Original Article

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Heterozygous mutation of c.3521C>T in *COL1A1* may cause mild osteogenesis imperfecta/Ehlers-Danlos syndrome in a Chinese family

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Summary **Osteogenesis imperfecta (OI) is an inheritable connective tissue disorder with a broad clinical heterozygosis, which can be complicated by other connective tissue disorders like Ehlers-Danlos syndrome (EDS). OI/EDS are rarely documented. Most OI/EDS mutations are located in the N-anchor region of type I procollagen and predominated by glycine substitution. We identified a c.3521C>T (p.A1174V) heterozygous mutation in *COL1A1* gene in a four-generation pedigree with proposed mild OI/EDS phenotype. The affected individuals had blue sclera and dentinogenesis imperfecta (DI) was uniformly absent. The OI phenotype varied from mild to moderate, with the absence of scoliosis and increased skin extensibility. Easy bruising, joint dislocations and high Beighton score were present in some affected individuals. EDS phenotype is either mild or unremarkable in some individuals. The mutation is poorly conserved and *in silico* prediction support the relatively mild phenotype. The molecular mechanisms of the mutation that leads to the possible OI/EDS phenotype should be further identified by biochemical analysis of N-propeptide processing and steady state collagen analysis.**

Keywords: Osteogenesis imperfecta, Ehlers-Danlos syndrome, type I collagen, mutations, *in silico* prediction

1. Introduction

Mutations in the *COL1A1* or *COL1A2* genes that encode proα1 and proα2 chains of type I procollagen have been shown to be the main cause of osteogenesis imperfecta, a genetic connective tissue disorder characterized by brittleness of bones, blue sclerae, impediments of teeth, hearing and sight (*1,2*). *COL1A1* and *COL1A2* genes also lead to the arthrochalasis type of Ehlers-Danlos syndrome (EDS type VIIA and B). The clinical spectrum of OI is broad, ranging from mild, moderate, severe to lethal forms (*1*).

More than 1000 mutations in the *COL1A1* and *COL1A2* genes have been reported in OI mutation database and the largest majority of these mutations exist in the triple helix domain of procollagen type I genes (*3,4*). Glycine substitution is the most common mutation in the OI and glycine to serine substitution is the predominate one.

In comparison with mutations identified in OI, only

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a limited number of mutations have been reported in EDS (*3,4*). The arthrochalasis type of EDS is caused by mutations leading to skipping of exon 6 in type I procollagen, resulting in the removal of the cleavage site for N-proteinase (*5,6*). The combination of OI and EDS is very rare and only 26 cases have been recorded worldwidely (*3,4*). Most OI/EDS mutations are located at the N-terminal (exon 6 to 11) of the type I procollagen genes (*5,7-11*). C-propeptide mutation of c.3790A>G (p.M1264V) of *COL1A1* was recorded once (*12*). Arginine to cysteine substitution at position 1036 and 1066 in the helix exon 44 was also reported (*13,14*).

We describe here a Chinese pedigree from four generations with a tendency of mild OI/EDS phenotype, in which the symptoms of OI and EDS are both mild. Heterozygous mutation of c.3521C>T (A1174V) in *COL1A1* was identified and this mutation was once reported in one type III Chinese OI patient (*15*).

2. Materials and Methods

2.1. *Clinical findings of the probands and family members*

The study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Ethical Committee of Shandong Academy of Medical Sciences. Informed Consent was obtained from the proband who had OI family history (Figure 1). Clinical phenotypes and bone mineral density were described in Table 1.

2.2. *Identification of type I collagen gene mutations*

Genomic blood DNA was extracted using the E.Z.N.A.® Blood DNA Kit (Omega Bio-Tek, Georgia, USA). PCR reactions were performed according to previously described and PCR products were submitted for Sanger sequencing. Genetic variations were evaluated by both Mutation Surveyor 4.0 software (SoftGenetics LLC, Pennsylvania, USA) and human collagen mutation database (*http://www.le.ac.uk/ genetics/collagen*).

2.3. *In silico prediction of mutation effects and alignment analysis*

Polyphen (*http://genetics.bwh.harvard.edu/pph*) (*16*), Align GVGD (*http://agvgd.iarc.fr/agvgd_input.php*) (*17*) and SIFT human coding snps (*http://sift.jcvi.org/www/sift_chr_coords_submit.html*) (*18*) softwares were adopted to predict the mutation effects on function. ClustX2 were used for the conservation analysis.

3. Results

3.1. *Clinical descriptions*

The pedigree was a four-generation family with 46 family members, including seven affected males, four affected females and three dead fetuses. Spontaneous abortion occurred at 10th week of the pregnancy for the first fetus. Medical abortion was performed at week of 23 and 22 respectively for limb abnormality observed by 4D color Doppler ultrasound. Gracile ribs and a narrow chest apex were observed in the third dead fetus. A bilateral asymmetry in shortened and bent lower limbs was obvious (Figure 2). The length of bilateral femur was 27.4 mm and 32.3 mm respectively. The size of biparietal diameter (BPD), head circumference, and abdominal circumference, cerebellar diameter, posterior fossa pool and transparent compartment was 51 mm, 195 mm, 175 mm, 23.6 mm, 6.7 mm and 4.8 mm respectively when tested by 4D color Doppler ultrasound of the third fetus at 21 weeks and 5 days.

At the time of delivery, the proband was 52 cm tall and weighed 3.9 kg. No limb malformation was observed despite frequent fractures occurring before the age of twelve. The proband also had some clinical symptoms resembling a mild form of EDS, including easy bruising, smooth skin and joint laxity. Easy bruising and joint dislocations were also observed in the affected family member (PIII-5). Though there's no fracture history, family member PIII-3 had low bone mineral density (BMD) (Table 1) and he complained of tooth pain when eating hard food and had an ankle sprain history.

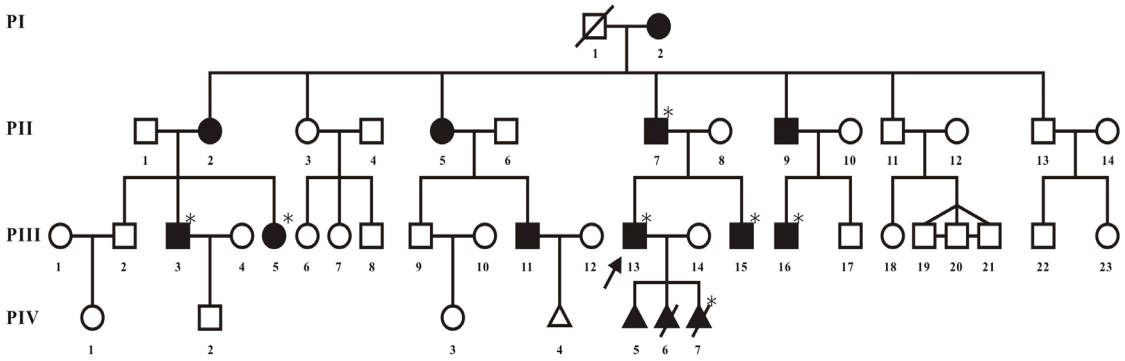

Figure 1. Pedigree map for the paiteint. "*" noted the patients tested by type I collagen gene analysis.

Table 1. Clinical characteristics of the proband and his affected family members

Items	PIII-3	PIII-5	PIII-13	PIII-15	PIII-16	PIV-7
Age (years) gender	27/M	23/F	26/M	22/M	23/M	21weeks/F
Height (cm)	170	148	173	169	155	28
Weight (kg)	75	42	80	57	65	0.463
Blue sclerae	slight	*+	+	+	+	N.A
Dentinogenesis imperfecta	**-	-	+	-	-	N.A
Spine deformities	-	-	-	-	-	-
Chest deformities	-	+	+	-	+	-
Limb deformities	-	+	-	-	+	+
Age (years) at first fracture	-	4 mon.	2 mon.	-	2 y	N.A
Number of fractures	0	5	10	1	3	N.A
Joint laxity	***N.A	+	+	+	+	N.A
Skin	normal	normal	Smooth, velvety skin	Smooth, velvety skin	N.A	N.A
Easy bruising	-	+	+	N.A	N.A	N.A
Joint dislocation	-	patella and finger	Shoulder, finger	N.A	left elbow joint, shoulder	N.A
Beighton score	2	3	5	5	0	N.A
other	Slight ptosis, flatfoot, have history of ankle sprain	Have history of ankle sprain	Congenital cataracts, thin corneal	-	Pinched nose, ptosis	-
DEXA z-score (L1-L4/Hip)	-1.6/-1.5	-2.5/-1.4	N.A	N.A	N.A	N.A

*+, presence of trait ; **-, absence of trait; ***N.A, not available

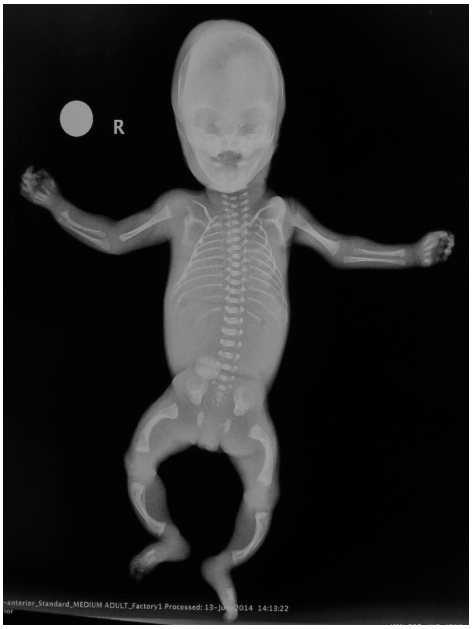

Figure 2. Radiographic examination of the patient PIV-7 at 22 weeks.

The typical clinical symptoms of the pedigree are shown in Table 1, the phenotypes of affected family members vary from mild to moderate clinical severity. All affected members had blue sclera, with nearly normal dentition. No affected members of the pedigree have scoliosis. Skin extensibility, atrophic scars and increased transparency were unremarkable. In some affected family members, fractures and joint dislocations were noticed especially at early ages and became less frequent with age. High Beighton score existed in both the proband and his brother, but was not obvious in all other patients. Upper eyelid drooping was observed in two patients.

3.2. *Molecular analysis of COL1A1 and COL1A2 genes*

Heterozygous c.3521C>T mutation resulting in alanine to valine substitution at position 1174, was identified in all tested affected family members, but not healthy family members (Figure 3A).

3.3. *In silico prediction of mutation effects and alignment analysis*

Align GVGD predicted the high Class values of C65, which indicated that the mutation has a pathogenic effect on protein function. Polyphen and SIFT software predicted a benign and tolerated effect of the mutation. Multiple sequence alignment discovered the relative poorly conserved variation of residue 1174 of pro-α1(I) in different organisms and different types of collagen (Figure 3B).

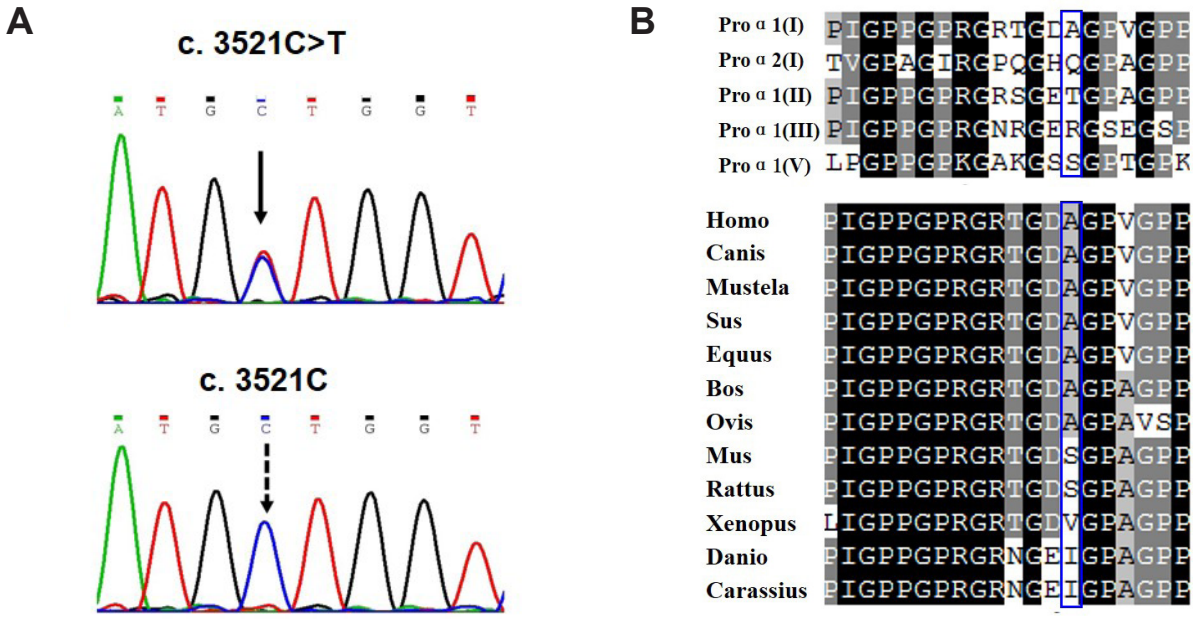

Figure 3. Molecular anlysis for type I collagen genes. **(A)** Heterozygous mutation of c.3521C>T was found in all tested affected patients, but not in the healthy family individuals **(B)** Alignment of the variant between different human fibrillar procollagen chains and different species.

4. Discussion

Substitution of alanine to valine at position 1174 was reported once in one Chinese type III OI patient (*15*). We identified this mutation in a second Chinese family with mild or moderate clinical phenotypes, which resembled OI type I and type IV. Besides the blue sclera and fracture history, joint dislocation, myopia, hypermobility value Beighton score and skin abnormities in the proband and his affected family members are highly suspected that the OI patient was complicated with Elhers-Danlos syndrome (EDS).

OI mutation severity is related with many factors, including location, mutation type and mutated residues. Heterozygous mutation of c.3521C>T (A1174V) located in exon 48 of *COL1A1* is near the lethal region (*2*). Substitution of glycine to serine/cystein at position 1175 and glycine to arginine or aspartic acid are related to severe OI type II or III clinical phenotypes(*3,4*). Residue 1174 is poorly conserved in different species and different types of collagen and it lies in the helix region of proα1(I), which binds interleukin 2 and amyloid precursor protein (*2*). *In silico* prediction of Align GVGD support that A1174V substitution is damaging while PolyPhen and SIFT predicted a benign and tolerated effect on protein.

Reduction of elastic modulus of tropocollagen is depicted as the severe OI (*19*). Alanine displayed a low value of Young's modulus for their softer tropocollagen mechanical properties than valine. Decrease of adhesion energy and increased equilibrium intermolecular spacing could describe the increase of severity of the OI mutation (*20*). All these values support that the severity of alanine to valine substitution is less severe than that of aspartate, glutamate and arginine.

OI/EDS patients are rarely reported and most of the mutations are located at the amino terminal N-anchor region of type I procollagen (*21*). The molecular mechanism of the mutation is related to the interference with N-propeptide processing and thus the defective procollagen and cross-linking are formed (*6,11*). M1264V substitution located at the proα1(I) of C-propeptide found in the OI/EDS patient was supposed to impede C-propeptide folding and chain association (*12,22,23*). R1066C non-glycine substitution near the C-terminal of type I procollagen helix region, could result in OI/EDS phenotype. The introduction of cysteine could cause helix kinking, resulting in the propagated register shift and delayed N-proteinase cleavage (*13*). This is the first time reporting alanine substitution at the end of the C-terminal procollagen helix region and it is unclear how this substitution leads to abnormal function of collagen I and OI/EDS phenotype still needs further study.

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