Original Article

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Complex heterozygous *WNT1* mutation in severe recessive osteogenesis imperfecta of a Chinese patient

Yanqin Lu[1,2,§], Yunzhang Dai[1,2,§], Yanzhou Wang[3], Naixiang Zhai[1,2], Jian Zhang[1,2], Junlong Liu[4], Xiaoli Yin[1,2],Tianyou Li[3], Xiuzhi Ren[4,], Jinxiang Han[1,2,**]**

[1] *Key Laboratory for Biotech-Drugs Ministry of Health, Key Laboratory for Rare & Uncommon Diseases of Shandong Province, Shandong Medicinal Biotechnology Centre, Shandong Academy of Medical Sciences, Ji'nan, China;*
[2] *School of Medicine and Life Sciences, University of Jinan-Shandong Academy of Medical Sciences, Ji'nan, China;*
[3] *Department of Paediatric Surgery, Shandong Provincial Hospital, Ji'nan, China;*
[4] *Department of Orthopaedic Surgery, The People's Hospital of Wuqing District, Tianjin, China.*

Summary **Osteogenesis imperfecta (OI) is a heritable connective tissue disorder with a predominately autosomal-dominant inheritance pattern. Recessive forms of OI are rare and involve many different causative genes. *WNT1* mutations were found to cause either autosomal-recessive OI or dominantly inherited early-onset osteoporosis. Here we describe a 32-year-old boy with severe osteopenia and deformity of the extremities. The relative long thumb and ring finger are obvious. We identified a novel combination of complex heterozygous *WNT1* mutation of c.397 A>T (p.Ala133Thr) and c.506dupG (p.Cys170Leufs*) in the proband, both parents and young brother were shown to be heterozygous asymptomatic carriers of the mutation. This is the eleventh family and the thirteenth patient we have ever found in China. Mutation of c.397 A>T (p.Ala133Thr) was found for the third time following our previous findings in two individual families with four patients in total, and may be a hotspot mutation in Chinese *WNT1*-related OI patients. *In silico* programs supported the damaging effects for both mutations. The three-D structure demonstrated the severely destroyed stability of WNT1. Serum levels of WNT1, LRP5, and β-catenin were decreased, while higher levels of GSK-3β were detected. The molecular mechanisms of the complex heterozygous mutations need further study.**

Keywords: Osteogenesis imperfecta, *WNT1* mutation, Wnt signaling pathway, *in silico* prediction, three-dimensional structure

1. Introduction

Osteogenesis imperfecta (OI) is a group of genetic connective tissue diseases characterized by the occurrence of frequent fractures and reduced bone mass. Blue or grey sclera and dentinogenesis imperfecta are also common (*1*). The primary inheritance pattern of OI is autosomal dominant and most causative mutations are in *COL1A1* and *COL1A2*, which encode collagen type I α chains (*2,3*). Recessively inherited forms of OI are rare and are caused by mutations in many different genes (*4,5*).

WNT1 was identified as a pathogenic gene for autosomal-recessive OI (*6-9*), or early-onset osteoporosis (*7,8,10*). The molecular pathogenesis of *WNT1*-induced OI differs from that of other recessive OI types. *In vitro* study showed that the *WNT1* mutant interferes with the *LPR5*-mediated β-catenin signaling pathway, although the exact mechanism is currently unclear (*7*). Meanwhile, decreased mTORC1-dependent osteoblast function due to loss of WNT1 signaling in osteocytes is part of the reason for WNT1-related OI and osteoporosis according to mouse studies (*11*). In addition, OI patients with *WNT1* mutations do

[§]These authors contributed equally to this work.
**Address correspondence to:*
Dr. Jinxiang Han, Shandong Medicinal Biotechnology Centre, Shandong Academy of Medical Sciences, 18877 Jingshi Road, Ji'nan 250062, China.
E-mail: samshjx@sina.com

Dr. Xiuzhi Ren, Department of Orthopaedic Surgery, The People's Hospital of Wuqing District, 100 Yogyang West Rd, Tianjin 301700, China.
E-mail: xiuzhiren@hotmail.com

not gain a substantial therapeutic effect using oral and intravenous bisphosphonate therapy, which is effective in OI patients with type I collagen mutations and anti-sclerostin antibody is a potential option for OI patient's treatment (*11*). Thus far, no more than 30 *WNT1* mutations are identified and listed in the OI mutation database (*6,7,9,12-18*). In this study, we present the genetic and functional changes of a patient with compound heterozygous mutation of *WNT1* gene in a 32-year-old Chinese boy with severe autosomal-recessive OI.

2. Materials and Methods

2.1. *Clinical phenotype and mutation detection of OI genes*

This study was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study was approved by the Ethical Committee of Shandong Medicinal Biotechnology Central and informed consent was obtained from the patient.

Peripheral blood was drawn from the patient and his parents, and genomic DNA was extracted using the E.Z.N.A.® Blood DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Initially, we sequenced only *COL1A1* and *COL1A2* genes (*19*), but later we sequenced all reported causative genes of recessive OI with the exception of *MBTPS2* and *PLS3*. A total of four PCR reactions were conducted to cover the entire genetic coding region and intron-exon boundaries of *WNT1*. PCR products were subjected to Sanger sequencing (Beijing Genomics Institute, Qingdao, China), and genetic variations were analyzed by Mutation Surveyor 4.0 software (SoftGenetics LLC, State College, PA, USA).

2.2. *Molecular and 3-D structural predication of the WNT1 mutation*

Predication of the mutational effect on protein function was performed using Polyphen (*20*), Align-GVGD (*21*), SIFT (*22*), and Mutation Taster (*23*) software. Alignment of 19 different human Wnt family members and *WNT1* from different animal species was conducted by the Clustal X2 program (*24*). The *WNT1* mutation was located on the N-terminal domain (PDB ID 4F0A) of the WNT1 protein by the PyMOL (Schrodinger LLC, Portland, OR, USA) program.

2.3. *Serum ELISA assay of Wnt signaling pathway molecules*

Serum ELISA assays for WNT1, LPR5, and β-catenin were performed according to the manufacturer's instructions (Beinglay Biotech Co. Ltd., Wuhan, China). Three age-matched healthy serums were included in the assay as controls for the patient (control 1) and patient's father (controls 2 and 3). Absorbance values were measured at 450 nm on a Synergy HT multiplate reader (Bio-tek, Winooski, VT, USA).

3. Results

3.1. *Clinical description of the proband*

The patient was born with normal height and weight at delivery. He was around 1 meter tall and weighed 26kg. He was born to non-consanguineous parents with no obvious phenotypic abnormalities. His sclerae were normal in color, vision, and intellectual function was normal. Teeth were sparse and progressive hearing loss was obvious after 28 years old. Pectus cranatum and scoliosis was obvious. Patient has a long face, with abnormal facial features including tip head, narrowing forehead, widening distance between the eyes and eyelashes and hypogynous ears. His head leans backward slightly. A relatively long thumb and ring finger was obvious (Figure 1A) His first left humerus fracture was documented over 1 year after birth. He then sustained recurrent bilateral femur fractures (n = 7-8), and angled malformation was obvious in upper and lower extremities. Lower extremities are in a frog position (Figure 1B). Most of his fractures occurred before 10 years old and no fracture has occurred after 13 years old, and this may partly due to long-term bed life. Cortical bone was extremely thin according to parents description, but X imaging was unavailable.

3.2. *Molecular and 3-D structural analysis*

The compound heterozygous mutation of c.397C>T (p.Ala133Thr) and c.506dupG (p.Cys170Leufs*) in the N-terminal α-helical domain (NTD) of *WNT1* were identified in the patient and was present in both parents, sibling has a heterozygous mutation (Figure 1C). SIFT and Mutation Taster programs predicted these two mutations to have a damaging effect, and class values of C55 and C65 were predicted by Align-GVGD in c.397C>T and c.506dupG, respectively. The Polyphen program predicted both mutations to have a probably damaging effect. Both alignments of different types of human Wnt family members (Figure 1D) and WNT1 among different species (Figure 1E) show high degrees of conservation.

Figure 2D shows the 3D structural model of WNT1. No obvious difference was observed between the wild-type and mutant with respect to p.Ala133Thr change (Figure 2A). Change of p.Cys170Leufs* induced the loss of C-terminal domain, which is responsible for Wnt binding and signaling transduction. Compared with the wild-type residues, a large amount of β-sheet structure was displaced by a loop region, with a loose and truncated structure (Figure 2B).

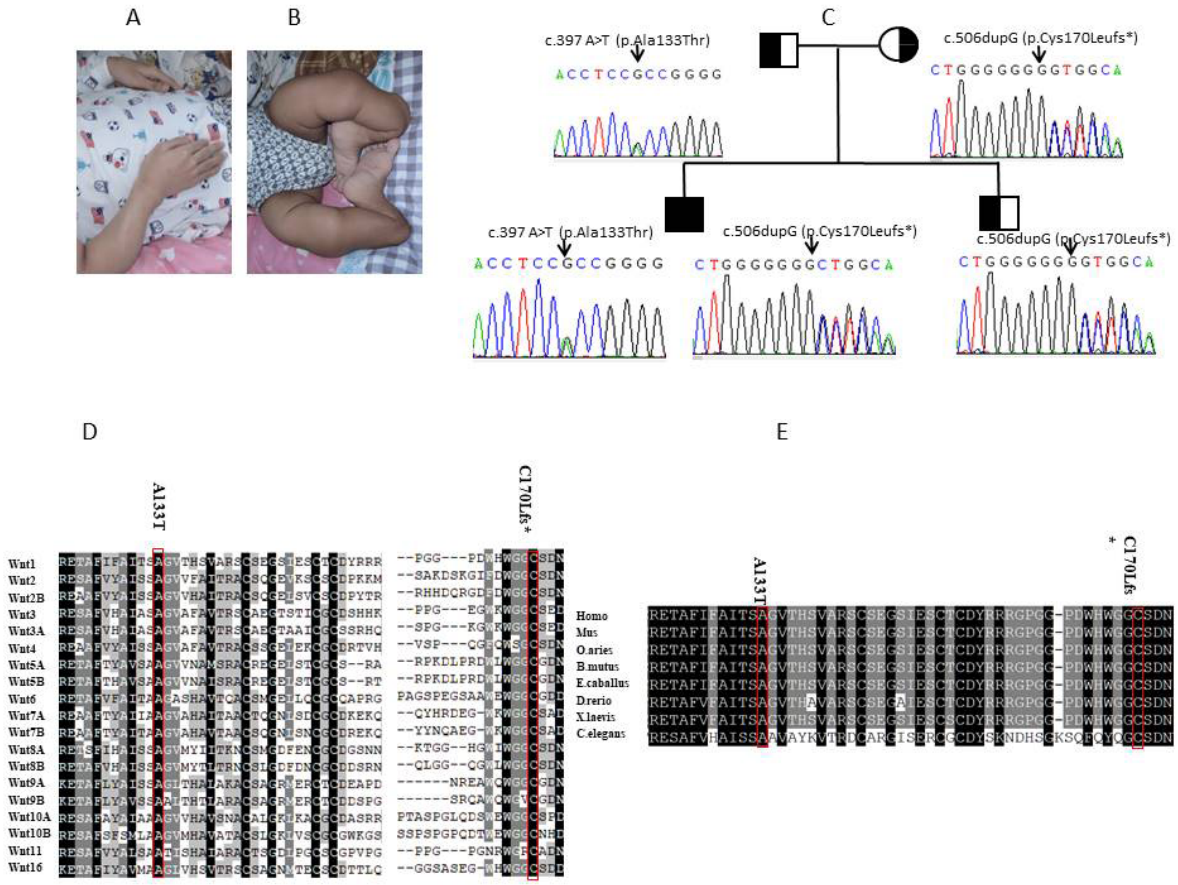

Figure 1. Clinical phenotype and Sanger sequencing results. (A) Abnormal finger, **(B)** Deformity in lower extremities, **(C)** Pediagree map and identified mutations of patient and their family members, **(D-E)** Highly conserved mutation in WNT family members and different species by alignment.

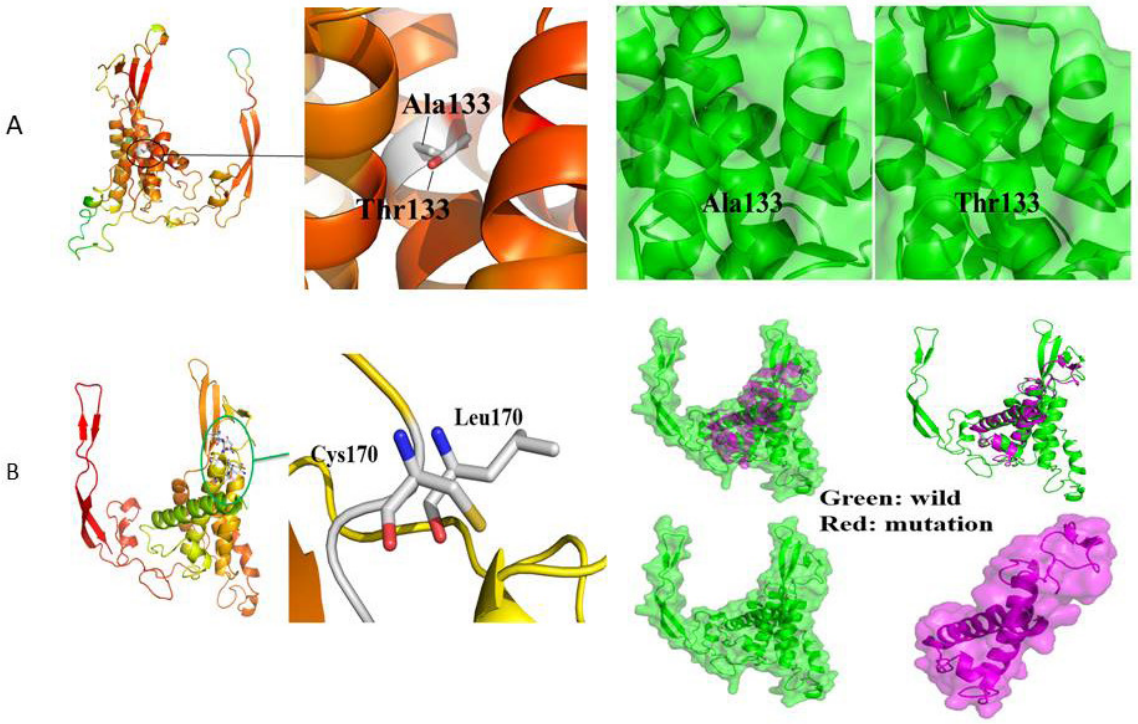

Figure 2. Location and 3-D structure of WNT1 variants. (A) p.Ala133Thr, (B) p.Cys170Leufs*.

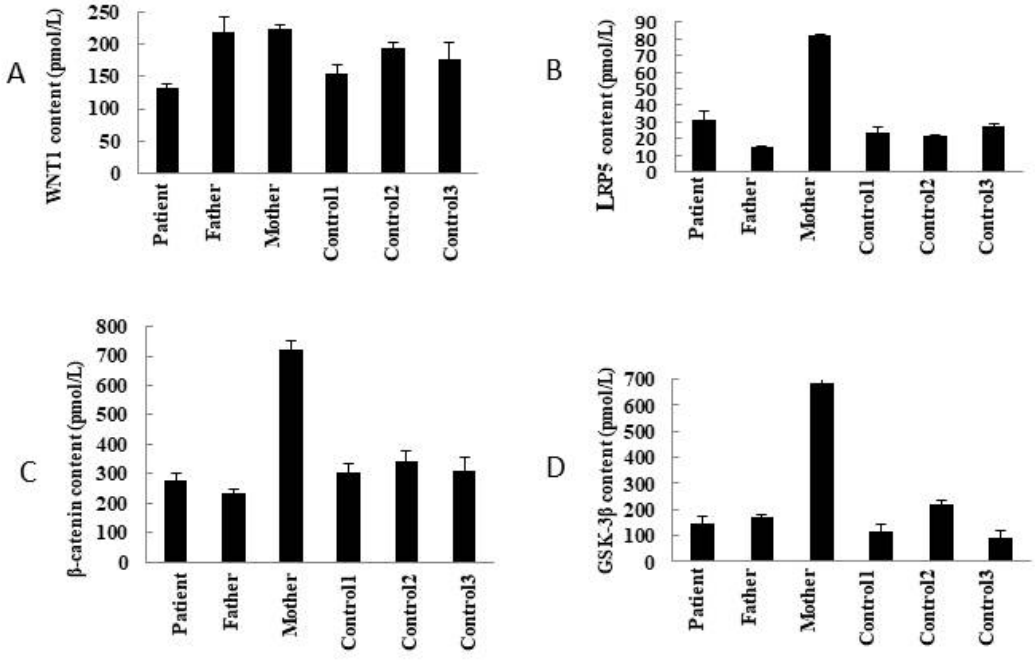

Figure 3. ELISA assay for serum quantification. (A) WNT1, (B) LRP5, (C) β-catenin, (D) GSK-3β.

3.3. *Wnt signaling pathway molecules*

Serum ELISA assays showed a significant difference in the WNT signaling pathway molecule. The expression of WNT1, β-catenin, GSK-3β were decreased and the level of LRP5 increased (Figure 3).

4. Discussion

We report one Chinese OI patient with an autosomal-dominant *WNT1* mutation following our previously identified 11 mutations (9 novel) from 10 individual Chinese families, which was the largest group of patients with recessive OI ever reported (revision data). The patient in our present paper has complex heterozygous of c.397C>T (p.Ala133Thr) and c.506dupG (p.Cys170Leufs*). Abnormalities in facial and finger characteristics extended the phenotype spectrum of OI patients with *WNT1* mutations. Serum ELISA analysis revealed decreased WNT1 and impaired canonical Wnt/β-catenin pathway.

For heterozygous c.397C>T (p.Ala133Thr) mutation, it was the third time that we found and the fifth patient from three individual families. It was identified to be combined with heterozygous c.667C>T (p. Ser226Leu) and c.774C>A(p.Tyr258*) in two individual families, respectively. This mutation may be a hotspot mutation (with 27.3%) in Chinese type XV OI patient. For c.506dupG (p.Cys170Leufs*), it was recorded in the list of Osteogenesis Imperfecta Variant Database (*http://www.le.ac.uk/ge/collagen*) (*12,13*) four times, including two homozygous mutations (both type III) and two heterozygous mutations in correlated c.506G>A (*17*), and c.259C>T (p.Gln87*). For the patient carrying c.506dupG and c.259C>T mutations, multiple fractures, delayed development, recurrent infections, ptosis, and high arched palate were seen (*9*). However, no ptosis and multiple fractures were observed in our patient, though these are common phenotypes in related type XV OI according to the data from our previous 10 WNT1 OI families. *WNT1* knockout mouse model and spontaneous *WNT1* mutations have demonstrated that it is necessary for the formation of the cerebellum and midbrain (*25,26*), and severe bone fragility (*11*).

Three D analysis predicated that the p.Ala133Thr change would nearly have no effect on protein structure. Same results were obtained from expression of WNT1 by Western blot (unpublished data). The change of cysteine to leucine at position of 170 leads to formation of a loop region instead of the original β-sheet, and hence, the stability of WNT1 is severity destroyed as predicted. Premature termination codon mutations mediate mRNA degradation through nonsense-mediated RNA decay (*27*). Wnt has an N-terminal α-helical domain (NTD) and C-terminal cysteine-rich domain (CTD). The NTD contains seven α-helices with five disulfide bonds formed by conserved cysteine residues, while the CTD has two β-sheets stabilized by disulfide bonds. Both the thumb loop from the NTD and index finger from the CTD grasp the frizzled cysteine-rich domain (Fz-CRD), which is responsible for Wnt binding and signaling transduction. Wnts are post-translationally acylated by the presence of palmitate and/or palmitoleic acid at particular conserved serine residues and further lipidation at the cysteine residue

of the mature secreted protein. The truncated protein caused by p.Cys170Leufs* leads to the loss of the lipidation site and CTD domain. Functional Wnt signaling pathway impaired and this is in line with our unpublished data and other group's finding (*8*).

Variable clinical phenotypes are notable in OI patients with *WNT1* mutations. One of the most striking phenotypes, compared with type I collagen gene related OI is that no deformities or fracture were noted at birth according to our unpublished Wnt1 OI population. Severe vertebral compressions was also obvious according to a report (*28*) and our unpublished data. Neurological problems can either be present or absent (*29*). Other characteristics such as ptosis, recurrent chest infections, hypotonia, and developmental delay have all been documented in these previous OI populations (*9,14*). The present study shows the obvious abnormality in facial characteristics and fingers in OI patient with identified *WNT1* mutations.

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References

1. Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet. 1979; 16:101-116.
2. Forlino A, Marini JC. Osteogenesis imperfecta. Lancet. 2016; 387:1657-1671.
3. Trejo P, Rauch F. Osteogenesis imperfecta in children and adolescents-new developments in diagnosis and treatment. Osteoporos Int. 2016; 27:3427-3437.
4. Bardai G, Moffatt P, Glorieux FH, Rauch F. DNA sequence analysis in 598 individuals with a clinical diagnosis of osteogenesis imperfecta: Diagnostic yield and mutation spectrum. Osteoporos Int. 2016; 27:3607-3613.
5. Lu Y, Ren X, Wang Y, Han J. Molecular mechanisms of osteogenesis imperfecta. Prog.Biochem.Biophys. 2015; 42:511-518
6. Fahiminiya S, Majewski J, Mort J, Moffatt P, Glorieux FH, Rauch F. Mutations in WNT1 are a cause of osteogenesis imperfecta. J Med Genet. 2013; 50:345-348.
7. Keupp K, Beleggia F, Kayserili H, *et al*. Mutations in WNT1 cause different forms of bone fragility. Am J Hum Genet. 2013; 92:565-574.
8. Laine CM, Joeng KS, Campeau PM, *et a*l. WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. N Engl J Med. 2013; 368:1809-1816.
9. Pyott SM, Tran TT, Leistritz DF, Pepin MG, Mendelsohn NJ, Temme RT, Fernandez BA, Elsayed SM, Elsobky E, Verma I, Nair S, Turner EH, Smith JD, Jarvik GP, Byers PH. WNT1 mutations in families affected by moderately severe and progressive recessive osteogenesis imperfecta. Am J Hum Genet. 2013; 92:590-597.
10. Mäkitie RE, Niinimäki T, Nieminen MT, Schalin-Jäntti C, Niinimäki J, Mäkitie O. Impaired WNT signaling and the spine-Heterozygous WNT1 mutation causes severe age-related spinal pathology. Bone. 2017; 101:3-9.
11. Joeng KS, Lee YC, Lim J, Chen Y, Jiang MM, Munivez E, Ambrose C, Lee BH. Osteocyte-specific WNT1 regulates osteoblast function during bone homeostasis. J Clin Invest. 2017; 127:2678-2688.
12. Dalgleish R. The human type I collagen mutation database. Nucleic Acids Res. 1997; 25:181-187.
13. Dalgleish R. The Human Collagen Mutation Database 1998. Nucleic Acids Res. 1998; 26:253-255.
14. Faqeih E, Shaheen R, Alkuraya FS. WNT1 mutation with recessive osteogenesis imperfecta and profound neurological phenotype. J Med Genet. 2013; 50:491-492.
15. Stephen J, Girisha KM, Dalal A, Shukla A, Shah H, Srivastava P, Kornak U, Phadke SR. Mutations in patients with osteogenesis imperfecta from consanguineous Indian families. Eur J Med Genet. 2015; 58:21-27.
16. Aldinger KA, Mendelsohn NJ, Chung BH, Zhang W, Cohn DH, Fernandez B, Alkuraya FS, Dobyns WB, Curry CJ. Variable brain phenotype primarily affects the brainstem and cerebellum in patients with osteogenesis imperfecta caused by recessive WNT1 mutations. J Med Genet. 2016; 53:427-430.
17. Liu Y, Song L, Ma D, Lv F, Xu X, Wang J, Xia W, Jiang Y, Wang O, Song Y, Xing X, Asan, Li M. Genotype-phenotype analysis of a rare type of osteogenesis imperfecta in four Chinese families with WNT1 mutations. Clin Chim Acta. 2016; 461:172-180.
18. Caparros-Martin JA, Aglan MS, Temtamy S, *et al*. Molecular spectrum and differential diagnosis in patients referred with sporadic or autosomal recessive osteogenesis imperfecta. Mol Genet Genomic Med. 2016; 5:28-39.
19. Lu Y, Ren X, Wang Y, Li T, Li F, Wang S, Xu C, Wu G, Li H, Li G, Zhao F, Wang Z, Mo X, Han J. Mutational and structural characteristics of four novel heterozygous C-propeptide mutations in the proalpha1(I) collagen gene in Chinese osteogenesis imperfecta patients. Clin Endocrinol (Oxf). 2014; 80:524-531.
20. Sunyaev S, Ramensky V, Koch I, Lathe W, 3rd, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Hum Mol Genet. 2001; 10:591-597.
21. Tavtigian SV, Deffenbaugh AM, Yin L, Judkins T, Scholl T, Samollow PB, de Silva D, Zharkikh A, Thomas A. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet. 2006; 43:295-305.
22. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res. 2001; 11:863-874.
23. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010; 7:575-576.
24. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. Clustal W and Clustal X version 2.0. Bioinformatics. 2007; 23:2947-2948.
25. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell. 2012;149:1192-1205.
26. Inestrosa NC, Toledo EM. The role of Wnt signaling

in neuronal dysfunction in Alzheimer's Disease. Mol Neurodegener. 2008; 3:9.
27. Lejeune F. Nonsense-mediated mRNA decay at the crossroads of many cellular pathways. BMB Rep. 2017; 50:175-185.
28. Palomo T, Al-Jallad H, Moffatt P, Glorieux FH, Lentle B, Roschger P, Klaushofer K, Rauch F. Skeletal characteristics associated with homozygous and heterozygous WNT1 mutations. Bone. 2014; 67:63-70.
29. Won JY, Jang WY, Lee HR, Park SY, Kim WY, Park JH, Kim Y, Cho TJ. Novel missense loss-of-function mutations of WNT1 in an autosomal recessive Osteogenesis imperfecta patient. Eur J Med Genet. 2017; 60:411-415.
30. Janda CY, Waghray D, Levin AM, Thomas C, Garcia KC. Structural basis of Wnt recognition by Frizzled. Science. 2012; 337:59-64.

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