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COMMENTARY

WNT1 for the skeleton

David R Deyle[1] and Jennifer J Westendorf[2,3]

[1]Mayo Clinic, Department of Medical Genetics, Rochester, MN, USA. [2]Department of Orthopedic Surgery, Rochester, MN, USA. [3]Department of Biochemistry and Molecular Biology, Rochester, MN, USA.

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Keupp K, Beleggia F, Kayserili H, Barnes AM, Steiner M, Semler O, Fischer B, Yigit G, Janda CY, Becker J, Breer S, Altunoglu U, Grünhagen J, Krawitz P, Hecht J, Schinke T, Makareeva E, Lausch E, Cankaya T, Caparrós-Martín JA, Lapunzina P, Temtamy S, Aglan M, Zabel B, Eysel P, Koerber F, Leikin S, Garcia KC, Netzer C, Schönau E, Ruiz-Perez VL, Mundlos S, Amling M, Kornak U, Marini J, Wollnik B. Mutations in WNT1 cause different forms of bone fragility. *Am J Hum Genet* 2013;**92**:565–574;
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 and
Fahiminiya S, Majewski J, Mort J, Moffatt P, Glorieux FH, Rauch F. Mutations in WNT1 are a cause of osteogenesis imperfecta. *J Am Genet* 2013;**50**:345–348.

WNTs are secreted glycoproteins that regulate cell fate, proliferation and survival during tissue development and regeneration. There are 19 WNTs encoded by the human and mouse genomes. They bind to cell surface receptor complexes consisting of LRP and frizzled (FZ) molecules. More than a decade ago, it was discovered that the loss-of-function mutations in LRP5 are responsible for the autosomal recessive disease, osteoporosis–pseudoglioma syndrome (OPPG),[1] whereas an autosomal dominant gain-of-function mutation in LRP5 is responsible for high bone mass.[2,3] Shortly thereafter, it was realized that mutations or deletions in the *SOST* gene prevented the expression of the LRP5/6 antagonist, sclerostin, and caused high bone mass observed in sclerosteosis, van Buchem disease and autosomal dominant craniodiaphyseal dysplasia.[4–7] These seminal findings triggered a flurry of studies examining WNT signaling pathways in bone formation, repair and maintenance and have led to the development of new anabolic therapies (reviewed in Monroe *et al*.[8] and Baron and Kneissel[9]). Despite this plethora of data, the identity of WNT ligand(s) responsible for bone formation at any particular time or space is unknown. A general presumption has been that the many WNTs present in skeletal tissues have redundant and complementary roles in initiating canonical signaling through β-catenin and the TCF/LEF1 family of transcription factors, as well as in stimulating non-canonical pathways. Thus, the recent set of studies identifying WNT1 mutations as the cause of bone fragility in subsets of patients with osteogenesis imperfecta (OI) and early-onset osteoporosis are remarkable.[10–13]

OI is a rare inherited connective-tissue disorder characterized by bone fragility. The vast majority (~95%) of OI cases are caused by autosomal dominant mutations in type I collagen genes (COL1A1 or COL1A2). The remaining OI cases are recessively inherited. In some of these patients, mutations have been found in genes (for example, BMP1, CRTAP, FKBP10, LEPRE1, PPIB, SERPINF1 and SERPINH1) encoding proteins that are involved in collagen assembly, secretion and modification or in regulating intracellular calcium levels (for example, TMEM38B); however, the causative mutation(s) is still unknown for a subset of OI patients. Using genome-sequencing platforms, four independent groups identified mutations within the *WNT1* gene on chromosome 12 as a potential causative factor of OI in some of these families.[10–13] In some patients, homozygous missense or nonsense mutations were observed, whereas in other OI families compound heterozygous mutations were found.

Autosomal recessive WNT1 mutations were associated with moderate-to-severe OI and, in one case, early infant death. The phenotype of affected individuals includes typical OI findings of early-onset fractures, bone deformities, low bone density and short stature; however, hearing and tooth development were normal. The majority of OI patients had no intellectual disabilities, but Laine *et al.*,[12] Keupp *et al.*[11] and Pyott *et al.*[13] found that some affected individuals had developmental delays and learning disabilities with three having brain malformations. Similar findings have been described in mice where mutations in Wnt1 cause brain malformations and neurologic impairment.[14,15] Whether cognitive delays and brain abnormalities are part of the WNT1 OI phenotype remains to be determined as normal and abnormal brain and cognitive development was observed between individuals with identical mutations. Interestingly, Laine *et al.* and Keupp *et al.* also found autosomal dominant missense mutations in WNT1 in the genomes of male and female patients with early-onset osteoporosis.[11,12] These

individuals experienced multiple bone fractures at an early age and low bone formation and remodeling rates.

Based on the similarity of WNT1 to XWnt8, the only canonical Wnt whose structure has been solved, some of the mutations found in the OI patients and both pCys218Gly and pArg235Trp mutations in osteoporosis patients are predicted to alter the protein the structure of WNT1, decreasing its affinity for LRP5/6 or FZ receptors. However, in some OI families, the mutations destabilize WNT1 mRNAs and prevent production of WNT1 altogether.

Laine *et al.* and Keupp *et al.* performed a variety of *in vitro* cellular and molecular experiments to determine how mutant WNT1 proteins might perform in patients.[11,12] WNT1 (also known as INT1) activates the 'canonical' signaling pathway, which involves stabilization of β-catenin and activation of TCF/LEF1 transcription factors. Both groups found that WNT1 molecules harboring the mutations identified in their patient groups were unable to activate the TOP-FLASH reporter, a commonly used tool to measure canonical WNT signaling and the TCF/LEF1 activity. Laine *et al.* also showed that β-catenin was not stabilized or present at high levels in nuclei of cells treated with mutant WNT1 proteins. Furthermore, the wild-type WNT1, but not two mutant WNT1 proteins, could stimulate mineralization of MC3T3 osteoblast cultures *in vitro*. Together, these data demonstrate that the mutant WNT1 proteins found in OI and osteoporosis patients are poor activators of the canonical signaling pathways and osteoblastogenesis.

However, do mutant WNT1s have a role *in vivo*? Laine *et al.* asked an interesting question about whether or not the mutant WNT1 proteins could block wild-type WNT1 signaling but got mixed results. The mutant WNT1 protein that would be expressed in the osteoporosis patients and would theoretically need to work in a dominant manner did not block the wild-type WNT1 activity; whereas, the one mutation tested from OI patients had modest effects. As OI patients do not produce a functional WNT1 allele, this may not be important for disease progression. These results do not exclude the possibility that mutant WNT1 could compete with other WNT ligands for receptors. These data also raise the question of whether the wild-type WNT1 allele is silenced in early-onset osteoporosis patients. Both Laine *et al.* and Keupp *et al.* provide convincing evidence that mature osteoblasts and osteocytes produce WNT1 transcripts, although there may be other sources, such as B cells, in the marrow environment. This is important because locally produced WNTs appear to have the greatest effects on cell fate and processes such as proliferation and differentiation.[16]

A yet-to-be-resolved issue is whether or not WNT1 mutations are sufficient to cause bone fragility. An intriguing new study provides evidence that a subset of Wnts (including Wnt1 as well as Wnts 2/6/7a/7b/9a/10a/10b but excluding Wnt3/3a) that signal through the first extracellular YWTD-type β-propeller region of Lrp6 are anabolic in mice.[17] Two Wnt1 mutant mouse strains exist but their bone phenotypes have not yet been reported. Germline Wnt1 knockout mice die during embryogenesis at E9.5 (before skeletal formation begins) from cerebellum and midbrain defects.[14,15] The 'swaying' mouse harbors a natural mutation that creates a truncated Wnt1 protein,[18] which interestingly is identical to a mutation found in one of the OI patients.[11] The creation and analysis of conditional Wnt1 knockout mice where Wnt1 is specifically inactivated in osteoblasts and osteocytes will provide important mechanistic information on the role of WNT1 in bone fragility. These animal models will also aid in the evaluation of novel therapeutic strategies for OI patients.

The translational impact of these findings is potentially exciting. OI patients with WNT1 mutations are a small fraction of all OI cases and are characterized by recessive inheritance and poor responses to bisphosphonates. It is possible that agonists of the canonical WNT signaling pathway that are already in the drug development pipeline (for example, anti-sclerostin antibodies) may be effective at increasing bone strength in this small subset of patients with WNT1 mutations and OI or early-onset osteoporosis. However, neutralizing a WNT inhibitor may not be sufficient to drive osteoblastogenesis in these patients. New therapies that specifically replace lost WNT1 or neutralize mutant WNT1 proteins may also be needed to activate appropriate receptors and accelerate cellular responses. As OI manifests in children and canonical WNT signaling is a well-known oncogenic pathway, more studies are needed to determine the safety of therapies that stimulate canonical WNT signaling during development, and WNT-targeted therapies would not be warranted for OI patients lacking WNT1 mutations.

Conflict of Interest

The authors declare no conflict of interest.

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