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

Yanqin Lu1,2,§, Yunzhang Dai1,2,§, Yanzhou Wang3, Naixiang Zhai1,2, Jian Zhang1,2, Junlong Liu4, Xiaoli Yin1,2, Tianyou Li3, Xiuzhi Ren4,**, Jinxiang Han1,2,**

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
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 PL53. 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 (Beijing Biotech Co. Ltd., Wuhan, China). Three age-matched healthy sera 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) Pedigree 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*.
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 predicted 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 (29) 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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