Case Report

Skeletal disease in a father and daughter with a novel monoallelic WNT1 mutation

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ABSTRACT

Context: Most heritable causes of low bone mass in children occur due to mutations affecting type 1 collagen. We describe two related patients with low bone mass and fracture without mutations in the type 1 collagen genes.

Case description: We describe the index case of a 10-year-old girl with low-impact fractures in childhood and her 59-year-old father with traumatic fractures in adulthood, both with low bone mineral density. They were found to have the same heterozygous missense mutation in the WNT1 gene (p.Gly222Arg), occurring in a highly conserved WNT motif in close proximity to the Frizzled binding site.

Conclusions: The WNT-ligand WNT1, signaling through the canonical WNT-βcatenin pathway, plays a critical role in skeletal development, adult skeletal homeostasis, and bone remodeling. Biallelic mutations have been described and are associated with moderate to severe osteogenesis imperfecta, in some cases with extra-skeletal manifestations. Patients with monoallelic mutations, as in our case, seem to present with low bone mineral density and less severe disease. The phenotypic difference between biallelic and monoallelic mutations highlights that the aberrant protein in monoallelic mutations may exert a dominant negative effect on the wild type protein as heterozygous carriers in families with biallelic disease are usually asymptomatic. With better understanding of disorders associated with WNT1 mutations, therapies targeting this signaling pathway may offer therapeutic benefit.

1. Introduction

The most frequently encountered heritable disorder of low bone mass in children is osteogenesis imperfecta (OI) (Makitie et al., 2016). OI types I-IV occur due to mutations in the COL1A1 or COL1A2 gene, affecting type 1 collagen and altering matrix structure, resulting in low bone mass and fragility fracture. In this report, we describe the index case of a 10-year-old girl with osteoporosis without mutations in the type 1 collagen genes as well as findings in her 59-year-old father.

2. Case report

A 10-year-old girl presented with a history of multiple fractures over the previous year. Her gestation and delivery were uncomplicated, and she reached developmental milestones normally. At age 9, she sustained a right metatarsal fracture resulting from direct trauma to her foot. Five months later, she sustained a right distal radius fracture after falling on her outstretched hand. Two weeks prior to evaluation, she experienced sudden back spasm while brushing her hair, followed by acute back pain. She was evaluated in an emergency department and found to have marked osteopenia of her vertebral bodies, but no fracture. When seen at Yale, she reported a two-year history of back pain, exacerbated by jumping. Her diet contained approximately 400 mg/day of calcium. At initial evaluation, her height was at the 85th percentile and weight at the 61st percentile. She appeared healthy, was premenarchal, was wearing a back brace, and using a wheelchair. Physical examination revealed normal strength and normal range of motion at all joints. Her sclerae were white and her dentition was normal. The initial laboratory evaluation is summarized in Table 1 and was unremarkable except for an extremely low L-spine BMD.

Whole exome sequencing revealed a heterozygous mutation (p.Gly222Arg) in the WNT1 gene. She was treated with zoledronic acid

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normal and his height was 6′3″, weight 208 pounds, BMI 26kg/m². On initial evaluation, he was well-appearing, his vital signs were
asthma and bilateral hand fractures while playing basketball in college. The same WNT1 mutation. He had sustained multiple finger fractures as
during the first year of treatment. She did not reach menarche while on
participate in physical education. She gained approximately 5cm in height
After 1 year of zoledronic acid infusions, she was able to run and parti-
commended. She enrolled in an intensive physical therapy program
infusions 0.0125mg/kg every 3 months for two doses, then 0.05mg/kg
every 6 months thereafter. A dietary Ca of 1200mg/d was re-

Laboratory and DXA results of the proband and her father at initial evaluation

|                      | Initial Evaluation | Reference Range | Post- Treatment |
|----------------------|--------------------|-----------------|----------------|
| **Proband**          |                    |                 |                |
| Calcium              | 10.2 mg/dL         | 8.8–10.2        | 9.3 mg/dL      |
| Phosphorus           | 4.2 mg/dL          | 3.5–5.6         | 4.3 mg/dL      |
| Alkaline Phosphatase | 177 U/L            | 50–480          | 219 U/L        |
| Collage Type I C- Telopeptide | 934 pg/mL | 519–2415 | 957 pg/mL |
| Parathyroid hormone, (mid molecule assay) | 9 nL.Eq/mL | 10–25 | 26 nL.Eq/mL |
| 25-Hydroxy Vitamin D | 31 ng/mL           | 20–50           | 26 ng/mL       |
| L-spine, BMD (by DXA) | 0.349 g/cm² | 0.489 g/cm² |                |
| L-spine, Z-score (by DXA) | −4.7 | −2.9 |                |
| **Father**           |                    |                 |                |
| L-spine, BMD (by DXA) | 0.853 g/cm² |                |                |
| L-spine, T-score (by DXA) | −2.2 |                |                |

Laboratory and DXA results of the proband and her father at initial evaluation.

in summary, we identified two family members with low bone mass in the WNT-ligand, WNT1, have been
associated with a spectrum of skeletal dysplasias (Laine et al., 2013). The WNT gene family encodes signaling proteins that act in several
 crucial developmental and regenerative processes (Baron and Kneissel, 2013). The canonical WNT–β-catenin signaling pathway is required for
skeletal development, homeostasis, and remodeling. This pathway is activated by interaction of a WNT ligand with the transmembrane re-
ceptor Frizzled, and a co-receptor, LDL-receptor related protein (LRP). Ligand binding leads to accumulation of intracellular β-catenin, which
translocates to the nucleus where, in association with transcription factor 4 (TCF4) or lymphoid enhancer binding factor 1 (LEF1), it reg-
ulates a wide array of genes in a tissue-specific manner, and in bone,
leads to increased formation and reduced resorption (van Amerongen et al., 2008).

Initial reports of OI secondary to WNT1 mutations were associated with an autosomal recessive inheritance pattern (Laine et al., 2013; 
Keupp et al., 2013; Pyott et al., 2013; Fahimiinia et al., 2013; Aldinger et al., 2016; Faqieh et al., 2013; Won et al., 2017; Stephen et al., 2015).
Phenotypically, they manifested with moderate to severe OI (Table 2). Several patients also developed extra-skeletal manifestations, including 
nurologic and developmental abnormalities. A particularly severe phenotype was described in 2 sisters of a Lao Hmong family with severe 
ostenia, fractures in utero, and long bone deformities (Laine et al., 2013).

Autosomal dominant mutations have also been described (Laine et al., 2013; Keupp et al., 2013). Affected individuals have presented with
fragility fractures and low bone density without extra-skeletal features, as in the family described here. This case report highlights the
phenotypic spectrum of a heterozygous missense mutation in the WNT1 gene, in that the daughter had low-impact fractures in early childhood,
whereas the father sustained traumatic fractures primarily in young adulthood.

The differences in phenotypic expression of autosomal recessive and autosomal dominant WNT1 mutations points to a potential difference in
disease pathogenesis for these two types of mutations. One possibility is that heterozygous missense mutations encode an aberrant protein that interferes with signaling by the wild-type protein. Although much more severe phenotypes generally occur with biallelic mutations, there is usually no phenotype associated with heterozygous carriers in families with biallelic disease due to null mutations. This suggests that the product of only one wild type allele is sufficient for normal function, and that the mutation in our case is consistent with a dominant negative effect.

A heterozygous missense mutation in WNT1 previously described in a Finnish family (p.Cys218Gly) is in close proximity to the mutation in our case (Laine et al., 2013). Both mutations occur within the highly conserved WNT1 motif at residues 218–227 of the protein (C-[KR]-C-H-G-[LIVMT]-SG-x-C) (Fig. 1) (Laine et al., 2013). Four of the ten affected members of the Finnish family had lumbar spine z-scores of −2.0 or
less. The majority also had multiple vertebral fractures, low-impact peripheral fractures, and no extra-skeletal manifestations. A second
family has also been described with dominantly inherited osteoporosis due to a heterozygous mutation in close proximity to the WNT motif
(p.Arg235Trp) (Keupp et al., 2013). Affected members of this family had cortical and trabecular bone loss on high-resolution peripheral
quantitative CT and history of recurrent fractures.

Given the emerging understanding of bone disorders associated with mutations in WNT1, therapies specifically targeting this signaling
pathway offer potential therapeutic benefit. Clinical trials with a humanized anti-sclerostin antibody which activates WNT signaling have
shown beneficial effects on bone mineral density as well as significant fracture risk reduction in patients with osteoporosis (Krause et al.,
2010; Cosman et al., 2016). Other agents that enhance Wnt signaling, such as an anti-Dkk-1 antibody and SFRP inhibitors, continue to to be
evaluated for clinical efficacy (Kim et al., 2013).

In summary, we identified two family members with low bone mass and fractures associated with a heterozygous missense mutation in the
WNT1 gene. This specific mutation (p.Gly222Arg) has not been previously described, but occurs in a highly conserved region of the WNT1
molecule and is in close proximity to the Frizzled binding site.
### Table 2
Summary of WNT1 mutations.

| Reference | Gene mutation | Phenotype |
|-----------|---------------|-----------|
| **Monoallelic mutations** | | |
| Proband and her father | c.666G > A (p.Gly222Arg) | • 1 male, 1 female • Recurrent traumatic fractures • Low bone mineral density |
| Keupp et al. (2013) | c.703C > T (p.Arg235Trp) | • 2 males, 3 females • Recurrent fractures of vertebrae and ribs beginning in adolescence • Low bone turnover markers, and a reduction of trabecular and cortical bone |
| Laine et al. (2013) | c.652T > G (p.Cys218Gly) | • 4 males, 6 females • Severe early-onset osteoporosis • Low-impact vertebral and peripheral fractures |
| Fahiminiya et al. (2013) | c.946_949insAACA (p.Ser317Lysfs) and c.1063G > T (p.Val355Phe) | • 1 female • Short stature • Low bone density • Severe vertebral compression fractures • Multiple long bone fractures |
| Pyott et al. (2013) | c.506dupG (p.Cys170Leufs*6) and c.259C > T (p.Gln87*) | • 1 male • Multiple fractures • Poor feeding • Delayed development • Recurrent infections |
| Aldinger Et al (2016) | c.184C > T (p.Gln62*) and c.677C > T (p.Ser226Leu) | • 4 males, 2 females • Multiple fractures • Bone deformities • Intellectual disability • Neurological abnormalities including seizures, absence of speech, and inability to feed • Brainstem and cerebellar hypoplasia |
| Yeon Won et al. (2017) | c.369A > C (p.Glu123Asp) and c.457T > G (p.Cys153Gly) | • 1 female • Early onset recurrent fractures of vertebrae and extremities • Bone deformity • Short stature • Bluish sclera in some affected individuals |
| **Compound mutations** | | |
| Fahiminiya et al. (2013) | c.946_949insAACA (p.Ser317Lysfs) and c.1063G > T (p.Val355Phe) | • 1 female • Short stature • Low bone density • Severe vertebral compression fractures • Multiple long bone fractures |
| Pyott et al. (2013) | c.506dupG (p.Cys170Leufs*6) and c.259C > T (p.Gln87*) | • 1 male • Multiple fractures • Poor feeding • Delayed development • Recurrent infections |
| **Biallelic Mutations** | | |
| Keupp et al. (2013) | c.859dupC (p.His287Profs*30) | • 2 males, 1 female • Early onset recurrent fractures of extremities • Bone deformity • Reduction of bone density • Short stature • Blue sclera • Bluish sclera in some affected individuals |
| | c.529G > T (p.Gly177Cys) | • 1 female • Early onset recurrent fractures of vertebrae and extremities • Bone deformity • Short stature • Blue sclera |
| | c.624 + 4A > G | • 1 male • Multiple fractures of vertebrae and extremities • Bone deformity • Short stature • Blue sclera |
| | c.565G > T (p.Glu189*) | • 1 male • Early onset (in utero) recurrent fractures of vertebrae and extremities • Bone deformity • Short stature • Faint blue sclera |
| Pyott et al. (2013) | c.893T > G (p.Phe298Cys) | • 1 male, 2 females • Early onset of recurrent fractures of extremities and vertebrae • Bone deformity • Short stature • Faint blue sclera |
| | c.884C > A (p.Ser295*) | • 2 males • Early onset of recurrent fractures of vertebrae and extremities • Developmental delays • Brain deformities (1 proband) • Autism |
| | c.287_300delAGTCCGGAATCGC (p.Gln96Profs*54) | • 1 female • Multiple fractures, including lumbar vertebrae • Short stature • Developmental delays • Right ptosis • 2 females |

*(continued on next page)*
Table 2 (continued)

| Reference           | Gene mutation                                      | Phenotype                                                                 |
|---------------------|----------------------------------------------------|---------------------------------------------------------------------------|
| Fahiminiya et al. (2013) | c.428G > T (p.Cys143Phe)                          | Early onset multiple fractures, including vertebral compression            |
|                     |                                                    | Kyphoscoliosis                                                             |
|                     |                                                    | Severe short stature                                                       |
|                     |                                                    | Deformities of the long bones                                              |
|                     |                                                    | Intellectual disability                                                    |
|                     |                                                    | Absence of speech (one of the probands)                                   |
|                     |                                                    | Neurological deformities                                                    |
|                     |                                                    | Quadriplegic                                                               |
|                     |                                                    | 1 male, 1 female                                                           |
|                     | c.287_300del (p.Gln96Profs)                        | Short stature                                                              |
|                     |                                                    | Multiple long bone and vertebral compression fractures                     |
|                     |                                                    | 1 female                                                                  |
|                     |                                                    | Short stature                                                              |
|                     |                                                    | Multiple long bone and vertebral compression fractures                     |
| Faqeh et al. (2013)  | c.990C > A (p.Cys330*)                             | 1 male                                                                    |
|                     |                                                    | Short stature                                                              |
|                     |                                                    | Severe hypotonia                                                           |
|                     |                                                    | Disfigured skull                                                           |
|                     |                                                    | Bone deformities                                                           |
|                     |                                                    | Recurrent chest infection                                                  |
|                     |                                                    | Early recurrent fractures                                                  |
|                     |                                                    | Severe global developmental delay                                          |
|                     |                                                    | Short stature                                                              |
|                     |                                                    | Seizures                                                                  |
| Stephen et al. (2014) | c.525_536delCTTCGGCGGCT (p.Phe176_Leu179del)      | 2 males, 5 females                                                         |
|                     |                                                    | Early onset multiple fractures                                             |
|                     |                                                    | Bone deformity                                                             |
|                     |                                                    | Short stature                                                              |

Gene mutation and phenotypic correlation of reported monoallelic, compound and biallelic WNT1 mutations.

Fig. 1. Diagram of reported monoallelic and biallelic WNT1 mutations. Ribbon and space-filling diagram of the WNT1 molecule, depicted as a white ribbon in the grey space-filling model. WNT1 is engaged with Frizzled (yellow ribbon). The two views represent 180° rotations along a vertical axis. The conserved WNT domain (amino acids 218–227) is depicted in blue. The positions of bi-allelic mutations are depicted in green. Pink and purple represent the positions of known mono-allelic mutations (purple designates mutations within the conserved WNT domain, as detailed in Table 2). The white arrow points to the mono-allelic mutation described in the current report. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Conflict of interest

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