


ORIGINAL ARTICLE

Consanguineous-derived homozygous WNT1 mutation results in osteogenesis imperfect with congenital ptosis and exotropia

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Abstract

Background: Wnt signaling pathway plays an important role in promoting osteogenesis. WNT1 mutations have been considered as a major cause of osteogenesis imperfect (OI). We identified an OI patient with pathogenic consanguineous-derived homozygous WNT1 missense mutation.

Methods: We designed and applied a panel of known 261 genes associated with hereditary bone diseases for targeted next-generation sequencing to examine clinically diagnosed OI patients. Detected mutations were confirmed by Sanger sequencing.

Results: The female proband presented with severe OI with low bone density, multiple long bone fractures, short stature, and absence of dentinogenesis imperfect and brain malformation. She had congenital ptosis and exotropia with her left eye, and absence of blue sclera. The proband came from a consanguineous family and had a homozygous *WNT1* missense mutation (c.677C>T, (p.S226L)). In addition, three other compound heterozygous mutations (c.1729C>T in *FKBP10*, c.1958A>C in *FGFR3*, c.760G>C in *TRPV4*) were also detected in her family members.

Conclusion: We report the first identified case of consanguineous derived homozygous WNT1 mutation leading to severe osteogenesis imperfecta with congenital ptosis and exotropia.

KEYWORDS

congenital ptosis and exotropia, homozygous mutation, osteogenesis imperfect, WNT1

1 | INTRODUCTION

Osteogenesis imperfecta (OI) is an inherited disorder predominantly characterized by the occurrence of frequent fractures and osteoporosis. The phenotypic spectrum of OI patients can be variable from very mild types to severe and lethal forms.

Most cases of OI are autosomal-dominant inheritance caused by mutations in collagen type I gene encoding the predominant protein component of bone matrix (Aldinger et al., 2016; Forlino & Marini, 2016). Autosomal-recessive inheritance forms of OI are rare, accounting for about 20%–25%, and involve many different pathogenic mutations mainly encoding

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proteins related to collagen type I folding, modification, or matrix mineralization. *WNT1* is one of the genes causing recessive OI (Bardai, Moffatt, Glorieux, & Rauch, 2016).

Wnt signaling is a well-established pathway that regulates skeletal development and homeostasis. In human bone homeostasis, *WNT1* was identified as a key ligand in Wnt signaling pathway. Patients with homozygous *WNT1* mutations are found to have more frequent fractures, while patients carrying heterozygous *WNT1* mutations have early onset of osteoporosis (Baron & Gori, 2018).

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study was reviewed and approved by the ethics board committee at Enze Medical Center Group. All family members signed informed consent before blood sample collection.

2.2 | Study subjects

The female proband presented with severe OI was admitted to Enze Hospital of Enze Medical Center Group, Taizhou, Zhejiang Province. She had low bone density, multiple long bone fractures, short stature, and absence of dentinogenesis imperfect and brain malformation. She had congenital ptosis and exotropia with her left eye, and absence of blue sclera. The proband came from a consanguineous family. Other family members did not have phenotype of OI.

2.3 | DNA sequencing

Genomic DNA was isolated from sodium citrate anticoagulated blood by using DNA blood-extracting kits (TIANGEN, Beijing, China). Next-generation sequencing was performed by using Illumina MiSeq and NextSeq technologies. Subsequent enrichment and sequencing were done for 261 genes known to be associated with hereditary bone diseases. Post sequencing, high-quality data were used for downstream analysis. Additionally, subsequently Sanger sequencing was also performed with standard methodology to confirm the mutations of *WNT1* [NM_005430.3], *FKBP10* [NM_021939.3], *FGFR3* [NM_000142.4], and *TRPV4* [NM_021625.4].

2.4 | Clinical examinations

Bone mineral density (BMD) was measured on GE Medical Systems Lunar dual-energy x-ray absorptiometry (DXA). X-ray radiographs were performed on GE Definium 6000

DR. Computer tomography (CT) was performed on a GE Brightspeed 16 Power CT Scanner. MRI was performed on GE medical systems Sigan HDxt 1.5T. 25-OH Vitamin D level was analyzed by LC-MS/MS with AB SCIEX 3200MD.

3 | RESULTS

The proband, now 28 years old, was the second daughter of consanguineous parents. She had an asymptomatic female sibling of 36-year-old (Figure 1). On general physical examination, her head circumference as well as her weight was normal, but her height was less than 80 cm and more than 2-SD below the mean value. Her left eye was born with eyelid ptosis accompanied by high arched eyebrows, constant exotropia, and slow to light reflection (Figure 1). Half of her left pupil was covered by the drooping eyelid. Her uncorrected visual acuity index was 25 cm. Over time, radiographs showed severe osteopenia, vertebral compression fractures, kyphoscoliosis, multiple fractures, and long bones deformities, with sequelae (Figures 2 and 3). She was wheelchair-bound and unable to perform most daily living activities due to her bone abnormality. Hearing and ophthalmic evaluations showed no neurological findings. Brain MRI revealed some cerebra atrophy, but apparently normal cerebellum and brainstem. No significant asymmetry was observed in the brain MRI images (Figure 2). Radiographs showed she had mild restrictive airway disease. Her serum calcium, phosphorous, and alkaline phosphatase levels were 2.08 mmol/L, 1.05 mmol/L, and 107 U/L, respectively, all within the normal range. Her β -CTX level was significantly increased, indicating that type I collagen was degraded. However, her 25-OH VitD level was only 5.4 ng/ml and in severe deficient range (sufficient should be >30 ng/ml) (Table 1).

In this case, the proband was from a consanguineous family and had a homozygous missense *WNT1* [MIM: 164820] mutation. Next generation sequencing with known 261 genes associated with hereditary bone diseases revealed that her father, mother, sister, and sister's two children had *WNT1* mutation (c.677C>T, p.S226L). In addition, her family members had compound heterozygous mutations c.1729C>T in *FKBP10* [MIM: 607063], c.1958A>C in *FGFR3* [MIM: 134934], and c.760G>C in *TRPV4* [MIM: 605427]. Her 62-year-old father was found to have low BMD on dual-energy x-ray absorptiometry (DXA) but with normal spinal radiographs. Her 56-year-old mother also had early onset osteoporosis and the spinal radiographs showed a mild compression deformity. Her mother's height was normal at 160 cm, but her height dropped by 4 cm in recent years. She had chronic/recurrent back ache with slipped disc, bone spurs, and a history of two bone fractures, right shin bone, and carpal after trivial trauma, respectively.

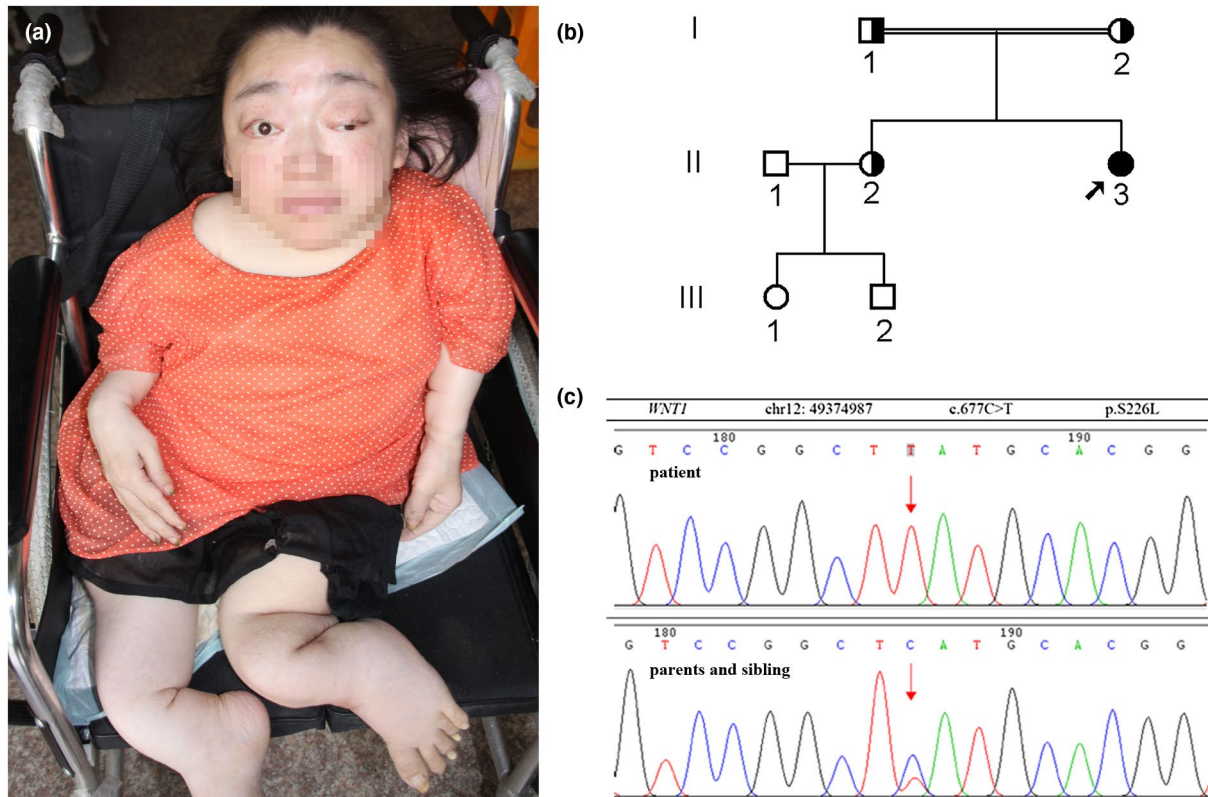


FIGURE 1 Clinical phenotype and Sanger sequencing results of the proband

4 | DISCUSSION

In this report, we investigated a homozygous missense *WNT1* mutation (c.677C>T, p.S226L) in a Chinese lady from a consanguineous family with additional compound heterozygous mutations (c.1729C>T in *FKBP10*, c.1958A>C in *FGFR3*, c.760G>C in *TRPV4*) segregating clinical features of OI. However, her father, mother, sister, and sister's two children, all with these heterozygous mutations, showed no features of osteogenesis imperfecta. We conclude that the homozygous *WNT1* mutation c.677C>T (p.S226L) is responsible for her severe osteogenesis imperfecta.

Wnt signaling pathway is involved in cell differentiation, proliferation, and migration. It was also reported that *WNT1* regulates gene expression in osteoblasts by activating canonical LDL related protein 5 and the WNT/ β -catenin signaling pathway (Gong et al., 2001; Li et al., 2018). There is evidence that classical *WNT1* signaling is essential for normal bone development and skeletal homeostasis (Keupp et al., 2013; Laine et al., 2013). Animal studies also indicate that overexpression of *WNT1* gene in osteocytes increases bone mass, whereas specific deletion of *WNT1* gene in osteocytes results in severe osteoporosis (Joeng et al., 2014, 2017). Patients with *WNT1* mutations were reported to have decreased bone density, thin ribs, recurrent fractures, short stature, and bluish sclerae (Faqeih, Shaheen, & Alkuraya, 2013; Stephen et al., 2015). Apart from severe bone fragility, a subset of these

patients also have learning disabilities, developmental delay, and central nervous system defects (Fahiminiya et al., 2013; Pyott et al., 2013).

Aldinger reported compound heterozygous *WNT1* mutations c.184C>T and c.677C>T in a Chinese boy with central nervous system defects. It was speculated that the p.S226L substitution in *WNT1* could possibly change the binding cavity around the mutant residues (Aldinger et al., 2016; Lu et al., 2018). *WNT1* mutations were reported to result in moderate to severe OI and some patients have ptosis (Lu et al., 2018; Nampoothiri et al., 2019). It was reported that patients with autosomal recessive *WNT1*-associated osteogenesis imperfecta have striking congenital ptosis (Nampoothiri et al., 2019). We identified the homozygous *WNT1* mutation leading to bone fragility and severe recessive forms of OI regarded as OI type XV. The proband came from a consanguineous family and the deformities of her eyes or fractures in her legs were noted at birth. To our knowledge, this is the first report of homozygous *WNT1* mutation leading to severe osteogenesis imperfecta and severe congenital ptosis and exotropia.

In this family, the mother with homozygous missense mutation in *TRPV4* (c.760G>C) experienced fractures twice, lost two teeth, began to show significant kyphoscoliosis, and dropped height by 4 cm in recent years. It is very possible that the homozygous missense mutation in *TRPV4* (c.760G>C) results in early onset kyphoscoliosis and backache. More studies are needed for the pathogenesis of

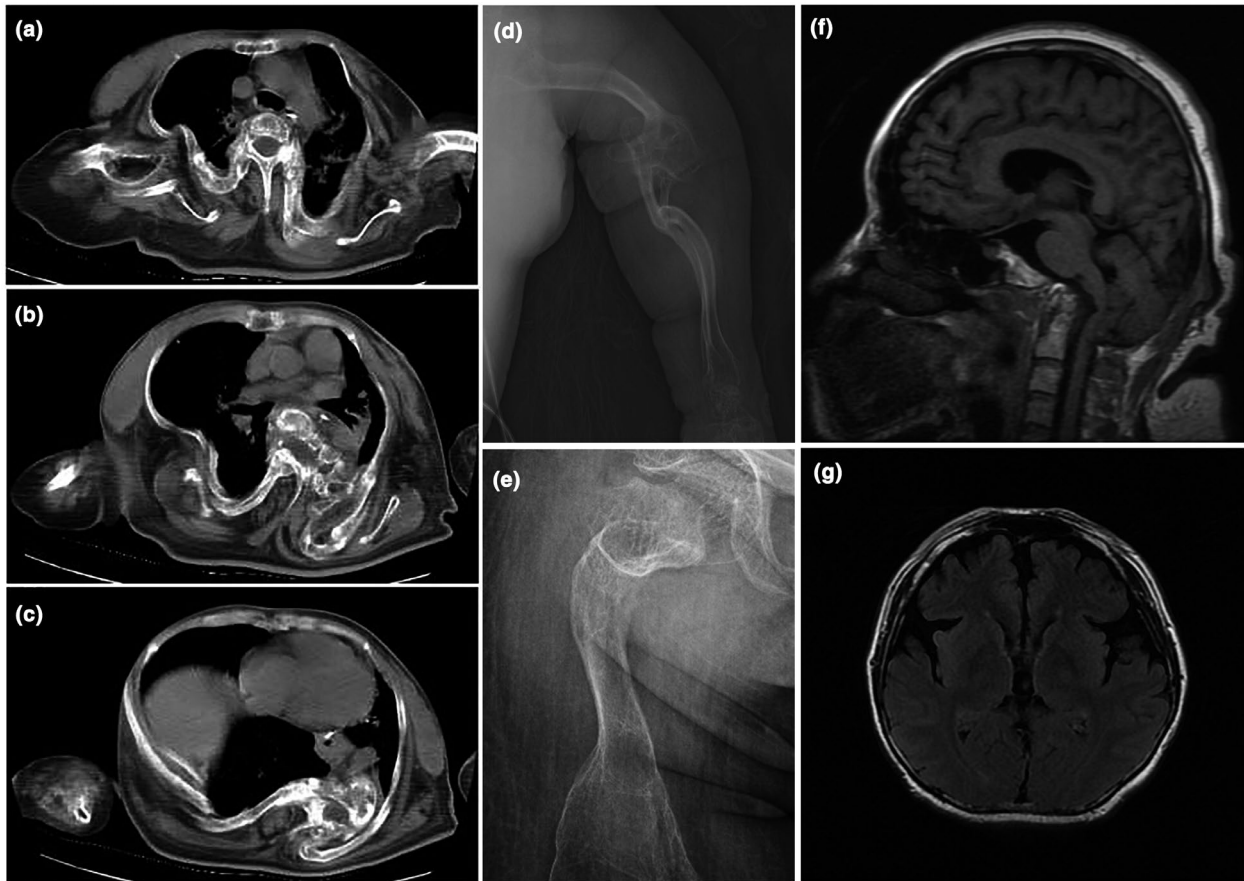


FIGURE 2 Results of radiographs and MRI examination

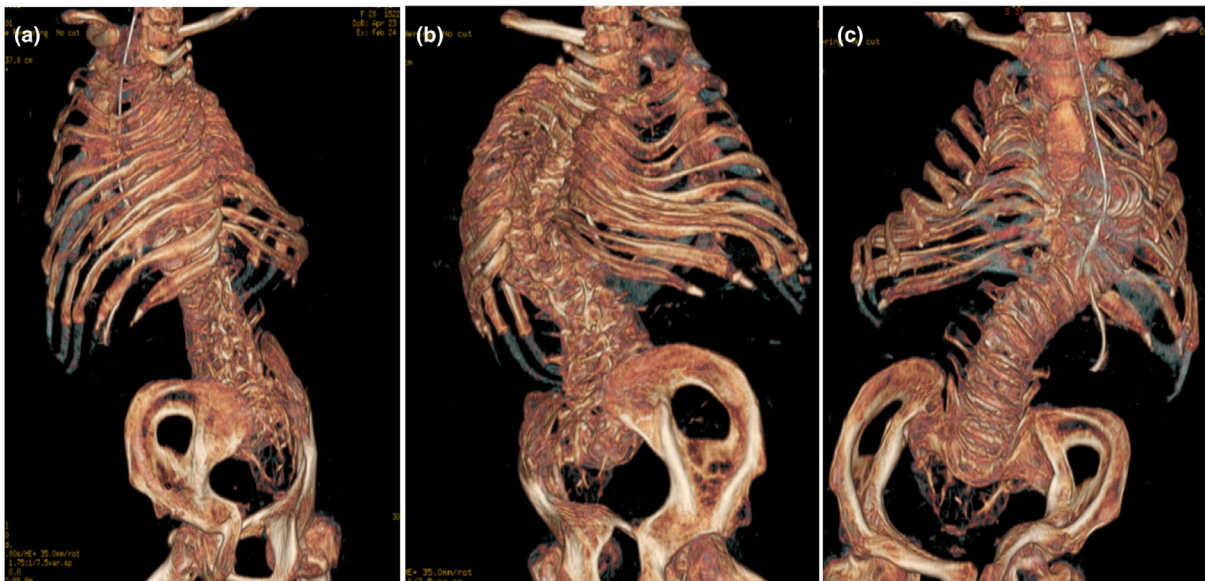


FIGURE 3 Computer tomography three-dimensional reconstruction of the upper body

kyphoscoliosis in patients with *WNT1* compound heterozygous mutations.

OI type XV is inherited in autosomal recessive form and there should be 25% higher risk of occurrence for siblings.

They should be evaluated by a multidisciplinary team including orthopedic surgeon, endocrinologist, and growth and development expert. Detection of carrier is possible once the DNA variants have been identified in the index

TABLE 1 Clinical findings of affected individuals in the present investigation

Affected individuals	I-1	I-2	II-1	II-2
Sex	Male	Female	Female	Female
Age	62	56	36	28
<i>Gene findings</i>				
c.677C>T in WNT1	Het	Het	Het	Hom
c.1729C>T in FKBP10	N	Het	N	Het
c.1958A>C in FGFR3	N	Het	Het	Het
c.760G>C in TRPV4	Het	Hom	Het	Het
<i>Skeletal findings</i>				
Multiple fractures	–	+	–	+
Vertebral fractures	–	+	–	+
Bowing of upper extremities	–	–	–	+
Bowing of lower extremities	–	–	–	+
Loss of tooth	–	+	+	+
<i>BMD z score</i>				
Lumbar spine	–2.5	–0.4	NA	–7.5
Hip	–1.9	0.2	NA	–7.5
<i>Laboratory findings</i>				
Vitamin D (ng/ml)	30.5	24.2	24.6	5.4
PTH (ng/ml)	33.5	25.3	31.3	23.7
β-CTX (ng/ml)	277.5	226.8	266.2	687
N-MID (ng/ml)	12.5	17.9	18.3	4.6
Ca (mmol/L)	2.44	2.39	2.35	2.08
P (mmol/L)	1.04	0.96	1.08	1.05
ALP (U/L)	119	132	125	107

case. Fortunately, the compound heterozygous mutations of c.677C>T in *WNT1*, c.1958A>C in *FGFR3* and c.760G>C in *TRPV4* have not been inherited to the next generation from other siblings in this family.

In summary, consanguineous derived homozygous *WNT1* mutations result in severe OI with severe congenital ptosis and exotropia.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Bo Shen conceived this study. Peng Chen recruited the patient. Yang Lu, Liping Shen, Kai Zhou, and Shenyi Ye performed the experiments. Jiayi Chen and Zhantao Yang interpreted and analyzed the data, and constructed and wrote the manuscript. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data supporting this study are available upon request.

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REFERENCES

- Aldinger, K. A., Mendelsohn, N. J., Chung, B. H., Zhang, W., Cohn, D. H., Fernandez, B., ... Curry, C. J. (2016). Variable brain phenotype primarily affects the brainstem and cerebellum in patients with osteogenesis imperfecta caused by recessive *WNT1* mutations. *Journal of Medical Genetics*, *53*, 427–430. <https://doi.org/10.1136/jmedgenet-2015-103476>
- Bardai, G., Moffatt, P., Glorieux, F. H., & Rauch, F. (2016). DNA sequence analysis in 598 individuals with a clinical diagnosis of osteogenesis imperfecta: Diagnostic yield and mutation spectrum. *Osteoporosis International*, *27*, 3607–3613. <https://doi.org/10.1007/s00198-016-3709-1>
- Baron, R., & Gori, F. (2018). Targeting *WNT* signaling in the treatment of osteoporosis. *Current Opinion in Pharmacology*, *40*, 134–141. <https://doi.org/10.1016/j.coph.2018.04.011>
- Fahiminiya, S., Majewski, J., Mort, J., Moffatt, P., Glorieux, F. H., & Rauch, F. (2013). Mutations in *WNT1* are a cause of osteogenesis imperfecta. *Journal of Medical Genetics*, *50*, 345–348. Retrieved from <https://jmg.bmj.com/content/50/5/345>
- Faqeih, E., Shaheen, R., & Alkuraya, F. S. (2013). *WNT1* mutation with recessive osteogenesis imperfecta and profound neurological phenotype. *Journal of Medical Genetics*, *50*, 491–492. <https://doi.org/10.1136/jmedgenet-2013-101750>
- Forlino, A., & Marini, J. C. (2016). Osteogenesis imperfecta. *Lancet (London, England)*, *387*, 1657–1671. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S014067361500728X>
- Gong, Y., Slee, R. B., Fukai, N., Rawadi, G., Roman-Roman, S., Reginato, A. M., ... Warman, M. L. (2001). LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell*, *107*, 513–523. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S0092867401005712>
- Joeng, K. S., Lee, Y. C., Jiang, M. M., Bertin, T. K., Chen, Y., Abraham, A. M., ... Lee, B. H. (2014). The swaying mouse as a model of osteogenesis imperfecta caused by *WNT1* mutations. *Human Molecular Genetics*, *23*, 4035–4042.
- Joeng, K. S., Lee, Y. C., Lim, J., Chen, Y., Jiang, M. M., Munivez, E., ... Lee, B. H. (2017). Osteocyte-specific *WNT1* regulates

- osteoblast function during bone homeostasis. *The Journal of Clinical Investigation*, 127, 2678–2688. Retrieved from <https://www.jci.org/articles/view/92617>
- Keupp, K., Beleggia, F., Kayserili, H., Barnes, A. M., Steiner, M., Semler, O., ... Wollnik, B. (2013). Mutations in WNT1 cause different forms of bone fragility. *American Journal of Human Genetics*, 92, 565–574. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S0002929713000827>
- Laine, C. M., Joeng, K. S., Campeau, P. M., Kiviranta, R., Tarkkonen, K., Grover, M., ... Makitie, O. (2013). WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. *The New England Journal of Medicine*, 368, 1809–1816. <https://doi.org/10.1056/NEJMoa1215458>
- Li, X., Yang, J., Bao, M., Zeng, K., Fu, S., Wang, C., & Ye, L. (2018). Wnt signaling in bone metastasis: Mechanisms and therapeutic opportunities. *Life Sciences*, 208, 33–45. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S0024320518303771>
- Lu, Y., Ren, X., Wang, Y., Bardai, G., Sturm, M., Dai, Y., ... Han, J. (2018). Novel WNT1 mutations in children with osteogenesis imperfecta: Clinical and functional characterization. *Bone*, 114, 144–149. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S8756328218302503>
- Nampoothiri, S., Guillemin, B., Elcioglu, N., Jagadeesh, S., Yesodharan, D., Suresh, B., ... Malfait, F. (2019). Ptosis as a unique hallmark for autosomal recessive WNT1-associated osteogenesis imperfecta. *American Journal of Medical Genetics Part A*, 179, 908–914. Retrieved from <https://onlinelibrary.wiley.com/doi/abs/10.1002/ajmg.a.61119>
- Pyott, S. M., Tran, T. T., Leistritz, D. F., Pepin, M. G., Mendelsohn, N. J., Temme, R. T., ... Byers, P. H. (2013). WNT1 mutations in families affected by moderately severe and progressive recessive osteogenesis imperfecta. *American Journal of Human Genetics*, 92, 590–597. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S0002929713000815>
- Stephen, J., Girisha, K. M., Dalal, A., Shukla, A., Shah, H., Srivastava, P., ... Phadke, S. R. (2015). Mutations in patients with osteogenesis imperfecta from consanguineous Indian families. *European Journal of Medical Genetics*, 58, 21–27. Retrieved from <https://linkinghub.elsevier.com/retrieve/pii/S1769721214001906>

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