

A Novel Mutation of COL2A1 in a Large Chinese Family with Avascular Necrosis of the Femoral Head

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Abstract

Avascular necrosis of the femoral head (ANFH) is a debilitating bone disease, characterized by collapse of the femoral head and subsequent loss of hip joint function. Heterozygous mutations in COL2A1 have been identified to cause familial ANFH. Here we report on a large Chinese family with ANFH and a novel heterozygous mutation (c.3517 G > A, Gly1173Ser) in exon 50 of COL2A1 in the Gly-X-Y domain. Previously, only five different COL2A1 mutations have been described in patients with familial ANFH. Therefore, our findings provide significant clues to the phenotype-genotype relationships in familial ANFH and may be helpful in clinical diagnosis. Furthermore, these results should assist further studies of the mechanisms underlying collagen diseases.

1. Introduction

Type II collagenopathies represent a group of chondrodysplasias which are expressed as a continuous spectrum of phenotypes, ranging from perinatally lethal (Achondrogenesis II; OMIM 200610) to severe (Spondyloepiphyseal dysplasia congenita; OMIM 183900) to those with only mild arthropathy (Stickler dysplasia; OMIM 108300)[1–4]. The common molecular bases of the type II collagenopathies are heterozygous mutations in the type II collagen gene (COL2A1), which encodes the precursor of the type II collagen α 1 chain, the most abundant cartilage component[5].

Avascular necrosis of the femoral head (ANFH) is characterized by collapse of the femoral head and subsequent loss of hip joint function. Its clinical manifestations include progressive pain in the groin, pain on exertion, a limping gait, and a discrepancy in leg length. Most cases of ANFH are sporadic, and several etiologic factors (including trauma, alcohol, steroids) have been reported to be implicated[6, 7]. Besides, there are familial cases of ANFH, which may be related to genetic factors. Actually, Liu et al. identified that heterozygous mutations in COL2A1 caused familial ANFH[8]. Thus, familial ANFH belongs to type II collagenopathies and represents the mild end of spectrum.

However, only five different COL2A1 mutations have been described in patients with familial ANFH[9–13]. The genotype-phenotype relationship is still poorly understood. Therefore, the studies of more patients about the novel mutations in COL2A1 will be needed for further research to clarify the genotype-phenotype relationship. Here we identify one novel mutation in the COL2A1 gene that causes ANFH in a large Chinese family.

2. Materials And Methods

2.1. Human subjects

This study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

All the participants signed informed consent documents according to the Declaration of Helsinki before entering the study.

A large ANFH pedigree (Fig. 1) with a total of 19 subjects was recruited in the present study. The proband (IV9), a female diagnosed with ANFH and premature hip osteoarthritis, visited our hospital due to groin pain and restricted motion of the both hip joints, which started when the patient was 25 years old. A comprehensive survey was conducted to obtain detailed information of the patient's medical history, physical examination and laboratory examination. The X-ray and MRI revealed that collapsed femoral heads with cystic degeneration and premature hip osteoarthritis in both hips (Fig. 2A and B). The patient was treated with total hip arthroplasty (Fig. 2C). The X-ray of the patient's spine (Fig. 2D and E) was normal and facial features were unremarkable. There were no obvious abnormalities in the patient's neurological system or limbs.

A total of 10 family members were clinically diagnosed with ANFH. Another two affected family members (IV3 and IV7) also received total hip arthroplasty, and the pre-operation and post-operation radiographs were shown in Fig. 3A-D. The X-ray and MRI also revealed that collapsed femoral heads with cystic degeneration and premature hip osteoarthritis in both hips. There were no obvious abnormalities in the facial features, neurological system or limbs. None of the family members had a history of trauma, alcohol, steroid use or any other risk factors.

2.2. Mutation analysis

Informed consent was obtained from the family and from 250 healthy volunteers before blood sampling and DNA analysis.

The DNA was extracted from peripheral white blood cells using conventional methods. The DNA sequence for the COL2A1 gene was obtained from the available online database (GenBank accession No. NC_000012). Primers of the COL2A1 gene were designed using the Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). All exons and their exon-intron boundaries in the COL2A1 gene were amplified via polymerase chain reaction (PCR). Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1 (Applied Biosystems, Foster, CA, USA), and the sequencing was analyzed with an ABI Prism 3130 automated sequencer.

3. Results

We screened for the COL2A1 mutation in the proband using PCR followed by direct sequence analysis. The sequencing revealed a heterozygous 1 bp missense (c.3517G > A) in exon 50, which resulted in Gly1173Ser (Fig. 4A). It is non-conservative, affects evolutionarily highly conserved amino acids from fish to mammals (Fig. 4B). Exon and nucleotide numbering was based on RefSeq NM_001844.4, starting at the ATG translation initiation codon. This mutation was identified in all available patients, and was not found in either non-affected family members or 250 healthy volunteers, indicating that this mutation may associate with the disease.

4. Discussion

The present study identified a novel heterozygous c.3517G > A mutation (Gly1173Ser) in the COL2A1 gene in a large Chinese family. The main clinical characteristics of the affected patients include ANFH and premature hip osteoarthritis, which has been described by the previous studies[10, 11]. In recent decades, > 200 mutations have been identified in the COL2A1 gene, including single substitution, splice-site mutations, insertions and deletions[1]. COL2A1 mutations have been associated with various human disorders, which are collectively termed type II collagenopathies[14, 15].

Until now, only six different COL2A1 mutations have been identified in patients with ANFH. As Table 1 shows, five of the six mutations are glycine to serine substitutions in the Gly-X-Y triple-helix, and Gly1170Ser is the hot spot, which has been identified in four families with ANFH.

Table 1
COL2A1 mutations have been identified in patients with ANFH

Protein	cDNA	Region	Race	References
Gly582Ser	c.1744G >A	Gly-X-Y	Japanese	Kishiya et al. 2014
Gly630Ser	c.1888G >A	Gly-X-Y	Chinese	Li et al. 2014
Gly717Ser	c.2149G >A	Gly-X-Y	Chinese	Liu et al. 2005
Gly1170Ser	c.3508G >A	Gly-X-Y	Chinese	Liu et al. 2005; Su et al. 2008; Wang et al. 2014; Liu et al. 2018
Gly1173Ser	c.3517G >A	Gly-X-Y	Chinese	The present study
Thr1383Met	c.4148G >A	C- propeptide	Unknown	Kannu et al. 2011

The variants shown are described using the NM_001844.4 transcript reference sequence.

Table 2
Characteristics of the family members

	Sex	Age	Disease condition	Age of onset	Height	Genotype
III1	F	73	Not affected	/	166	G/G
IV1	M	49	affected	36	161	G/A
IV2	M	51	affected	30	160	G/A
IV3	M	48	affected	25	163	G/A
IV4	F	58	Not affected	/	156	G/G
IV5	F	52	affected	40	155	G/A
IV6	M	49	Not affected	/	180	G/G
IV7	M	62	affected	40	163	G/A
IV8	M	53	Not affected	/	165	G/G
IV9	F	51	affected	38	157	G/A
IV10	F	49	affected	20	155	G/A
IV11	M	52	affected	40	158	G/A
IV12	M	50	affected	19	160	G/A
IV13	M	50	Not affected	/	172	G/G
IV14	F	48	Not affected	/	165	G/G
V1	F	25	Not affected	/	167	G/G
V2	F	35	affected	26	156	G/A
V3	F	34	Not affected	/	159	G/G
V4	M	27	Not affected	/	165	G/G

The nature of the mutations and their localizations in the protein seem to explain the phenotypic differences, at least to a certain extent[15]. Truncating mutations leading to reduced amounts of normal type II collagen are related with mild phenotypes. In contrast, missense mutations, which replace one Gly residue in the Gly-X-Y repeating pattern, are usually related with severe phenotypes. The Gly-X-Y triple-helix motif is crucial for the proper crosslinking of the pro- α 1 peptide chain to form functional type II collagen. Mortier et al. reported that there are numerous excessive post-translational modifications in type II collagen in patients carrying a Gly-substituted mutation[16].

The exception is glycine to serine substitutions. Glycine to serine substitutions, unlike glycine to nonserine residue substitutions, produced variable phenotypes, with both inter- and intra-familial

phenotypic variation[17, 18]. In type I collagenopathies, the severity of the disease has been correlated with the size and charge of the substituted amino acid, specifically Ala < Ser < Cys < Arg < Glu < Asp < Val, in order from least to most disruptive[19]. The same domain-specific effect may exist in type II collagenopathies. Sobetzko et al. identified c.3517G > C mutation leading to Gly1173Arg in COL2A1 in a boy affected with a severe form of spondyloepiphyseal dysplasia[20]. The mutation position is exactly the same with the present study. However, glycine to arginine substitutions usually causes severe phenotypes.

Although most mutations associated with ANFH are glycine to serine substitutions in the Gly-X-Y triple-helix, there is one exception: c.4148G > A (Thr1383Met) in the C-propeptide of COL2A1 gene[12]. C-propeptide mutations typically cause spondyloperipheral dysplasia, characterized by vertebral body abnormalities, hip dysplasia and brachydactyly type E. Therefore, more cases need to be described and more mutations needs to be identified, to clarify the genotype-phenotype relationship.

In summary, we identified a novel heterozygous c.3517G > A mutation (Gly1173Ser) in the Gly-X-Y triple-helix motif of COL2A1 in a large Chinese family with ANFH. Our findings will provide clues to the phenotype-genotype relations and may assist not only in the clinical diagnosis of familial ANFH but also in the interpretation of genetic information used for prenatal diagnosis and genetic counseling.

Abbreviations

ANFH avascular necrosis of the femoral head

COL2A1 collagen type II alpha 1 chain

SEDC spondyloepiphyseal dysplasia congenital

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All the participants signed informed consent documents according to the Declaration of Helsinki before entering the study.

Consent for publication

All the authors consent for publication.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qi Wang designed the study. Zeng Zhang and Kechao Zhu contributed equally to this study. Zeng Zhang analyzed the data and wrote the main manuscript text. Kechao Zhu prepared figures 1-4. Kechao Zhu, Huiyong Dai, Changqing Zhang, Zhenlin Zhang and Qi Wang helped revise the manuscript. All the authors read and approved the final manuscript.

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Not applicable.

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Figures

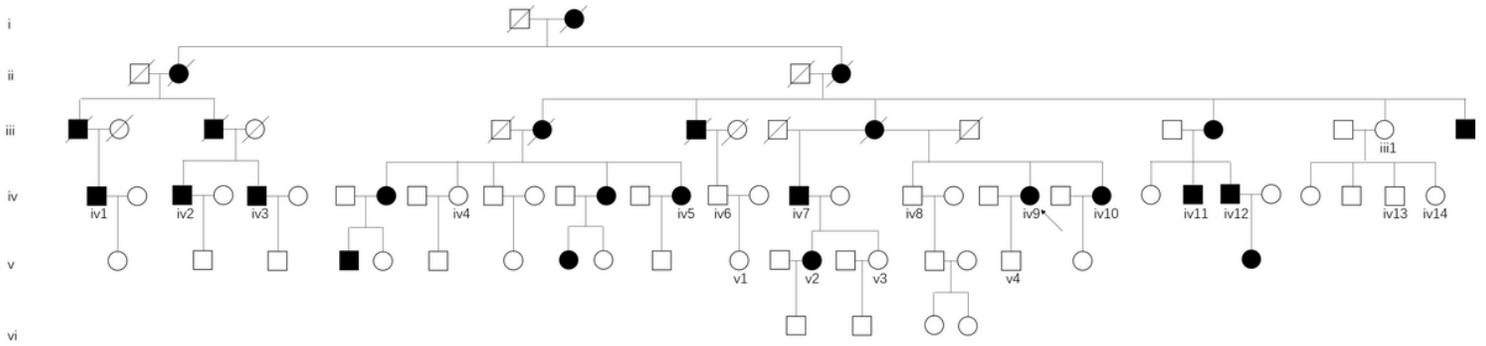


Figure 1

Pedigree of the family. Black symbols indicate affected individuals, and open symbols unaffected individuals.

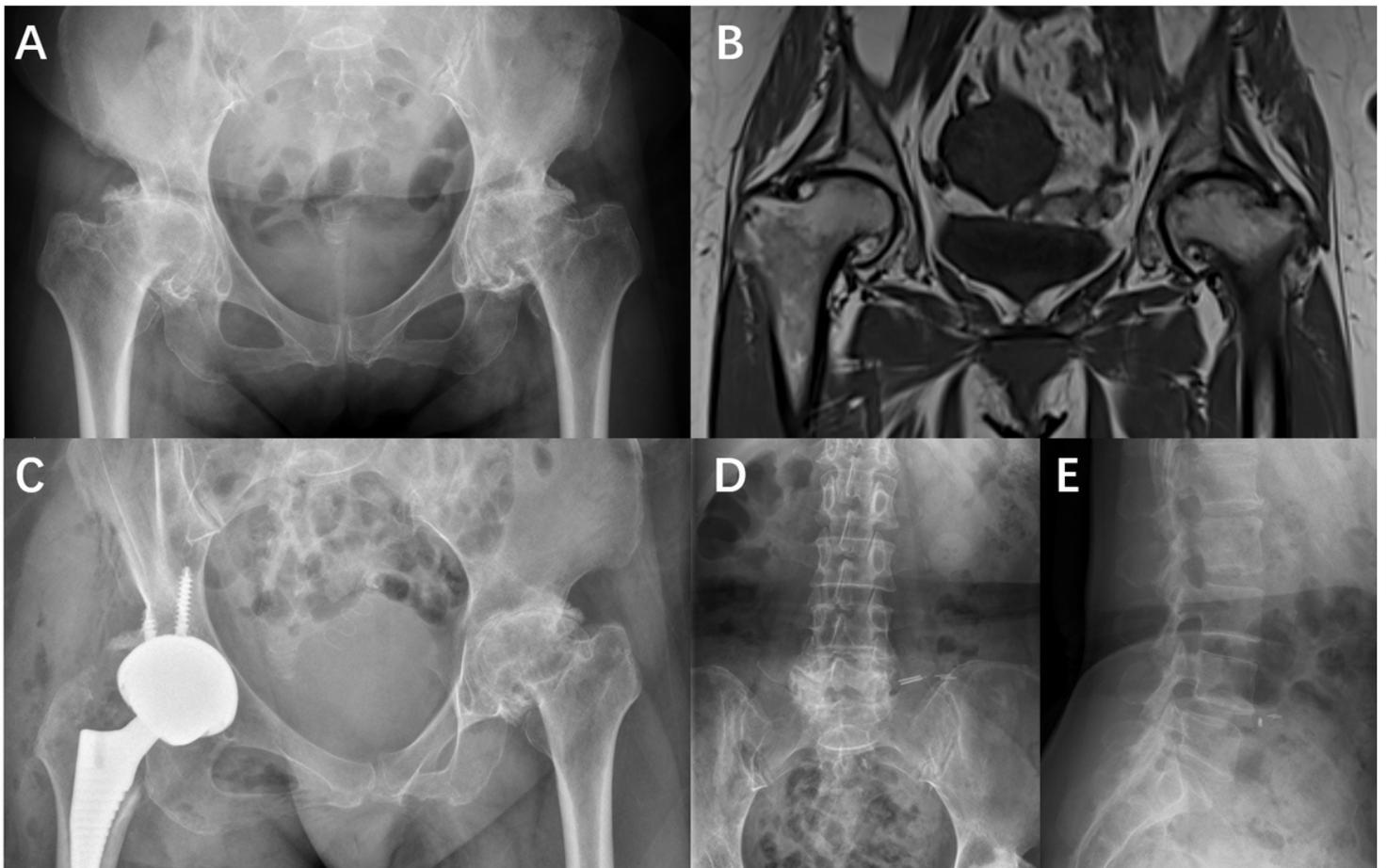


Figure 2

A and B: The X-ray and MRI revealed that collapsed femoral heads with cystic degeneration, and premature hip osteoarthritis in both hips of the proband (IV9). C: post-operation radiograph. D and E: The X-ray of the patient's spine indicated normal vertebrae.

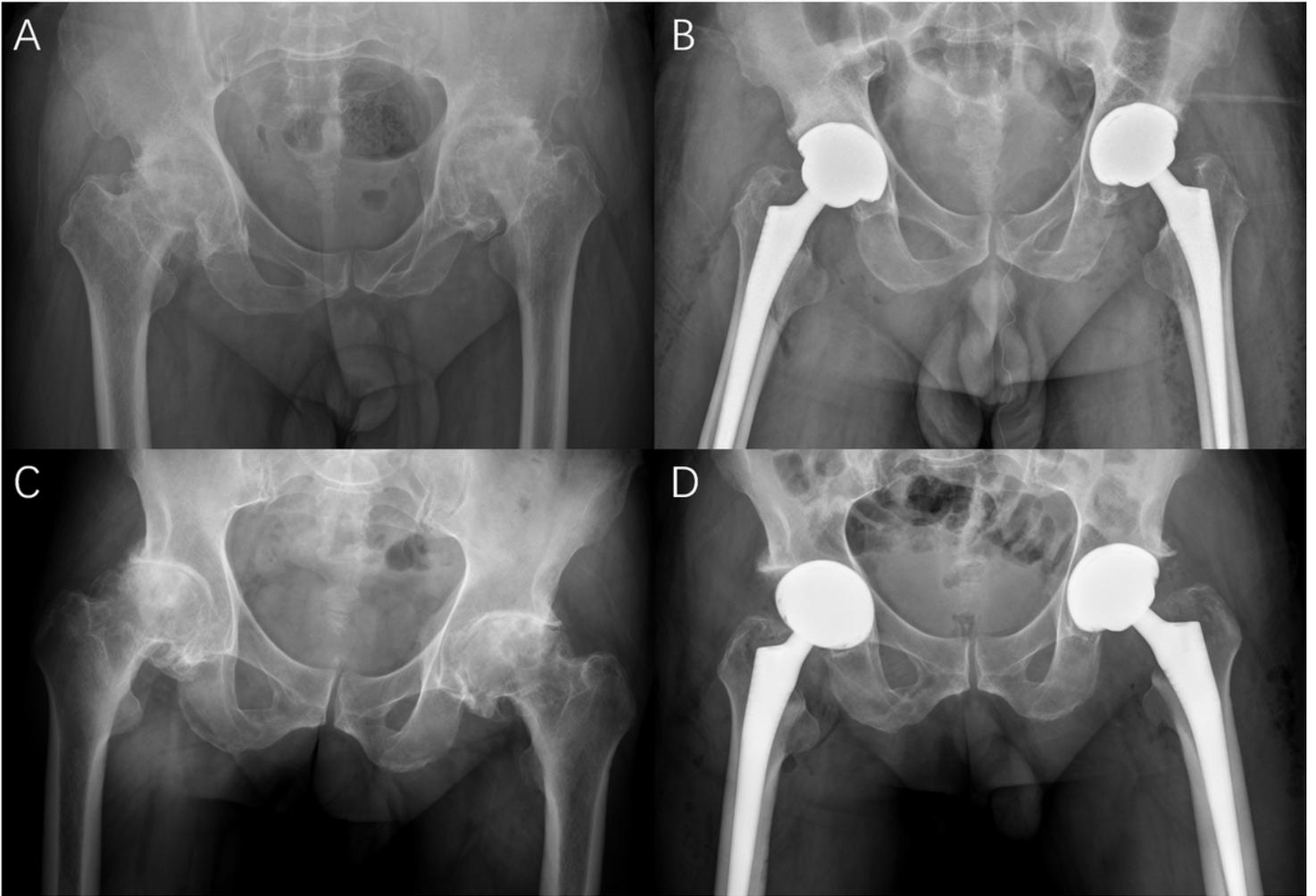


Figure 3

A and B: the pre-operation and post-operation radiographs of IV3. C and D: the pre-operation and post-operation radiographs of IV7.

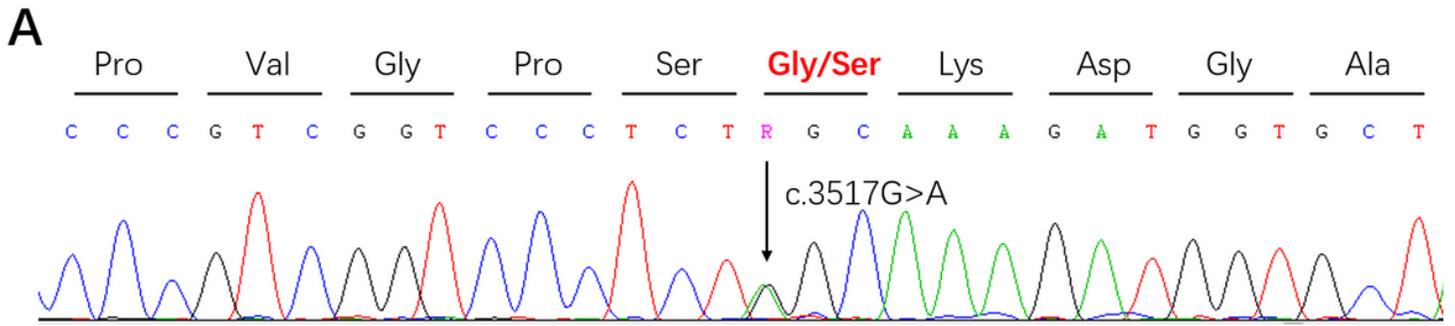


Figure 4

A: The sequencing revealed a heterozygous 1 bp missense (c.3517G>A) in exon 50, which resulted in Gly1173Ser. B: It is non-conservative, affects evolutionarily highly conserved amino acids from fish to mammals.