Effects of WNT1 c.110 T>C and c.505G>T mutations on osteoblast differentiation via the WNT1/β-catenin signaling pathway

Bashan Zhang 1*, Rong Li 1, Wenfeng Wang 1, Xueming Zhou 2, Beijing Luo 1, Zinian Zhu 1, Xibo Zhang 1 and Aijiao Ding 1

Abstract

Background: WNT1 c.110 T>C and c.505G>T missense mutations have been identified in patients with osteogenesis imperfecta (OI). Whether these mutations affect osteoblast differentiation remains to be determined. This study aimed to investigate the effects of WNT1 c.110 T>C and c.505G>T mutations on osteoblast function, gene expression, and pathways involved in OI.

Methods: Empty vector (negative control), wild-type WNT1, WNT1 c.110 T>C, WNT1 c.505G>T, and WNT1 c.884C>A (positive control) mutant plasmids were constructed and transfected into preosteoblast (MC3T3-E1) cells to investigate their effect on osteoblast differentiation. The expressions of osteoblast markers, including BMP2, RANKL, osteocalcin, and alkaline phosphatase (ALP), were determined using quantitative real-time polymerase chain reaction (RT-qPCR), western blotting (WB), enzyme-linked immunosorbent assay, and ALP staining assay, respectively. The mRNA and protein expression levels of WNT1 or the expression levels of the relevant proteins involved in the WNT1/β-catenin signaling pathway were also determined using RT-qPCR, WB, and immunofluorescence (IF) assays after the different plasmids were transfected into MC3T3-E1 cells.

Results: Compared with those in the wild-type group, in the mutation groups, the mRNA and protein expression levels of BMP2 were suppressed, the expressions of osteocalcin and ALP were inhibited, and the mRNA and protein expression levels of RANKL were enhanced in MC3T3-E1 cells. WB and IF assays revealed that the protein expression levels of WNT1 in MC3T3-E1 cells were downregulated in the mutation groups compared with those in the wild-type group. Furthermore, the expression levels of nonphosphorylated β-catenin (non-p-β-catenin) and phosphorylated GSK-3β (p-GSK-3β) were downregulated in the mutation groups compared with those in the wild-type group. However, no significant changes in the expression level of non-p-β-catenin or p-GSK-3β were observed in the mutation groups.

* Correspondence: zbsdgy@163.com

1 Clinical Laboratory, Affiliated Dongguan People’s Hospital, Southern Medical University, No.3 Xinguchong Wandao South Road, Wangjiang District, Dongguan 523059, China

Full list of author information is available at the end of the article.

© The Author(s). 2021 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Conclusions: WNT1 c.110 T>C and c.505G>T mutations may alter the proliferation and osteogenic phenotype of MC3T3-E1 linked to the progression of OI via the inhibition of the WNT1/β-catenin signaling pathway. This is the first study to confirm the effect of WNT1 c.110 T>C and c.505G>T missense mutations on osteoblast differentiation and propose a new molecular mechanism for OI development.

Keywords: Osteogenesis imperfecta, Osteoblast, WNT1, Mutation, β-Catenin

Introduction
Osteogenesis imperfecta (OI) is a group of heritable connective tissue disorders characterized by increased bone fragility during early childhood, reduced bone mass, and frequent fractures [1–4]. It is currently believed that approximately 90% of OI cases are caused by autosomal dominant mutations in COLIA1 and COLIA2. However, approximately 20–25% of patients with moderate-to-severe OI have pathogenic mutations in other genes [5]. Mutations such as SERPINF1, P3H1, CRTAP, PPILB, BMP1, FKBPI0, SP7, PLOD2, TMEM38B, PLOD2, P4HB, SPARC, and SEC24D have been considered closely related to autosomal recessive OI cases [6]. Moreover, researches have shown that WNT1 mutation affects osteoblast activity, leading to increased bone mass disorder, brittle fractures, and progressive bone abnormality in patients with OI [7, 8].

WNT1, a member of the WNT protein family, plays a vital role in regulating bone mass and maintains the homeostasis of bone metabolism. In vitro experiments have shown that WNT1 defects can result in significantly decreased bone formation, whereas WNT1 overexpression can promote bone formation [9]. In OI, studies have shown that biallelic mutations in WNT1 result in recessive OI, whereas heterozygous mutations in WNT1 are associated with early-onset osteoporosis in dominant hereditary families [10, 11]. More than 27 disease-causing WNT1 mutations have been discovered thus far [12]. However, the biological functions in most of the WNT1 mutations have not yet been elucidated.

The canonical WNT1 pathway initiates a signaling cascade by binding to the Frizzled and LRP5 receptors on the cell surface, resulting in the accumulation of non-phosphorylated β-catenin (non-p-β-catenin) in cells, and functions as a transcription factor to stimulate the transcription of the downstream target genes of the WNT signaling pathway [13]. A study has shown that WNT1 mutations can affect the activation of the canonical WNT pathway and the mineralization of osteoblasts [11]. Another study has also shown that a missense mutation in exon 3 (c.505G>T) of WNT1 resulted in the substitution of glycine by cysteine at position 169 (p.G169C); besides, the missense mutation in exon 2 (c.110G>T) of WNT1 resulted in the conversion of isoleucine to threonine at position 37 (p.I37T) in patients with OI [6]. However, whether the mechanism of the WNT1 c.110 T>C and c.505G>T mutations affect osteoblast differentiation remains to be determined.

For a better evaluation of the potential molecular mechanisms of WNT1 mutations, a nonsense mutation (c.884C>A) was set as a positive control. This mutation causes the truncation of the last 76 amino acids of WNT1, which has been confirmed by western blotting (WB). Hence, c.884C>A expression can be detected at a position below the molecular weight of the wild-type WNT1 [11]. Therefore, in this study, WNT1 c.110 T>C, WNT1 c.505G>T, and WNT1 c.884C>A (positive control) mutant plasmids were constructed and transfected into osteoblasts to investigate the effects of the mutations on cell viability, expression levels of osteoblast markers, and activation of the WNT1/β-catenin pathway in MC3T3-E1 cells. This study showed the effect of WNT1 c.110 T>C and c.505G>T mutations on osteoblast differentiation for the first time and proposed a new molecular mechanism for the development of OI.

Materials and methods
Cell cultures
Preosteoblast (MC3T3-E1) cells (ATCC, VA, USA) were cultured in α-DMEM supplemented with 10% fetal bovine serum. Cells were cultured at 37 °C in an incubator under 5% CO2 and 90% humidity.

Plasmid construction and transfection
Wild-type WNT1, WNT1 c.110 T>C, WNT1 c.505G>T, and WNT1 c.884C>A mutant plasmids were synthesized by General Biosystems, Inc (Anhui, China). Empty plasmids were used as the negative control, and c.884C>A was used as a positive control. The MC3T3-E1 cells were grown for 24 h in 6-well plates with an initial cell density of 5 × 105 cells/mL. Cells in each well were transfected with Vector, WNT1, c.110 T>C, c.505G>T, and c.884C>A using Lipofectamine 3000 (Invitrogen, CA, USA) according to the manufacturer’s instructions for subsequent quantitative real-time polymerase chain reaction (RT-qPCR) and WB experiments. MC3T3-E1 cell suspensions were transfected with different plasmids using an osteogenic differentiation medium (#MUXMT-90021, Cyagen Biosciences, Guangzhou, China). Cells were cultured in the osteogenic differentiation medium and seeded in coverslips of 6-well plates at a density of 2 × 104 cells/mL, and then the plasmids were transfected...
into the MC3T3-E1 cells every 72 h for a total of 14 days. Samples were then collected for the enzyme-linked immunosorbent assay (ELISA) and alkaline phosphatase (ALP) staining assay. Three independent assays were performed.

**3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay**

Before transfection, cells were seeded into a 96-well plate for 24 h at a density of $1 \times 10^4$ cells/well. Next, cells were transfected with empty vector, wild-type WNT1, WNT1 c.110 T>C, WNT1 c.505G>T, and WNT1 c.884C>A mutant plasmids. After transfection, the cells were seeded in 96-well plates overnight, and then 100 μl of α-DMEM supplemented with 20 μl of CellTiter 96 AQueous One Solution Reagent (Promega, WI, USA) were added into the wells, which contained MTS and phenazine ethosulfate. The cell viability was determined at 492 nm using a 96-well plate reader (Bio-Rad Laboratories, CA, USA). Three independent assays were performed.

**ELISA and ALP activity assay**

During the cultivation of MC3T3-E1 cells in the osteogenic differentiation medium for 14 days, the supernatants were collected for the ELISA experiments. The Mouse OT/BGP ELISA Kit (#CSB-E06917m) provided by Cusabio (Wuhan, China) was used. Briefly, cell culture media were added to 96-well plates, incubated with 100 μl of biotinylated antibodies for 60 min at room temperature, washed five times, incubated with 100 μl of HRP-conjugated streptavidin for 20 min at room temperature in the dark, incubated with 3,3′,5,5′-tetramethylbenzidine solution for 20 min, and incubated with the termination solution. The relative expression levels were then determined by measuring the absorbance at 450 nm. Three independent assays were performed.

Similarly, during the cultivation of cells in the osteogenic differentiation medium for 14 days, the cells were also collected and examined. Cells were stained with ALP according to the instructions of the BCIP/NBT Alkaline Phosphatase Color Development Kit (#C3206, Beyotime Biotechnology, Jiangsu, China). The coverslips were removed, washed twice with phosphate buffer saline, and fixed with 95% ethanol for 8 min. Subsequently, the cells were air-dried and incubated with a substrate solution at 37 °C for 30 min in the dark. After the reaction, the samples were washed with double-distilled water, counterstained with methyl green for 2 min, washed three times with double-distilled water, and air-dried. Five sections per sample were randomly selected for analysis under a microscope (OPTEC, TP510, Chongqing, China). Three independent assays were performed.

**RT-qPCR**

Total RNA was isolated from the MC3T3-E1 cells using TRIzol reagent (Invitrogen) according to the standard protocol. Total RNA was reverse transcribed into cDNA using M-MLV Reverse Transcriptase (Promega, WI, USA) with random primers. WNT1, BMP2, and RANKL were amplified using SYBR Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan) and specific primers as follows: WNT1 forward, 5′-CGATGGTGGGATTGTG TGAAC-3′; WNT1 reverse, 5′-CCGGATTTTGCGT ATCAGAC-3′; BMP2 forward, 5′-GGGACCCGCT GTCTCTAGT-3′; BMP2 reverse, 5′-TCAACTCAAA TTTGCTGAGGAC-3′; RANKL forward, 5′-AGGC TGGGCCAAGATCTCTA-3′; and RANKL reverse, 5′- GTCTGTAGTACGCTTCCC-3′. The relative expression levels of WNT1, BMP2, and RANKL were calculated using the 2−ΔΔCT method. GAPDH was used as an internal control: GAPDH forward, 5′-ATCAAG TGGGGGTATGCTGG-3′, reverse, 5′-CCTGCTTCAC CACCTTCCTGA-3′. Three independent assays were performed.

**WB**

Cells were lysed using radioimmunoprecipitation assay buffer (Beyotime, Shanghai, China) supplemented with protease inhibitors and phosphatase inhibitors (Roche, Mannheim, Germany). Protein concentration was quantified using a Bradford kit (Pierce, IL, USA). Proteins (40 μg/sample) were separated on a 10% SDS-polyacrylamide gradient gel and transferred to PVDF membranes (Millipore, MA, USA). After blocking with 5% bovine serum albumin (BSA), the membranes were incubated with primary WNT1 (ab15251, Abcam, USA), β-catenin (sc7199, Santa, USA), non-p-β-catenin (19807 T, Cell Signaling Technology, USA), GSK-3β (ab32391, Abcam, USA), p-GSK-3β (9323 s, Cell Signaling Technology), BMP2 (9323 s, Proteintech, China), and RANKL (ab45039, Abcam, USA) antibodies and corresponding HRP-conjugated secondary antibodies. The blots were visualized using a chemiluminescence reagent (Millipore, CA, USA). The relative expressions of the proteins were normalized to that of GAPDH (60004-1-lg, Proteintech, China) using Image-Pro software. Three independent assays were performed.

**Immunofluorescence (IF) assay**

The cells cultured on the coverslips of 6-well plates were transfected with empty vector, wild-type WNT1, c.110 T>C, WNT1 c.505G>T, and WNT1 c.884C>A mutant plasmids. After 72 h of transfection, the cells were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton-100 (Calbiochem, CA, USA), and blocked with 5% BSA. Next, cells were incubated with WNT1 (ab15251, Abcam), β-catenin (sc7199, Santa), non-p-β-
catenin (19807 T, Cell Signaling Technology), GSK-3β (ab32391, Abcam), and p-GSK-3β (9323 s, Cell Signaling Technology) primary antibodies followed by FITC-conjugated secondary antibodies (Sangon, Shanghai, China). Positive signals were observed using a fluorescent microscope, and samples were analyzed using Image-Pro software. DNA was stained with 4′,6-diamidino-2-phenylindole (Sigma-Aldrich, MO, USA) for 5 min. Three independent assays were performed.

**Statistical analysis**

GraphPad Prism 7 was used for visualization. One-way analysis of variance followed by Tukey’s post hoc test for multiple comparisons was performed to compare differences between groups. P < 0.05 was considered to indicate a statistically significant difference.

**Results**

*WNT1* c.110 T>C and c.505G>T mutations affect MC3T3-E1 cell proliferation

We transfected empty vector, wild-type *WNT1*, *WNT1* c.110 T>C, *WNT1* c.505G>T, and *WNT1* c.884C>A plasmids into MC3T3-E1 cells for 24 h individually, and then detected the expression levels of *WNT1*. The RT-qPCR results showed that the mRNA expression levels of *WNT1* were upregulated in the mutation and wild-type groups compared with those in the empty vector (Vector) group and that the mRNA levels of *WNT1* were downregulated in the *WNT1* c.110 T>C and c.505G>T mutation groups compared with those in the wild-type *WNT1* group, indicating that these plasmids were successfully transfected into the MC3T3-E1 cells and the *WNT1* mutations in MC3T3-E1 cells were successfully established (Fig. 1A). To investigate the effects of *WNT1* c.110 T>C and c.505G>T mutations on osteoblast proliferation, empty vector, wild-type *WNT1*, *WNT1* c.110 T>C, *WNT1* c.505G>T, and *WNT1* c.884C>A plasmids were transfected into the MC3T3-E1 cells for 24 and 48 h, respectively, and the cell viability in each group was measured using the MTS assay. Cell viability was found to be increased in the wild-type *WNT1* group compared with that in the Vector group. However, cell viability in the mutation groups was significantly lower than that in the wild-type *WNT1* group at 24 and 48 h, suggesting that *WNT1* mutations inhibited the viability of MC3T3-E1 cells (Fig. 1B).

**WNT1** C.110 T>C and C.505G>T mutations affect the expression of osteoblast markers

For further investigation of the effects of *WNT1* c.110 T>C and c.505G>T mutations on osteoblast characteristics, the expression levels of the osteoblast differentiation marker *BMP2* and osteoclast differentiation marker *RANKL* were analyzed using RT-qPCR and WB in MC3T3-E1 cells transfected with empty vector, wild-type *WNT1*, *WNT1* c.110 T>C, *WNT1* c.505G>T, and *WNT1* c.884C>A plasmids. Compared with the Vector group, the cells transfected with wild-type *WNT1* plasmid had upregulated mRNA and protein expression levels of *BMP2* and downregulated mRNA and protein expression levels of *RANKL* (Fig. 2A–E). In addition, compared with the cells transfected with wild-type *WNT1* plasmid, the *WNT1* c.110 T>C, *WNT1* c.505G>T, and *WNT1* c.884C>A mutation groups showed downregulated mRNA and protein expression levels of *BMP2* and upregulated mRNA and protein expression levels of *RANKL* (Fig. 2A–E). The ELISA results showed that after induction for 14 days, the content of osteocalcin in the osteogenic differentiation medium in the wild-type *WNT1* group was significantly higher than that in the Vector group and also higher than that in the mutation groups (Fig. 2F). The ALP staining results showed that...
the ALP content in the osteogenic differentiation medium in the wild-type WNT1 group was significantly higher than that in the Vector group and also higher than that in the mutation groups (Fig. 2G), suggesting that the c.110 T>C and c.505G>T mutation sites of WNT1 inhibited the expression of osteocalcin and ALP during osteogenic induction and affected the osteogenic differentiation of the cells.

**WNT1 c.110 T>C and c.505G>T mutations affect the WNT1/β-catenin signaling pathway**

For further evaluation of WNT1 expression in the mutation and wild-type groups and the possible mechanism underlying the effects of WNT1 mutations on the differentiation of osteoblasts, MC3T3-E1 cells were transfected with WNT1 and c.110 T>C, c.505G>T, and c.884C>A plasmids. Next, the expression levels of proteins related to the WNT1/β-catenin signaling pathway were validated in transiently transfected cells. WB and IF assays revealed that wild-type WNT1 plasmids were able to upregulate WNT1 expression at the protein level in MC3T3-E1 cells compared with that in the Vector group, but mutation plasmids downregulated WNT1 expression at the protein level in MC3T3-E1 cells compared with that in the wild-type WNT1 group. The highest expression occurred in the wild-type cells (Figs. 3A, B; 4; and 5A). Compared with wild-type WNT1 cells, cells transfected with WNT1 c.110 T>C, WNT1 c.505G>T, and WNT1 c.884C>A mutant plasmids showed downregulated expressions of non-p-β-catenin and p-GSK-3β (Figs. 3C, D; 4; and 5B, C), demonstrating that WNT1 c.110 T>C and c.505G>T mutations might inhibit osteoblast proliferation and the expression of BMP2 by weakening the WNT1/β-catenin signaling pathway.
Discussion

In a previous study, four new heterozygous WNT1 mutations (c.110 T>C, c.505G>T, c.385G>A, and c.506G>A) were found to be associated with OI in four independent pedigree peripheral blood samples [6]. Of these, a missense mutation (WNT1 c.110 T>C) located in exon 2 caused the conversion of isoleucine to threonine. Further, a missense mutation in exon 3 (WNT1 c.505G>T) allowed the substitution of glycine by cysteine. However, currently, the physiological significance of these mutations remains unclear. Therefore, in the present study, we specifically analyzed the effects of WNT1 c.110G>T and c.505G>T mutations on osteoblast differentiation in MC3T3-E1 cells for the first time. The results revealed that WNT1 c.110 T>C and c.505G>T mutations had effects on osteoblast proliferation and the WNT1/β-catenin signaling pathway. Wild-type WNT1 could induce the expression of osteoblast differentiation markers such as BMP2, osteocalcin, and ALP; suppress the expression of the osteoclast differentiation marker RANKL; and activate the WNT1/β-catenin signaling pathway; however, these effects were considerably impaired in the presence of the WNT1 c.110 T>C and c.505G>T mutations.

In healthy humans, bone resorption of osteoclasts and bone formation of osteoblasts contribute to the maintenance of homeostasis. The disruption of the balance between bone resorption and bone formation of osteoblasts leads to the development of bone diseases such as osteoporosis, which is a critical feature in patients with OI [14, 15]. Research has been shown that BMP2 and RANKL induce osteoblast and osteoclast differentiation, respectively [16–20], and that osteoblast cell viability in WNT1-deficient mice is reduced and associated with the fracture phenotype [21]. In this regard, our study found that WNT1 c.110G>T and c.505G>T mutations inhibited the cell viability, weakened the mRNA and protein expression levels of BMP2, and enhanced the mRNA and protein expression levels of RANKL, indicating that WNT1 c.110G>T and c.505G>T mutations can lead to osteoblast phenotype dysplasia, inhibit bone formation, and easily lead to an increased risk of osteoporosis and fracture.
A study has also revealed that WNT1 mutations could lead to changes in the bone structure by deactivating the canonical WNT pathway [12]. Although some mutant forms have induced the activation of the WNT1 signaling pathway, the expressions of the downstream target genes of the WNT signaling pathways are dysregulated and osteoblast mineralization is impaired [11]. The canonical WNT/β-catenin signaling pathway is activated by a combination of WNT and the Frizzled/LRP5/6 complex, which mediates the activation of β-catenin and then activates downstream gene expression. Furthermore, a functional study of essential receptors in the WNT signaling pathway has revealed that loss-of-function mutations in LRP5 can lead to reduced bone formation and decreased bone mass [22]. However, when there is no extracellular WNT, GSK-3β adds a phosphate group to β-catenin, resulting in the hydrolysis of β-catenin, decreased expression of β-catenin in cells
and, ultimately, inhibition of the WNT/β-catenin signaling pathway. When the WNT signal is present, GSK-3β-mediated β-catenin hydrolysis is inhibited and downstream target genes of β-catenin can be activated [23].

Thus, the present study evaluated the effects of c.110G>T and c.505G>T mutations on WNT1 expression. The WB results revealed that the WNT1 c.110G>T and c.505G>T mutations decreased the levels of p-GSK-3β and non-p-β-catenin compared with that in the wild-type WNT1 group, suggesting that WNT1 c.110G>T and c.505G>T mutations are associated with decreased WNT1 activation and decreased activation of the canonical WNT1/β-catenin signaling pathway. However, the precise mechanisms require further study for better understanding.

Conclusion
This study is the first to confirm the effect of WNT1 c.110 T>C and c.505G>T mutations on osteoblast differentiation via the WNT1/β-catenin signaling pathway, and consequently, a possible cause of OA at the cellular level. These findings suggest a new molecular mechanism for OA development.

Acknowledgements
Not applicable.

Authors’ contributions
BZ conceived and designed the study and critically revised the manuscript. BZ performed the experiments, analyzed the data, and drafted the manuscript. RL, WW, XZ, BL, ZZ, XZ, and AD participated in the study design, study implementation, and manuscript revision. The authors read and approved the final manuscript.

Funding
This work was supported by Dongguan Social Science and Technology Development Project (grant number 2018507150011653).

Availability of data and materials
The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Clinical Laboratory, Affiliated Dongguan People’s Hospital, Southern Medical University, No.3 Xingchong Wanda South Road, Wangjiang District, Dongguan 523059, China. 2Department of Orthopedic, Affiliated Dongguan People’s Hospital, Southern Medical University, Dongguan 523059, China.

Received: 1 March 2021 Accepted: 23 May 2021
Published online: 02 June 2021

References
1. Lindahl K, Astrum E, Rubin CL, Grigelioniene G, Malmgren B, Ljunggren O, et al. Genetic epidemiology, prevalence, and genotype-phenotype correlations in the Swedish population with osteogenesis imperfecta. Eur J Hum Genet. 2015;23(8):1042–50. https://doi.org/10.1038/ejhg.2015.81.
2. Tournis S, Dede AD. Osteogenesis imperfecta - A clinical update. Metabolism. 2018;80:27–37. https://doi.org/10.1016/j.metabol.2017.06.001.
3. Vonderlin HD, Jessel M, Knobel A, Juergensen I, Strewer J. Late onset hyperplastic callus formation in osteogenesis imperfecta type V simulating osteosarcoma-A case report. Int J Surg Case Rep. 2020;69(8):6–6. https://doi.org/10.1016/j.ijscr.2020.03.024.
4. Numbere N, Weber DR, Porter G Jr, Iqbal MA. A 235 Kb deletion at 17q21.33 encompassing the COL1A1, and two additional secondary copy number variants in an infant with type I osteogenesis imperfecta: a rare case report. Mol Genet Genomic Med. 2020;8(6):e1241.
5. 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(12):3607–13. https://doi.org/10.1007/s00198-016-3709-1.
6. Liu Y, Song L, Ma D, Lv F, Xu X, Wang J, et al. Genotype-phenotype analysis of a rare type of osteogenesis imperfecta in four Chinese families with WNT1 mutations. Clin Chim Acta. 2016;461:172–80. https://doi.org/10.1016/j.cca.2016.07.012.
7. Kausar M, Siddiqi S, Yaqoob M, Mansoor S, Makite O, Mir A, et al. Novel mutation G324C in WNT1 mapped in a large Pakistani family with severe recessively inherited Osteogenesis Imperfecta. J Biomed Sci. 2018;25(1):1–10.
8. Keupp K, Beleggia F, Kayserili H, Barnes AM, Steiner M,SEMler O, et al. Mutations in WNT1 cause different forms of bone fragility. Am J Hum Genet. 2013;92(4):565–74. https://doi.org/10.1016/j.ajhg.2013.02.010.
9. Joeng KS, Lee YC, Lim J, Chen Y, Jiang MM, Munivez E, et al. Osteocyte-specific WNT1 regulates osteoblast function during bone homeostasis. J Clin Invest. 2017;127(7):2678–88. https://doi.org/10.1172/JCI92617.
10. Pyott SM, Tran TT, Leistritz DF, Pepin MG, Mendelsohn NJ, Temme RT, et al. WNT1 mutations in families affected by moderately severe and progressive recessive osteogenesis imperfecta. Am J Hum Genet. 2013;92(4):590–7. https://doi.org/10.1016/j.ajhg.2013.02.009.
11. Laine CM, Joeng KS, Campeau PM, Khivanta R, Tarkkonen K, Grover M, et al. WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. N Engl J Med. 2013;368(19):1809–16. https://doi.org/10.1056/NEJMoa1215458.
12. Lu Y, Ren X, Wang Y, Bardai G, Sturm M, Dai Y, et al. Novel WNT1 mutations in children with osteogenesis imperfecta: clinical and functional characterization. Bone. 2018;114:144–9. https://doi.org/10.1016/j.bone.2018.05.018.
13. Kang H, Aryal ACS, Marini JC. Osteogenesis imperfecta: new genes reveal novel mechanisms in bone dysplasia. Transl Res. 2017;181:27–48. https://doi.org/10.1016/j.trsl.2016.11.005.
14. Landsmeer-Beker EA, Massa GG, Maaswinkel-Mooy PD, van de Kamp JJ, Papapoulos SE. Treatment of osteogenesis imperfecta with the bisphosphonate olpadronate (dimethylaminolhydroxypropyldene bisphosphonate). Eur J Pediatr. 1997;156(10):792–6. https://doi.org/10.1007/s00198-016-3709-1.
15. Ward LM, Weber DR, Munns CF, Hogler W, Zemel BS. A contemporary view of the definition and diagnosis of osteoporosis in children and adolescents. J Clin Endocrinol Metab. 2020;105(5):e2088–97. https://doi.org/10.1210/clinem/dg204.
16. Collin-Osdoby P, Yu X, Zheng H, Osdoby P. RANKL-mediated osteoclast formation from murine RAW 264.7 cells. Methods Mol Med. 2003;80:153–66.
17. Ramazzotti G, Baveloni A, Blalock W, Piazz M, Cocco L, Faenza L. BMP-2 Induced Expression of PCBeta1 That is a positive regulator of osteoblast differentiation. J Cell Physiol. 2016;231(3):625–9. https://doi.org/10.1002/jcp.25107.
18. Kim KM, Jeon WJ, Kim EJ, Jang WG. CRTCC2 suppresses BMP2-induced osteoblastic differentiation via Smurf1 expression in MC3T3-E1 cells. Life Sci. 2018;214:70–6. https://doi.org/10.1016/j.lfs.2018.10.052.
19. Imai R, Sato T, Iwamoto Y, Hanada Y, Terao M, Ohta Y, et al. Osteoclasts modulate bone erosion in cholesteatoma via RANKL signaling. J Assoc Res Otolaryngol. 2019;20(5):449–59. https://doi.org/10.1007/s10162-019-00727-1.
20. Vonica A, Bhat N, Phan K, Guo J, Iancu L, Weber JA, et al. Apcdd1 is a dual BMP/Wnt inhibitor in the developing nervous system and skin. Dev Biol. 2020;464(1):71–87. https://doi.org/10.1016/j.ydbio.2020.03.015.

21. Joeng KS, Lee YC, Jiang MM, Bertin TK, Chen Y, Abraham AM, et al. The swaying mouse as a model of osteogenesis imperfecta caused by WNT1 mutations. Hum Mol Genet. 2014;23(15):4035–42. https://doi.org/10.1093/hmg/ddu117.

22. Koay MA, Brown MA. Genetic disorders of the LRP5-Wnt signalling pathway affecting the skeleton. Trends Mol Med. 2005;1(3):129–37. https://doi.org/10.1016/j.molmed.2005.01.004.

23. Baarsma HA, Konigshoff M, Gosens R. The WNT signaling pathway from ligand secretion to gene transcription: molecular mechanisms and pharmacological targets. Pharmacol Ther. 2013;138(1):66–83. https://doi.org/10.1016/j.pharmthera.2013.01.002.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.