Genetic diagnosis for osteogenesis imperfecta: abridged secondary publication

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KEY MESSAGES

1. Osteogenesis imperfecta (OI) is a rare hereditary connective tissue disorder, with an incidence of one in 15 000 to 20 000 newborns.
2. Identification of pathogenic mutations and genotype-phenotype correlations in Chinese patients with OI would expand the mutational spectrum of causative genes and enhance our understanding of bone development, enabling optimisation of targeted molecular-based therapies.
3. In patients with OI in southern China, variants in *COL1A1*, *COL1A2*, *IFITM5*, *FKBP10*, *WNT1*, and *P4HB* were identified. Correlations between genotype and clinical manifestations were also investigated to improve medical care.

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Introduction

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a rare hereditary bone dysplasia typically manifesting as skeletal fragility, deformity, and growth deficiency, with an incidence of 1 in 15 000 to 20 000 newborns. The clinical features of this connective tissue disorder affect multiple organs including pulmonary dysfunction, cardiac defects, dentinogenesis imperfecta, joint hypermobility, blue sclerae, and hearing loss.[1,2] OI is an autosomal dominant disease caused by *COL1A1* and *COL1A2*; other dominant and recessive genes related to diverse aspects of bone metabolism can also be involved, such as *CRTAP*, the first recessive gene known to cause lethal OI.[3] Newly identified recessive genes are designated with additional numbers for a more adaptive and comprehensive classification system. Current clinical therapies for OI are symptom-based and dependent on clinical severity. However, unclear effects on fracture occurrence and a lack of systemic clinical guidance for OI hinder their efficacy.[4] Identification of pathogenic mutations and genotype-phenotype correlations in Chinese patients would expand the mutational spectrum of causative genes and enhance our understanding of bone development, enabling the optimisation of targeted molecular-based therapies. This study aimed to identify causative mutations in an OI cohort to promote patient-centred medical care.

Methods

We recruited >200 patients with OI (including 80 patients and their parents/siblings) at The University of Hong Kong–Shenzhen Hospital for genetic diagnosis. We performed targeted amplicon sequencing to identify variants in genes present in most patients. Variants were identified based on specific variant filtration criteria. Detailed clinical features were documented by a panel of clinicians. We examined risk variant pathogenicity using a variety of cellular assays, including minigene splicing, luciferase, and scrambled RNase A assays. After the necessary procedures, human samples were collected for histological analyses.

Results

An OI genetic diagnosis workflow was used for variant identification (Fig). Considering the cost-effectiveness of genetic testing for most families, we first performed targeted amplicon sequencing to identify variants in genes present in most patients. We found that 119 patients carried pathogenic mutations in *COL1A1* (n=61), *COL1A2* (n=56), or both (n=2). These OI-COL1 patients predominantly demonstrated clinical subtypes I and IV. Six patients harbouring biallelic variants in *FKBP10* were identified: three children from a consanguineous family had a homozygous variant of *FKBP10* (c.918-3C>G) and another three patients diagnosed with OI had biallelic variants of *FKBP10*. Additionally, 23 patients were confirmed to have the *IFITM5* c.-14C>T mutation. Type V patients accounted for 10.6% of our cohort, comparable to the reported prevalence of type V OI among individuals of Chinese ethnicity. Of 25 (10.3%) patients harbouring pathogenic variants in the *WNT1* locus, 19 displayed

compound heterozygous variants and six showed homozygous variants. Moreover, two *de novo* heterozygous *P4HB* missense variants (c.524C>A, p.Ala175Glu and c.1200C>G, p.Cys400Trp) were identified in two unrelated patients with moderate OI.

Twenty critical clinical traits for OI patients, including blue sclerae, dentinogenesis imperfecta, hearing loss, and joint and skeletal abnormalities, were recorded. Bones from affected individuals displayed increasing porosity with disorganised collagen alignment from type I to types III and IV, suggesting that the severity of clinical manifestations was positively correlated with the degree of abnormal bone geometry. Missense mutations in *COL1A1/2* were associated with more severe clinical phenotypes.

The homozygous variant of *FKBP10* (c.918-3C>G) caused retention of intron 5 and deletion of exon 6 in the *FKBP10* mRNA isoforms. Compared with control cells, no FKBP65 protein could be detected in the mutant osteoblasts. These findings suggest that abnormal mRNA splicing results in a truncated FKBP65 protein. Compared with bone histology from a normal sample, the increased porosity in the cortical bones of *FKBP10* patients resulted in abnormal bone geometry, reduced mechanical toughness, and skeletal deformity.

WNT1 proteins could be detected after transfection with wild-type and mutant vectors. Four intact shifting bands indicated post-translational N-glycosylation required for WNT1 secretion.[5] Other than p.C151Y and p.S295L, most variants substantially affected WNT1 secretion capacity. WNT1 functions as an important ligand to activate the WNT/β-catenin signalling pathway. We measured WNT signalling activity induction by various mutant forms of WNT1. The transactivation function of this ligand was severely compromised by different amino acid substitutions. We measured the transcription level of *Axin2* to examine the cellular impact of WNT1 variants. Variants including p.G169D and p.L257P retained partial WNT1 transactivation activity. Other variants considerably reduced the mRNA level of *Axin2*.

To characterise *P4HB* variants, we first overexpressed PDI-Myc fusion protein in MC3T3 cells to observe the mutational impact on protein expression. An obvious additional band with high molecular weight (>170 kDa) appeared in PDI-Glu175 under both reducing and non-reducing conditions. Such strong intermolecular forces could only be disrupted after prolonging the boiling time during protein sample preparation. We next performed a co-immunoprecipitation assay to clarify the nature of the macromolecular complex. Consistent molecular weight band patterns suggested that the relevant component of the macromolecular

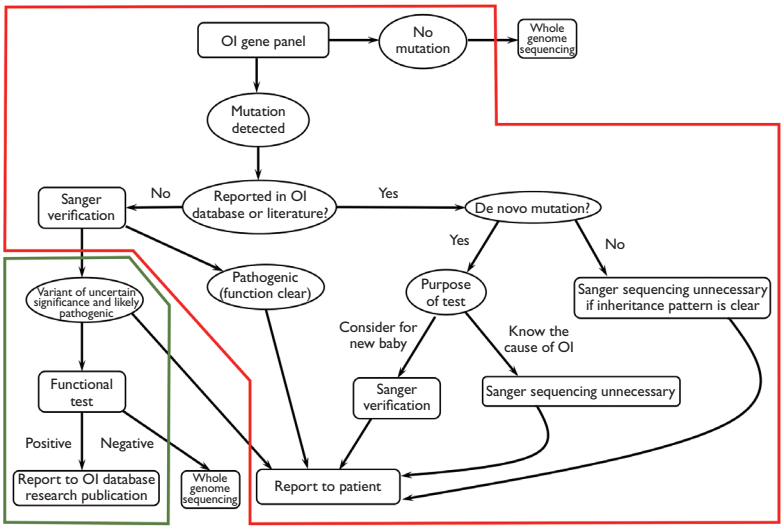

FIG. Flowchart depicting genetic diagnosis for patients with osteogenesis imperfecta (OI).

complex is a tetramer. The catalytic and chaperone functions of PDI homopolymers are substantially diminished due to the covering of substrate-binding sites. Isomerase activity was considered to indicate its biochemical function. We directly used proteins competitively eluted from A/G agarose beads (retaining the presence of tetramers) to reactivate scrambled RNase A. The results indicated that the isomerase activities of these two mutant forms were both reduced, but the function of PDI-Trp400 was more severely impaired.

Discussion

Considering the complex clinical and genetic features of OI, genotyping facilitates accurate diagnosis and informed genetic counselling. We detected a correlation between genotype and phenotype. We identified 102 unique mutations in *COL1A1* and *COL1A2*—most patients (63.6%, 119/187) carried variants in type I collagen. The relationship between genetic mutations and clinical severity in OI patients is complex, such that glycine substitutions and splicing mutations are the dominant variants, due to either structural defects (qualitative) or quantitative changes in type I collagen. Mild OI cases were caused by both quantitative and qualitative defects in type I collagen. Bisphosphonate treatment improved bone mineral density, particularly in patients aged 10 to 15 years; however, the effects of treatment on height were minimal.

We found that type V OI caused by the *IFITM5* (c.-14C>T) mutation was present in 10.6% of patients. Six patients with autosomal recessive variants

of *FKBP10* were identified; two of the variants (c.745C>T and c.825dupC) have not been previously reported. The c.918-3C>G variant resulted in intron retention and exon skipping during *FKBP10* mRNA splicing.

Our genetic analysis also identified 25 patients harbouring *WNT1* variants and two patients harbouring *P4HB* variants. Patients with *WNT1* variants primarily displayed moderate (type IV) to severe (type III) symptoms, whereas those with *COL1A1* and *COL1A2* variants predominantly exhibited types I and IV symptoms. This supports the association of autosomal recessive OI with more severe phenotypes. Secreted WNT ligands are cysteine-rich lipoproteins that bind to the Frizzled receptor and co-receptors (LRP5/6), triggering downstream pathways. Our mutagenesis assay indicated that amino acid substitutions in the WNT1 protein altered its secretory capacity and signalling activity, resulting in a wide spectrum of skeletal deformities. A more pronounced discrepancy was observed in the luciferase assay, implying that the variants have detrimental effects on WNT1 binding affinity and signalling activation.

P4HB encodes PDI, which can function as a monomeric oxidoreductase and molecular chaperone to catalyse the formation of inter-chain disulfide bonds for collagen fibril stabilisation and to prevent aggregation of premature triple helices, respectively. Moreover, PDI is identical to the beta subunit of prolyl 4-hydroxylase, a crucial enzyme responsible for proline residue hydroxylation during post-translational modification of collagen alpha chains. A stable complex was formed after substitution of the amino acid p.A175E. These two variants disrupted the biochemical functions of PDI. Dimers and even tetramers of PDI have repeatedly been reported, but the monomer is the most active form, suggesting that multimerisation weakens its substrate-binding capabilities. The variant p.C400W substitutes the second cysteine residue of the CGHC catalytic motif in PDI, leading to disruption of the disulfide bond between the two cysteine residues in the oxidised state. Thus, its deleterious effect on the function of PDI is more pronounced. Conceivably, therefore, *WNT1* and *P4HB* variants identified in our cohort might contribute to dysfunctions regarding bone formation and differentiation through multiple pathways.

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Disclosure

The results of this research have been previously published in:

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