Research & Reviews: Journal of Biology e-ISSN: 2322-0066

Mutation of COL2A1 in a Chinese Family with Presentations of Legg-Calvé-Perthes Disease

Denglu Yan[1]*, Zhaojie Wang[2], Zhi Zhang[3]

[1]Department of Orthopedics, First People's Hospital of Zhaoqing, Guangzhou, China
[2]Department of Orthopaedics, Twelve People's Hospital of Guangzhou, Guangzhou, China
[3]Department of Orthopedics, Third Hospital of Guangzhou Medical University, Guangzhou, China

Research Article

Received: 22-Jul-2022, Manuscript No. JOB-22-69914; **Editor assigned:** 26-Jul-2022, PreQC No. JOB-22-69914 (PQ); **Reviewed:** 09-Aug-2022, QC No. JOB-22-69914; **Revised:** 16-Aug-2022, Manuscript No. JOB-22-69914 (R); **Published:** 26-Aug-2022, DOI: 10.4172/2322-0066.10.6.004.

***For Correspondence:** Denglu Yan, Department of Orthopedics, First People's Hospital of Zhaoqing, Guangzhou, China

E-mail: yandenglu@163.com

Keywords: Genetics; Osteonecrosis; Femoral Head; COL2A1

Abbreviations: ONFH: Osteonecrosis of the Femoral Head; LCPD: Legg-Calvé-Perthes Disease; HD: Hip Dysplasia; SNP: Single Nucleotide Polymorphism

ABSTRACT

Background: The aim of this study was to identify genetic factors and chromosomal regions contributing to Osteonecrosis of the Femoral Head (ONFH) in a Chinese family with presentations of Legg-Calvé-Perthes Disease (LCPD).

Methods: In this study, we performed whole exon sequencing of a Chinese family with LCPD for mutation detection. Ten members had ONFH in twenty-seven family members in four generations family, 5 unaffected members of the studied family and 5 normal peoples as control were underwent whole exome sequencing for mutation detection. Structural modeling test was applied to analyze the potential structural changes caused by the missense substitution.

Results: In this Chinese family affected by LCPD, the mutation (c.3508 G>A, p. Gly1170Ser) in exon 50 of COL2A1 in the Gly–X–Y domain was present in 10 patients but absent in 5 unaffected members of the studied family and in 5 control chromosomes from unaffected individuals of matched geographical ancestry. The COL2A1 gene mutation was further validated by Sanger sequencing, confirmed that were heterozygous for the mutation. Then, we identified the p.Gly1170Ser mutation in exon 50 of COL2A1 in a Chinese family with LCPD.

Conclusions: This study maps the mutation of mutation (c.3508 G>A, p. Gly1170Ser) in exon 50 of COL2A1 in the Gly–X–Y domain in a Chinese family of LCPD, which causes osteonecrosis of femoral head.

INTRODUCTION

Osteonecrosis of the Femoral Head (ONFH) was seen in adult and children, and the osteonecrosis of the femoral head that occurs in children was called Legg-Calvé-Perthes Disease (LCPD). Although LCPD was described over 100 years ago, its etiology is still controversial. LCPD can be associated with abnormalities in factor V Leiden mutation [1].The Factor V Leiden gene mutation in children with LCPD. Described a family with three-generation transmission of factor V Leiden and LCPD developed in three siblings in this family [2]. Report heterozygosity for factor V Leiden was the only inherited risk factor associated with the development of LCPD [3]. Suggest that the homozygous form of Factor V Leiden mutation has some role in the clinical course of LCPD [4,5].

Although it has recently been postulated that thrombophilia may have a role in the aetiology of LCPD, it remain conflicting [6].Report no association between factor V Leiden and LCPD[7]. Report thrombotic component in the etiology of LCPD [8]. Study did not find an increased rate of factor V or prothrombin mutations in children with en with LCPD compared to the natural incidence [9]. Found no genetic association between LCPD and increased genetic thrombophilia among patients compared with the control group[10]. The prothrombin II mutation is located in 11p11–q12 at position G20210A and activates the coagulation cascade which increases the risk of thrombosis. Report methylenetetrahydrofolate not associated with LCPD[11-13].

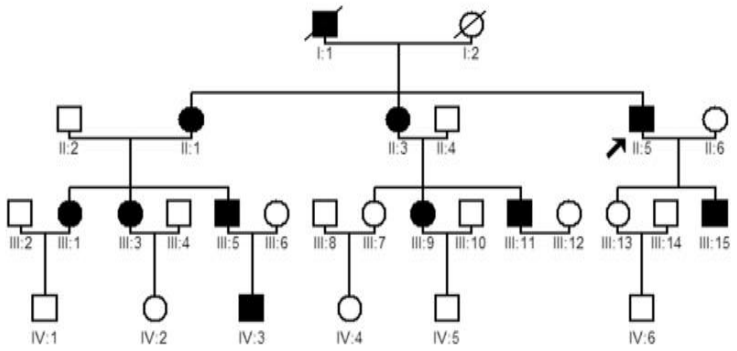
Previous studies showed that LCPD aggregates strongly in families, and the genetic predisposition to osteonecrosis is well documented in COL2A1 [14-18]. Report the cases of two children who presented with abnormal development of both hips and in whom novel mutations in the COL2A1 gene were found. Report 2 generations of 4 male family members with LCPD-like features and mutation of the COL2A1 gene of the 12q13 chromosome. Identify a heterozygous mutation (c.1888 G>A, p. Gly630Ser) in exon 29 of COL2A1 in the Gly–X–Y domain, in a Chinese family affected by LCPD and ANFH. An understanding of the causes of LCPD is important for its prevention, diagnosis, and treatment. Although its obscure etiology is still under discussion, recent studies have focused on the importance of genetic factors in the development of LCPD because genetic factors can provide insight into the pathophysiology of ONFH and point to new treatments. We evaluated a large family with a dominantly inherited of ONFH in Chinese family with LCPD.

MATERIALS AND METHODS

Patient recruitment/sample collection

Patients of a Chinese family with LCPD suffered severe hip pain were diagnosed ONFH in the Zhaoqing first people's hospital. The study protocol was approved by the Committee on Clinical Investigation at the Zhaoqing first people's hospital. Informed written consent was obtained from all study participants and the study was conducted in accordance with the Declaration of Helsinki II and approved by the local Ethical Committees. Twenty-seven members of this Chinese family with LCPD participated in the study, of which ten showed clinical symptoms of ONFH shown in Figure 1.

Figure 1: Pedigrees of the four generation LCPD family. Patients with LCPD are shown by darkened symbols. An arrow indicates the proband.



Clinical examination showed that none of these patients has any other associated bone and joint signs, visual dysfunction or diabetes mellitus. All patients complained of pain in the affected hip with limited range of motion and walking distance, and the first symptoms of hip pain were self-reported at ages ranging from 1 to 13 years old (Table 1).

Table 1: Summarization of clinical manifestations of the affected family members.

Patients	Age	Gender	Height	Dwarfism	Myopia	Hearing	Spinal	Skeletal		
-	(years)	-	(cm)	-	-	loose	changes	ONFH	HD	Other abnormal
II:1	59	F	156	N	N	N	N	Y	N	N
II:3	57	F	157	N	N	N	N	Y	N	N
II:5	53	M	163	N	N	N	N	Y	Y	N
III:1	34	F	160	N	N	N	N	Y	N	N
III:3	32	F	158	N	N	N	N	Y	N	N
III:5	30	M	164	N	N	N	N	Y	N	N
III:7	36	F	160	N	N	N	N	-	N	N
III:9	31	F	159	N	N	N	N	Y	N	N
III:11	29	M	165	N	N	N	N	Y	N	N
III:13	26	F	161	N	N	N	N	-	Y	N
III:15	13	M	159	N	N	N	N	Y	Y	N
IV:1	9	M	138	-	N	N	N	-	-	-
IV:2	12	F	145	N	N	N	N	-	-	N
IV:3	3	M	89	-	N	N	N	Y	-	-
IV:4	14	F	153	N	N	N	N	-	-	N
IV:5	8	F	132	-	N	N	N	-	-	-
IV:6	4	M	97	-	N	N	N	-	-	-

Note: N=None; Y=Years; ONFH=Osteonecrosis of Femoral Head; HD=Hip Dysplasia

Whole exome Sequencing

There were ten members had ONFH in twenty-seven family members in four generations family. The ten family members of ONFH, 5 unaffected members of the studied family and 5 normal peoples as control were underwent whole exome sequencing for mutation detection. The DNA samples were extracted from peripheral blood

leukocytes using QIAamp Blood Mini DNA kit (Qiagen, Santa Clara, CA, USA). Before analysis of the samples, DNA aliquots were re-precipitated to remove proteins and fragments. A whole exome–enriched library was prepared from 3 µg of genomic DNA from the proband (II:6) using Agilent's SureSelect Human All Exon 50 Mb solution-based capture reagent. Exome capture was performed according to the manufacturer's protocol (Agilent, USA). The captured DNA was then sequenced using the Illumina HiSeq2000 platform. Raw image files were processed by Illumina Basecaller Software 1.7 (San Diego, CA, USA) for base calling with default parameters.

Variant annotation

The obtained sequence reads were aligned to the human genome (hg19) using the SOAP2 and BWA tools for Single Nucleotide Polymorphism (SNP) and insertion/deletion (indel), respectively. The percentages of read alignment to both the reference genome and the targeted exome were calculated using Perl scripts. Similarly, Perl scripts were used for the detection of mismatch frequencies and error positions. SNP calling was done with SOAPsnp, and indels were identified through the alignment result with GATK. Detailed annotation information was obtained from dbSNP, CCDS, UCSC Genome Browser, Ensembl, and Encode databases. Using these annotations, we screened the novel and likely deleterious variants for further study.

Sanger sequencing

Sanger sequencing was then conducted for mutation verification and prevalence test in 10 unrelated controls using a previously defined protocol. Specific primers were designed for the target region, and the PCR products were sequenced on an ABI 3730 DNA analyzer following standard procedures (Life Technologies, USA). The sequence reads were analyzed using the Sequencher software package (GeneCodes Inc, USA). The sequencing traces were visually inspected in Finch TV v1.4 (Geospiza Inc, USA).

Bioinformatics analysis

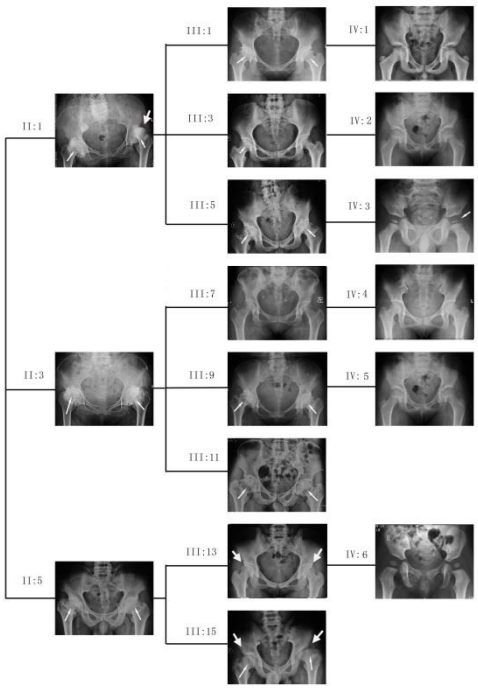
Microsatellite markers were selected based on ABI PRISM Linkage Mapping Set and the UCSC database. PCR products were electrophoresed on a 96-capillary automated DNA sequencer (MegaBACE 1000, Amersham, Germany) and were analyzed with Genetic Profiler software (version 1.5. Two-point LOD scores were calculated using MLINK from the LINKAGE package. Autosomal dominant inheritance, disease-gene frequency of 0.0001, and 95% penetrance were assumed. Haplotyping was constructed using Cyrillic.

RESULTS

All patients were contacted for clinical and radiological follow-up and had standard AP pelvic and a lateral hip radiographs. Skeletal radiographs including full spine, double hip, and full length of distal laxity x-ray film were reviewed, and no other abnormal skeletal disorders could be found. Overall, the abnormalities noted in the affected family members included nine patients had osteonecrosis of the femoral heads, one had ONFH combine hip dysplasia, and one had hip dysplasia roofs in Figure 2.

The average exome sequencing generated 7.42 Gb sequence data per sample as 90-bp paired-end reads. After quality control, 92.8% of sequence data was aligned to the UCSC human reference genome (build 37.1) and 62.6% of sequence data was mapped to target regions. The distribution of per-base sequencing depth in target regions approximated a Poisson distribution, which showed that the captured exome region was evenly sampled.

Figure 2: The Pedigrees anteroposterior hip radiograph of patients with LCPD, who had osteonecrosis of femoral head (small white arrow) and hip dysplasia (big white arrow).



Mean depth per base within the target regions was 111.85-fold, and 97.7% of these regions were covered by four or more reads (94.5% by 10 or more reads) by paired-end sequencing. A total of 1934 genetic variations, including non-synonymous mutations, splice site variations, and indels, were identified from the proband shown in Table 2.

Table 2: Variations identified by whole exome sequencing.

Mutation type		Number
SNP analysis	Missense	1382
-	Nonsense	53
-	Splice site	215
-	Readthrough	8
Indel analysis	indel	39
-	Splice site	42
-	Frameshift	53
-	5-UTR	89
-	3-UTR	46
-	Promoter	7
Total		1934

Because numerous mutations were detected, we combined whole exome sequencing and linkage analysis to sift through the potential causative mutations. According to the manifestation of ONFH of the femoral heads, most genetic skeletal disorders could be ruled out, sequence analysis of the candidate gene was subsequently carried out to further validate the underlying disorder of this family. A mutation of COL2A1, a known gene causing congenital skeletal disorders, was included in these mutations. In this Chinese family affected by LCPD, we identify heterozygous mutation (c.3301 G>A, p. Gly1101Ser) in exon 49 of COL2A1 in the Gly–X–Y domain, mutation (c.3508 G>A, p. Gly1170Ser) in exon 50, mutation (c.4006 G>A, p. Gly1336Ser) in exon 52, and mutation (c.4213 G>A, p. Gly1405Ser) in exon 53. The mutation (c.3508 G>A, p. Gly1170Ser) in exon 50 of COL2A1 in the Gly–X–Y domain was present in 10 patients but absent in 5 unaffected members of the studied family and in 5 control chromosomes from unaffected individuals of matched geographical ancestry. The COL2A1 gene mutation was further validated by Sanger sequencing, confirmed that were heterozygous for the mutation. Then, we identified the p.Gly1170Ser mutation in exon 50 of COL2A1 in a Chinese family with LCPD shown in Table 3.

Table 3: The mutation COL2A1 in the Chinese family with LCPD.

Patients	Age	Gender	Height	Skeletal		Mutation COL2A1			
-	(years)	-	(cm)	ONFH	HD	exon49	exon50	exon52	exon53
II:1	58	F	156	Y	N	Y	Y	N	N
II:3	56	F	157	Y	N	N	Y	Y	N
II:5	50	M	163	Y	Y	N	Y	N	N
III:1	34	F	160	Y	N	Y	Y	N	N
III:3	32	F	158	Y	N	N	Y	N	Y
III:5	30	M	164	Y	N	N	Y	Y	N
III:9	31	F	159	Y	N	N	Y	N	Y
III:11	29	M	165	Y	N	Y	Y	N	N
III:15	13	F	143	Y	Y	N	Y	Y	N
IV:5	3	M	85	Y	-	N	Y	N	-

Note: N=None; Y=Yes; ONFH=Osteonecrosis of femoral head; HP=Hip dysplasia

DISCUSSION

Genetic association studies suggest that mutations involved in cartilage constitution, bone metabolism, detoxification, coagulation system or vascular endothelium function might participate in the aetiology of osteonecrosis [19-22]. Based on an initial screening of exome sequencing and the subsequent confirmation of Sanger sequencing of this study, pathogenic mutations were identified in COL2A1 in a Chinese family with LCPD. The COL2A1 gene is localized at 12q13.11-q13.2 and about 30 kb in length with 54 exons, which provides a key element for the production of type II collagen [23,24]. Three a1 chains encoded by the COL2A1 gene are folded together in a triple-helical configuration to form the procollagen homotrimer [25,26]. The triple helical domain, containing about 300 amino acids, exists in the limited space of the procollagen, and is restricted so that every three amino acids is a glycine, generating a repeating (Gly–X–Y)n sequence pattern. If the Gly in this specific region is replaced by other amino acids, the structure of type II collagen will be destroyed [27,28]. Our findings indicate that the osteonecrosis of femoral head in a Chinese family with LCPD showed an inheritance pattern.

COL2A1 gene encodes a homotrimer of three identical a-chains that composes type II collagen. Mutations in COL2A1 gene can lead to a wide spectrum of skeletal disorders designated as type II collagenopathies. Recent

studies showed that the genetic predisposition to osteonecrosis is well documented in COL2A1 [29,30].Study showed alert clinicians to the possibility that children who present with bilateral Perthes-like disease of the hip might have an underlying mutation in the gene encoding type II collagen. Have located a missense mutation (p.G1170S) in the type II collagen gene (COL2A1) in a Japanese family with an autosomal dominant hip disorder manifesting as LCPD and showing considerable intra-familial phenotypic variation.

LCPD may result from a variety of mutations in the COL2A1 gene, most of which are located within the triple-helical domain of the protein [31-33]. It has been postulated that mutations in the triple-helical domain of procollagen a chains can delay the folding of the protein into the triple-helical conformation, thus leading to posttranslational over modification of the protein [34,35]. In this study, all affected individuals were found to carry p.Gly1170Ser mutation in exon 50 of COL2A1 gene, which resulted in a change from glycine to serine at codon 1170 (Gly1170Ser). A Gly1170Ser mutation potentially interrupts the mandatory Gly-X-Y triplet sequence required for the normal formation of stable triple-helical type II collagen molecules, which could further lead to the degradation of premature collagen molecules, or to the production of overmodified type II collagen [36,37]. Earlier molecular analysis has demonstrated that specific sites located within a triple-helical region of the collagen molecule serve as important binding sites of telopeptides in the early stages of fibril formation.

Variation in the phenotypic expression of different individuals suggests that some other genetic and environmental effects may play a role in the final clinical expression [38-40]. To date, the knowledge on the development of LCPD in humans is limited because of the vast number of mutations found in the COL2A1 gene and the variability in the clinical phenotype [41,42]. It is apparent that the process in which mutations in collagens alter connective tissues is complicated and cannot be described by one single pathway. In the future study, this pedigree can be extended by including the distal relatives of the family, which we believe can probably shed some light into the delineation of the pathological phenotype heterogeneity of this disease.

CONCLUSION

This study maps the mutation of mutation (c.3508 G>A, p.Gly1170Ser) in exon 50 of COL2A1 in the Gly–X–Y domain in a Chinese family of LCPD, which causes osteonecrosis of femoral head. Reported that the p.Gly1170Ser mutation of COL2A1 in the family described is responsible for pathology confined to the hip joint. As the previous study, the p.Gly1170Ser mutation in exon 50 of COL2A1 was identified in this study. However, this study has a large Chinese family of femoral head osteonecrosis compare to the previous reports

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

This research was funded by Science and Technology Department of Guangdong Province (2014A020212041) and Shenzhen science and Technology Innovation Committee (JCYJ20140411091151442).

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: Denglu Yan, Performed the experiments: Zhaojie Wang, Analyzed the data and wrote the paper: Zhi Zhang.

ACKNOWLEDGEMENTS

We sincerely appreciated the genial help from all scholars who contributed to our research and the support from Science and Technology Department of Guangdong Province (2014A020212041) and Shenzhen science and Technology Innovation Committee (JCYJ20140411091151442).

REFERENCES

1. Glueck CJ, et al. Resistance to activated protein C and Legg-Perthes disease. Clin Orthop Relat Res. 1997:139-152.
2. Gruppo R, et al. Legg-Perthes disease in three siblings, two heterozygous and one homozygous for the factor V Leiden mutation. J Pediatr. 1998;132:885-888.
3. Arruda VR, et al. Inherited risk factors for thrombophilia among children with Legg-Calve-Perthes disease. J Pediatr Orthop. 1999;19:84-87.
4. Szepesi K, et al. The most severe forms of Perthes' disease associated with the homozygous Factor V Leiden mutation. J Bone Joint Surg Br. 2004;86:426-429.
5. Glueck CJ, et al. Legg-Calve-Perthes disease, venous and arterial thrombi, and the factor V Leiden mutation in a four-generation kindred. J Pediatr Orthop. 2007;27:834-837.
6. Sirvent N, et al. Absence of congenital prethrombotic disorders in children with Legg-Perthes disease. J Pediatr Orthop B. 2000;9:24-27.
7. Lopez-Franco M, et al. Legg-Perthes disease and heritable thrombophilia. J Pediatr Orthop. 2005;25:456-459.
8. Vosmaer A, et al. Coagulation abnormalities in Legg-Calve-Perthes disease. J Bone Joint Surg Am. 2010; 92:121-128.
9. Schmitz A, et al. Incidence of thrombophilic factor V Leiden and prothrombin G20210A mutation in Perthes disease--A pilot study. Z Orthop Ihre Grenzgeb. 2001;139:143-146.
10. Kenet G, et al. Perthes' disease and the search for genetic associations: Collagen mutations, Gaucher's disease and thrombophilia. J Bone Joint Surg Br. 2008;90:1507-1511.
11. Royle NJ, et al. Human genes encoding prothrombin and ceruloplasmin map to 11p11-q12 and 3q21-24, respectively. Somat Cell Mol Genet. 1987;13:285-292.
12. Hayek S, et al. Does thrombophilia play an aetiological role in Legg-Calve-Perthes disease? J Bone Joint Surg Br. 1999;81:686-690.
13. Balasa VV, et al. Legg-Calve-Perthes disease and thrombophilia. J Bone Joint Surg Am. 2004;86:2642-2647.
14. Miyamoto Y, et al. A recurrent mutation in type II collagen gene causes Legg-Calve-Perthes disease in a Japanese family. Hum Genet. 2007;121:625-629.
15. Su P, et al. Age at onset-dependent presentations of premature hip osteoarthritis, avascular necrosis of the femoral head, or Legg-Calve-Perthes disease in a single family, consequent upon a p.Gly1170Ser mutation of COL2A1. Arthritis Rheum. 2008;58:1701-1706.

16. Kannu P, et al. Two novel COL2A1 mutations associated with a Legg-Calve-Perthes disease-like presentation. Clin Orthop Relat Res. 2011;469:1785-1790.
17. Al-Omran AK. Legg-Calve-Perthes disease in two generations of male family members: A case report. J Orthop Surg. 2013;21:258-261.
18. Li N, et al. A novel p. Gly630Ser mutation of COL2A1 in a Chinese family with presentations of Legg-Calve-Perthes disease or avascular necrosis of the femoral head. PloS one. 2014;9:100505.
19. Sakamoto Y, et al. Screening of the COL2A1 mutation in idiopathic osteonecrosis of the femoral head. J Orthop Res. 2016;35:768-774.
20. Tham E, et al. Autosomal recessive mutations in the COL2A1 gene cause severe spondyloepiphyseal dysplasia. Clin Genet. 2015;87:496-498.
21. Hildebrand B. Mutation in the type II collagen gene (COL2A1) as a cause of early osteoarthritis and juvenile idiopathic arthritis-A pseudorheumatoid arthritis variant. Semin Arthritis Rheum. 2015;4:22.
22. Walter K, et al. COL2A1-related skeletal dysplasias with predominant metaphyseal involvement. Am J Med Genet A. 2007;143:161-167.
23. Unguryte A, et al. Human articular chondrocytes with higher aldehyde dehydrogenase activity have stronger expression of COL2A1 and SOX9. Osteoarthritis Cartilage. 2016;24:873-882.
24. Takagi M, et al. Whole exome sequencing identified a novel COL2A1 mutation that causes mild Spondylo-epiphyseal dysplasia mimicking autosomal dominant brachyolmia. Am J Med Genet A. 2016;170:795-798.
25. Snelgrove TA, et al. Association of COL1A2, COL2A1 and COL9A1 and primary osteoarthritis in a founder population. Clin Genet. 2005;67:359-360.
26. Zhang Z, et al. Identification of one novel mutation in the C-propeptide of COL2A1 in a Chinese family with spondyloperipheral dysplasia. Gene. 2013;522:107-110.
27. Lewis R, et al. Disruption of the developmentally-regulated Col2a1 pre-mRNA alternative splicing switch in a transgenic knock-in mouse model. Matrix biol. 2012;31:214-226.
28. McAlinden A, et al. Nuclear protein TIA-1 regulates COL2A1 alternative splicing and interacts with precursor mRNA and genomic DNA. JBC. 2007;282:24444-24454.
29. Heinrich T, et al. Co-Occurence of reciprocal translocation and COL2A1 mutation in a fetus with severe skeletal dysplasia: Implications for genetic counseling. Cytogenet Genome Res. 2015;145:25-28.
30. Kannu P, et al. Avascular necrosis of the femoral head due to a novel C propeptide mutation in COL2A1. Am J Med Genet A. 2011;155:1759-1762.
31. Dikaiakou E, et al. Identification of an autosomal dominant mutation in the COL2A1 gene leading to spondyloepiphyseal dysplasia congenita in a greek family. Mol Syndromol. 2019; 9:241-246.
32. Dasa V, et al. Exome sequencing reveals a novel COL2A1 mutation implicated in multiple epiphyseal dysplasia. Am J Med Genet A. 2019;179:534-541.
33. Sangsin A, et al. Short stature, platyspondyly, hip dysplasia, and retinal detachment, an atypical type II collagenopathy caused by a novel mutation in the C-propeptide region of COL2A1: A case report. BMC Med Genet. 2016;17:96.
34. Chen J, et al. Recurrent c.G1636A (p.G546S) mutation of COL2A1 in a Chinese family with skeletal dysplasia and different metaphyseal changes: A case report. BMC Pediatr. 2017;17:175.
35. Hufeland M, et al. Trichorhinophalangeal syndrome type I: A novel mutation and Perthes-like changes of the hip in a family with 4 cases over 3 generations. J Pediatr Orthop. 2015;35:1-5.

36. Xiong Q, et al. A novel *de novo* mutation in COL2A1 leading to spondyloepiphyseal dysplasia congenita in a Chinese family. Hum Genome Var. 2018;5:17059.
37. Reinartz S, et al. Germline mutation within COL2A1 associated with lethal chondrodysplasia in a polled Holstein family. BMC Genomics. 2017;18:762.
38. Almatrafi A, et al. A heterozygous mutation in the triple helical region of the alpha 1 (II) chain of the COL2A1 Protein causes non-lethal spondyloepiphyseal dysplasia congenita. Genet Test Mol Biomarkers. 2019;23:310-315.
39. Kusano C, et al. A novel mutation in the C-propeptide of COL2A1 causes atypical spondyloepiphyseal dysplasia congenita. Hum Genome Var. 2017;4:17003.
40. Terhal PA, et al. A study of the clinical and radiological features in a cohort of 93 patients with a COL2A1 mutation causing spondyloepiphyseal dysplasia congenita or a related phenotype. Am J Med Genet A. 2015;167:461-475.
41. Liu F, et al. COL2A1 mutation (c.3508G>A) leads to avascular necrosis of the femoral head in a Chinese family: A case report. Mol Med Rep. 2018;18:254-260.
42. Sakamoto Y, et al. Screening of the COL2A1 mutation in idiopathic osteonecrosis of the femoral head. J Orthop Res. 2017;35:768-774.