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...
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], FKBPI0 [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 Signa 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 FKBPI0 [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 carpale after trivial trauma, respectively.
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 imperfect.

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 imperfect 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 imperfect 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 1 Clinical phenotype and Sanger sequencing results of the proband
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.
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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. Jiaxi 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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