long bone development (Anderson et al., 1990). When the function of type II collagen is affected, a disease known as type II collagenopathy occurs and SEDC is the most common type (Cui et al., 2008; Zhang et al., 2020).

Patients affected with SEDC may show skeletal manifestations such as short stature, abnormal epiphyses, and flattened vertebral bodies(Dikaiakou et al., 2019; Zheng et al., 2020), as well as extraskeletal manifestations such as cleft palate, retinal detachment, myopia and hearing loss(Saleem et al., 2019). A recent study has reported that SEDC patients with glycine-to-valine substitution often show scoliosis dysplasia, lumbar lordosis, femoral head dysplasia, vertebral body, kyphosis, and acetabular flattening. However, exoskeletal characteristics such as myopia, retinal detachment, nuclear cataract, and hearing impairment are not found (Zheng et al., 2020). The clinical

features of the proband in our study are consistent with this report.

For a long time, prenatal diagnosis of SEDC relied on ultrasound findings (Patel & Filly, 1995), which showed short long bones (Donnenfeld & Mennuti, 1987), short femurs (Chitty et al., 2006; Xia et al., 2008), a hypomineralised spine , a narrow chest, increased nuchal translucency(Chitty et al., 2006) and so on. In our study, the fetus showed a relatively small body, including short HL and short FL (Fig.3B), which were consistent with previous studies. Moreover, the fetus also showed scoliosis which was firstly found in a fetus with SEDC. However, the most abnormal ultrasound findings of a fetus are not obvious until the second trimester of pregnancy(Patel & Filly, 1995). The earliest gestation when FL was noted to be shortened was 16 weeks (Kirk & Comstock, 1990). Additionally, short long bones were found in a fetus at around 19 weeks (Donnenfeld & Mennuti, 1987). In our study, the abnormal structures of the fetus were found at 22^{+3} weeks' gestation, which had exceeded the best period for prenatal diagnosis. Therefore, the families affected with SEDC should be advised to complete prenatal molecular diagnosis as soon as possible once the wives are pregnant.

Conclusion

In conclusion, we identified a novel pathogenic mutation c.3392G>T (p. Gly1131Val) in exon 48 of *COL2A1* in a Chinese family affected with SEDC. Our findings expanded the genotypic and phenotypic spectrums of SEDC. Moreover, the findings would contribute to the genetic counseling among families affected with SEDC.

Acknowledgement

We are deeply grateful to all clinicians for referring the patient and laboratory technicians for their dedicated work to achieve accurate results.

Funding

The work was supported by the Zhejiang medical science and technology program (2021KY1085).

Availability of data and materials

The data supporting the conclusions of this article is included within the article.

Author contribution

L F, G S were involved in the conception and design of the study. L F, X S and K T were involved in the laboratory experiments. L F, L

J were involved in genetic counseling, data collection and data analyzation. J Y and Y X wrote the manuscript. L J revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the institutional review board of Huzhou Maternity & Child Health Care Hospital for clinical research. All the family members gave their informed consents.

Reference

Anderson, I. J., Goldberg, R. B., Marion, R. W., Upholt, W. B., & Tsipouras, P. (1990). Spondyloepiphyseal dysplasia congenita: genetic linkage to type II collagen (COL2AI). *Am J Hum Genet, 46*(5), 896-901.

Barat-Houari, M., Sarrabay, G., Gatinois, V., Fabre, A., Dumont, B., Genevieve, D., & Touitou, I. (2016). Mutation Update for COL2A1 Gene Variants Associated with Type II Collagenopathies. Hum Mutat, 37(1), 7-15. doi: 10.1002/humu.22915

Chitty, L. S., Tan, A. W., Nesbit, D. L., Hall, C. M., & Rodeck, C. H. (2006). Sonographic diagnosis of SEDC and double heterozygote of SEDC and achondroplasia--a report

of six pregnancies. *Prenat Diagn, 26*(9), 861-865. doi: 10.1002/pd.1525

Cui, Y. X., Xia, X. Y., Bu, Y., Zhou, G. H., Yang, B., Lu, H. Y., . . . Li, X. J. (2008). Rapid molecular prenatal diagnosis of spondyloepiphyseal dysplasia congenita by PCR-SSP assay. *Genet Test, 12*(4), 533-536. doi: 10.1089/gte.2008.0037

Dikaiakou, E., Vlachopapadopoulou E, A., Manolakos, E., Samelis, P., Margariti, R., Zampakides, C., & Michalacos, S. (2019). Identification of an Autosomal Dominant Mutation in the COL2A1 Gene Leading to Spondyloepiphyseal Dysplasia Congenita in a Greek Family. Mol Syndromol, 9(5), 241-246. doi: 10.1159/000492190

Donnenfeld, A. E., & Mennuti, M. T. (1987). Second trimester diagnosis of fetal skeletal dysplasias. *Obstet Gynecol Surv, 42*(4), 199-217.

Kirk, J. S., & Comstock, C. H. (1990). Antenatal sonographic appearance of spondyloepiphyseal dysplasia congenita. *J Ultrasound Med, 9*(3), 173-175. doi: 10.7863/jum.1990.9.3.173

Li, S., Zhou, H., Qin, H., Guo, H., & Bai, Y. (2014). A novel mutation in the COL2A1 gene in a Chinese family with Spondyloepiphyseal dysplasia congenita. *Joint Bone Spine, 81*(1), 86-89. doi: 10.1016/j.jbspin.2013.06.010

Miyoshi, K., Nakamura, K., Haga, N., & Mikami, Y. (2004). Surgical treatment for atlantoaxial subluxation with myelopathy in spondyloepiphyseal dysplasia congenita. *Spine (Phila Pa 1976), 29*(21), E488-491. doi: 10.1097/01.brs.0000143621.37688.f3

Nishimura, G., Haga, N., Kitoh, H., Tanaka, Y., Sonoda, T., Kitamura, M., . . . Ikegawa, S. (2005). The phenotypic spectrum of COL2A1 mutations. *Hum Mutat, 26*(1), 36-43. doi: 10.1002/humu.20179

Patel, M. D., & Filly, R. A. (1995). Homozygous achondroplasia: US distinction between homozygous, heterozygous, and unaffected fetuses in the second trimester. *Radiology, 196*(2), 541-545. doi: 10.1148/radiology.196.2.7617874

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., . . . Rehm, H. L. (2015). 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. *17*(5), 405-424. doi: 10.1038/gim.2015.30

Saleem, S., Anwar, A., Iftikhar, P. M., Anjum, Z., & Tariq, Z. (2019). Spondyloepiphyseal Dysplasia Congenita: A Rare Cause of Respiratory Distress. *Cureus, 11*(7), e5101. doi: 10.7759/cureus.5101

Terhal, P. A., van Dommelen, P., Le Merrer, M., Zankl, A., Simon, M. E., Smithson, S. F., . . . Mortier, G. R. (2012). Mutation-based growth charts for SEDC and other COL2A1 related dysplasias.
Am J Med Genet C Semin Med Genet, 160c(3), 205-216. doi: 10.1002/ajmg.c.31332

Xia, X. Y., Cui, Y. X., Huang, Y. F., Pan, L. J., Feng, Y., Yang, B., . . . Liang, Q. (2008). Molecular prenatal diagnosis in 2 pregnancies at risk for spondyloepiphyseal dysplasia congenita. *Clin Chim Acta, 387*(1-2), 153-157. doi: 10.1016/j.cca.2007.09.006

Xiong, Q., Liu, Y., Xue, Y., Liu, S., Wang, J., Li, P., . . . Xiao, H. (2018). A novel de novo mutation in COL2A1 leading to spondyloepiphyseal dysplasia congenita in a Chinese family. *5*, 17059. doi: 10.1038/hgv.2017.59

Zhang, B., Wang, C., Zhang, Y., Jiang, Y., Qin, Y., Pang, D., . . . Tang, X. (2020). A

CRISPR-engineered swine model of COL2A1 deficiency recapitulates altered early skeletal developmental defects in humans. *Bone, 137*, 115450. doi: 10.1016/j.bone.2020.115450

Zheng, W. B., Li, L. J., Zhao, D. C., Wang, O., Jiang, Y., Xia, W. B., . . . Li, M. (2020). Novel variants in COL2A1 causing rare spondyloepiphyseal dysplasia congenita. 8(3), e1139. doi: 10.1002/mgg3.1139

Figure 1. Pedigree of the family and radiographs of the proband (III_1). A: III_1 and IV_1 were affected with SEDC and all other family members were healthy; B: The proband showed flattened lower femur, narrowed upper tibia(B1), flattened bilateral femoral heads and shortened femoral neck (B2).

Figure 2. The schematic illustration, Sanger sequencing image and conservation analysis in COL2A1: c.3392G>T (p. Gly1131Val). A: The construction of mature type II collagen fibers and the approximate location of the gene mutation; B: Both proband and fetus had the mutation in the *COL2A1*: c.3392G>T (p. Gly1131Val) while other family members did not have the mutation. C: c.3392G>T (p. Gly1131Val) of COL2A1 gene was highly conserved among different

species (human, cattle, dog, horse, chimpanzee, rat, chicken and rabbit)

Figure 3. The karyotype and radiographic features of fetus (the final ultrasound at 22 weeks' gestation). A: Fetal karyotype was 46, XX and all the chromosomes had no obvious abnormalities. B1: The fetus is generally smaller than the fetus at the same gestational age; B2: Humerus length was equivalent to that of fetus at 20 weeks' gestation; B3: Femur length was equivalent to that of fetus at 19^{+1} weeks' gestation; B4: Fetus scoliosis could be seen.

Figures

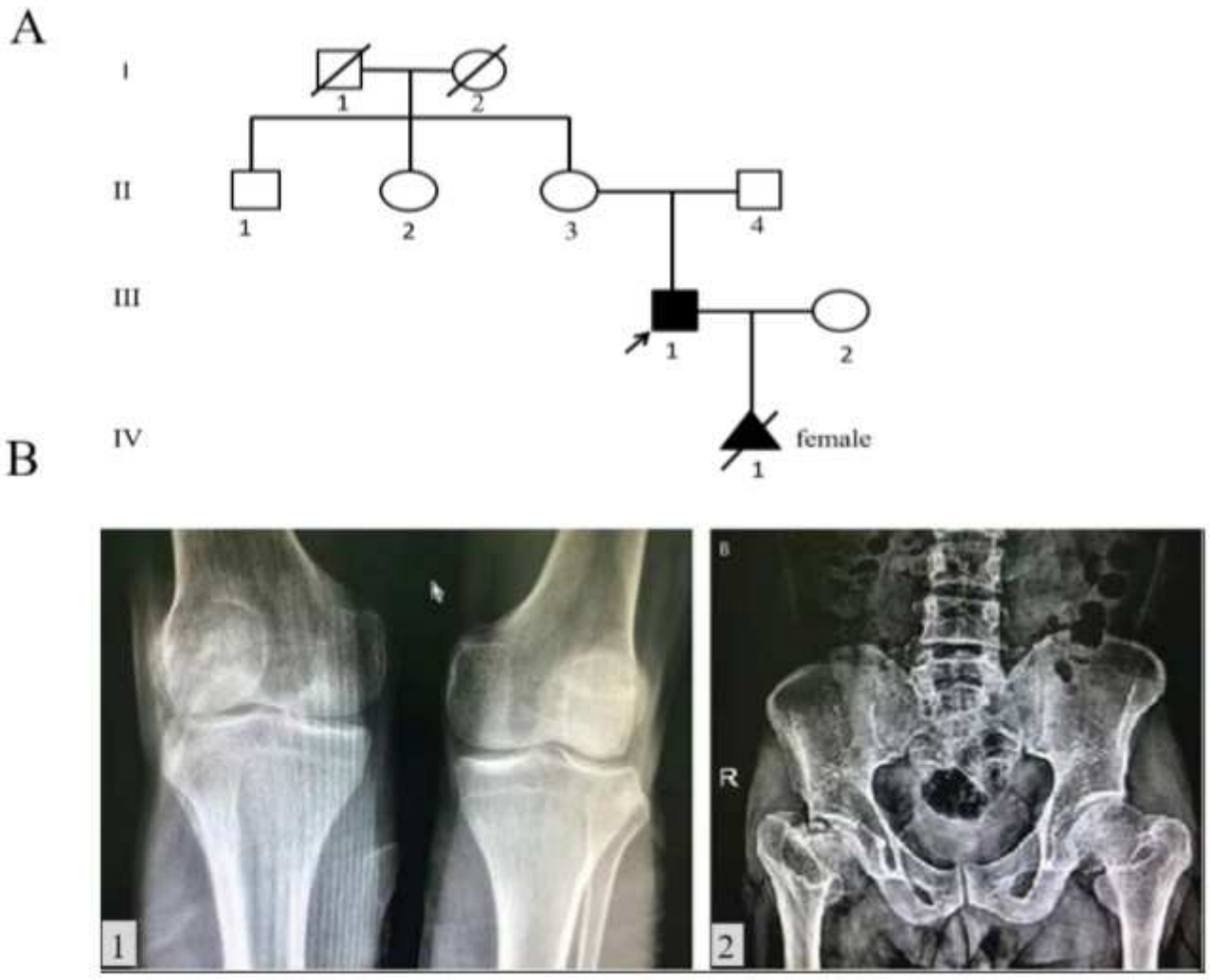

Figure 1

Pedigree of the family and radiographs of the proband (III1). A: III1 and IV1 were affected with SEDC and all other family members were healthy; B: The proband showed flattened lower femur, narrowed upper tibia(B1), flattened bilateral femoral heads and shortened femoral neck (B2).

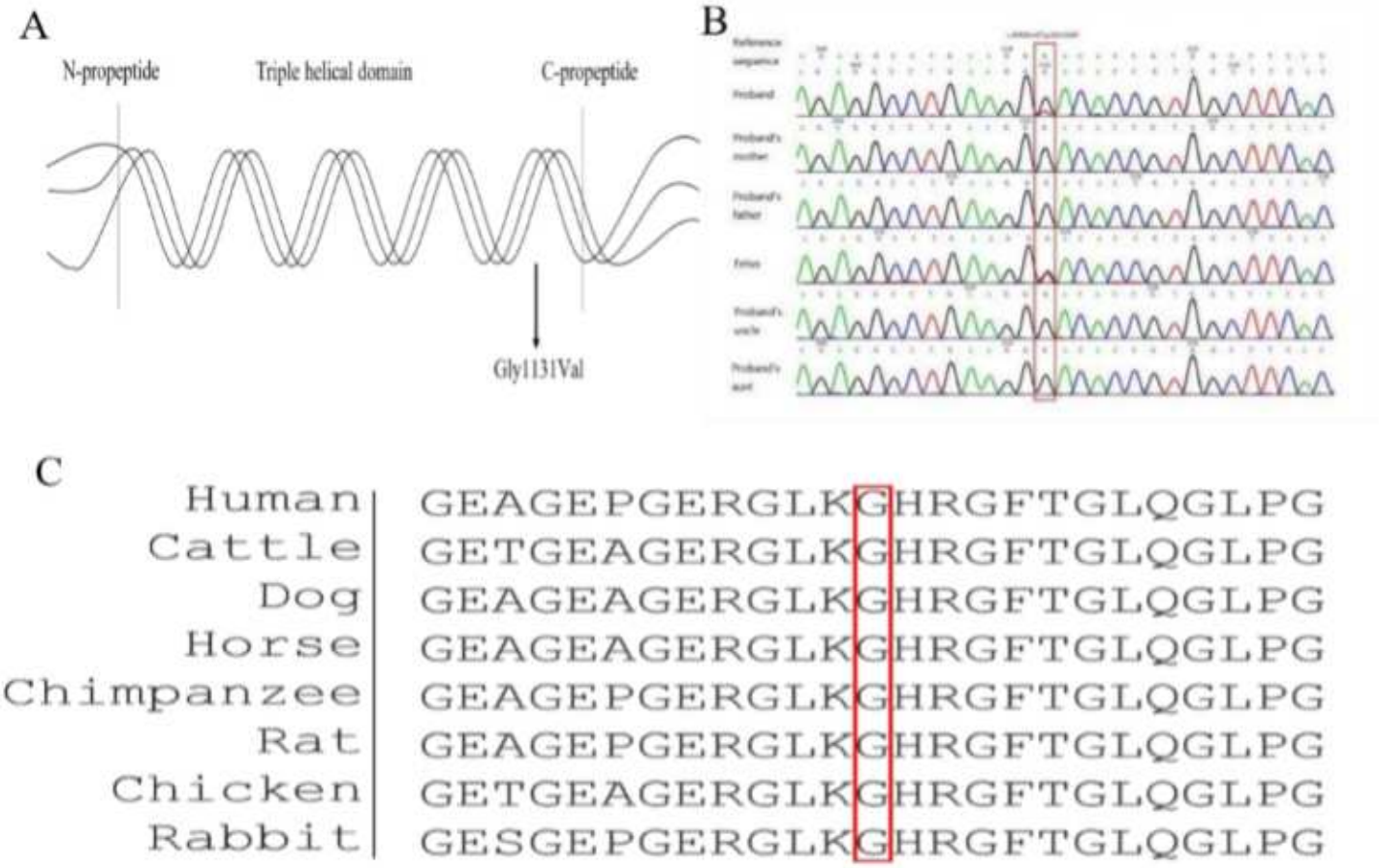

Figure 2

The schematic illustration, Sanger sequencing image and conservation analysis in COL2A1: c.3392G>T (p. Gly1131Val). A: The construction of mature type II collagen fibers and the approximate location of the gene mutation; B: Both proband and fetus had the mutation in the COL2A1: c.3392G>T (p. Gly1131Val) while other family members did not have the mutation. C: c.3392G>T (p. Gly1131Val) of COL2A1 gene was highly conserved among different species (human, cattle, dog, horse, chimpanzee, rat, chicken and rabbit)

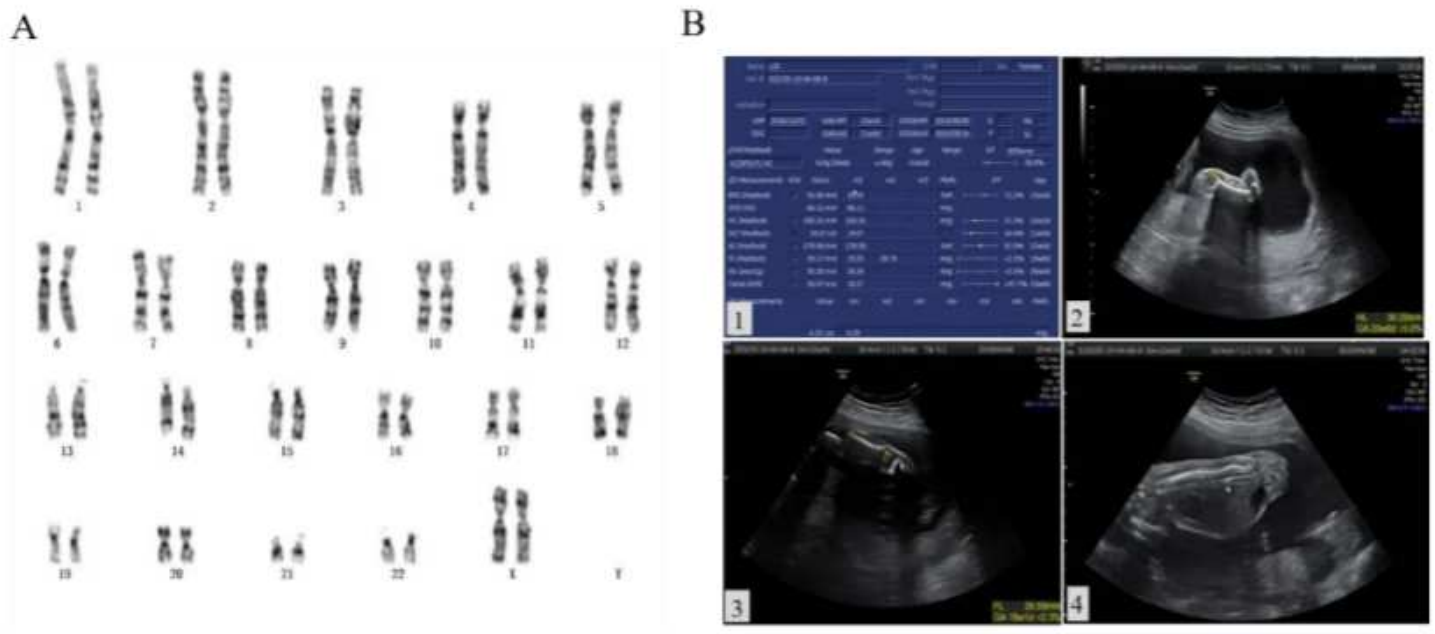

Figure 3

The karyotype and radiographic features of fetus (the final ultrasound at 22 weeks' gestation). A: Fetal karyotype was 46, XX and all the chromosomes had no obvious abnormalities. B1: The fetus is generally smaller than the fetus at the same gestational age; B2: Humerus length was equivalent to that of fetus at 20 weeks' gestation; B3: Femur length was equivalent to that of fetus at 19+1 weeks' gestation; B4: Fetus scoliosis could be seen.