



Review Article

Genotypes and Clinical Phenotypes of Osteogenesis Imperfecta

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Abstract

Osteogenesis Imperfecta (OI) is a rare genetic disorder clinically characterized by skeletal and bone deformity, low bone mass, impaired bone strength, connective tissue symptoms and several extraskelatal symptoms. Mutations in the two genes that encode type I collagen are the most common cause of OI. During the last decade, numerous novel causative genes involved in collagen biosynthesis, modification, and secretion, osteoblast development and function, and bone homeostasis have been linked to recessive and dominant forms of OI. As a result, OI has evolved into a group of hereditary disorders that shed light on the factors that influence both quantity and quality of bone. In this review the molecular genetics and the clinical phenotypes of all types of OI are described.

Keywords: Bone, Collagen type I, Fragility, Genetics, Osteogenesis imperfecta

Introduction

Osteogenesis Imperfecta (OI), also referred to as brittle bone disease, is an inherited skeletal disorder, that is caused by defective bone formation¹⁻². The term OI originates from Latin, which refers to imperfect bone formation and was introduced by Willem Vrolik who described a newborn with numerous fractures in 1840s³. It is a generalized connective-tissue disease caused by dominant or recessive mutations leading to reduced bone mass, susceptibility to fractures in the long bones and vertebrae and/or other skeletal manifestations such as substantial growth deficiency and variable malformation of long bones, ribs and spine⁴. Different secondary clinical features can also be developed during growth, often based on the underlying genetic mutation or the severity of the disease. These additional features include conductive or sensory hearing loss, abnormalities of tooth structure, called dentinogenesis imperfecta, blue or grey discoloration of the sclera, malocclusion, basilar invagination, scoliosis, pulmonary function impairment, cardiac valve abnormalities, muscle weakness and ligamentous laxity⁵. With the incidences being 1 in 15,000 in general population, OI is the most common skeletal disorder⁶.

Initially, the categorization of OI was solely based on

the skeletal characteristics. However, Smith and colleagues (1983) proposed a classification system that takes into consideration the age of the diagnosis. The patients that were born with fractures are considered to have congenital OI. When the diagnosis was made later and the fractures occurred after birth, the condition was categorized as OI tarda and were separated to gravis (fractures occurred in the first year) or levis (fractures occurred later in life). Later on, the Sillence classification, also known as the classic classification of OI, suggested 4 types of OI (I-IV). This classification was mainly based on the inherited mutations in *COL1A1* (OMIM #120150) and *COL1A2* (OMIM #120160) genes and the clinical characteristics of the patients⁷.

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Osteogenesis imperfecta	OMIM Phenotype	Gene	Inheritance	OMIM Gene	Location
Type I	166200	<i>COL1A1</i>	AD	120150	17q21.33
Type II	166210	<i>COL1A1</i>	AD	120150	17q21.33
		<i>COL1A2</i>	AD	120160	7q21.3
Type III	259420	<i>COL1A1</i>	AD	120150	17q21.33
		<i>COL1A2</i>	AD	120160	7q21.3
Type IV	166220	<i>COL1A1</i>	AD	120150	17q21.33
		<i>COL1A2</i>	AD	120160	7q21.3
Type V	610967	<i>IFITM5</i>	AD	614757	11p15.5
Type VI	613982	<i>SERPINF1</i>	AR	172860	17p13.3
Type VII	610682	<i>CRTAP</i>	AR	605497	3p22.3
Type VIII	610915	<i>P3H1</i>	AR	610339	1p34.2
Type IX	259440	<i>PPIB</i>	AR	123841	15q22.31
Type X	613848	<i>SERPINH1</i>	AR	600943	11q13.5
Type XI	610968	<i>FKBP10</i>	AR	607063	17q21.2
Type XII	613849	<i>SP7</i>	AR	606633	12q13.13
Type XIII	614856	<i>BMP1</i>	AR	112264	8p21.3
Type XIV	615066	<i>TMEM38B</i>	AR	611236	9q31.2
Type XV	615220	<i>WNT1</i>	AR	164820	12q13.12
Type XVI	616229	<i>CREB3L1</i>	AR	616215	11p11.2
Type XVII	616507	<i>SPARC</i>	AR	182120	5q33.1
Type XVIII	617952	<i>TENT5A</i>	AR	611357	6q14.1
Type XIX	301014	<i>MBTPS2</i>	XLR	300294	Xp22.12
Type XX	618644	<i>MESD</i>	AR	607783	15q25.1
Type XXI	619131	<i>KDELR2</i>	AR	609024	7p22.1
Unclassified Type	n/a	<i>PLS3</i>	XLR	300131	Xq23

n/a: not available

Table 1. Osteogenesis imperfecta types, genes involved in each type, location and inheritance.

Collagen type I is a crucial and the most prevalent structural protein in bone, tendon, and ligament. Type I procollagen is initially produced in the rough endoplasmic reticulum (rER) with C and N terminal propeptides. A triple helix is formed in the rER by the folding of the two alpha1 chains and one alpha2 chain, which are made up of Gly-X-Y triplets, where X and Y can be any amino acid but are often proline and hydroxyproline, respectively. Numerous enzymes modify collagen posttranslationally during folding, leading to the hydroxylation of lysine and proline residues and glycosylation of hydroxylysyl residues. Once the chain is completely folded, post-translational modification ends⁸. Proteases cleave the N- and C-terminal propeptides of procollagen molecule when folding is completed, and collagen molecules combine to form fibrils. The remaining

fibrillar molecule is integrated into the highly organized extracellular matrix, where it covalently links to nearby collagen molecules and telopeptide regions. This biological frame is then mineralized by hydroxyapatite, which makes the bone structure hard and compressible⁹.

The chains of type I collagen are encoded by the *COL1A1* and *COL1A2* genes, both composed of 52 exons. *COL1A1* is located on chromosome 17 and measures 18 kb, whereas *COL1A2* is located on chromosome 7 and measures 37 kb. Mutations in either *COL1A1* or *COL1A2* genes are responsible for most of the OI described cases¹⁰⁻¹². Quantitative and qualitative collagen deficiencies are the two main types of dominant collagen type I related OI. A quantitative deficiency with reduced but structurally normal collagen I due to a *COL1A1* haploinsufficiency is the most

common cause of OI type I. This may result from a premature termination codon or a frame-shift mutation following an insertion, deletion, or splice site mutation inducing nonsense mediated decay (NMD) of *COL1A1* mRNA¹³⁻¹⁴. On the other hand, mutations that involve the substitution of a glycine residue within the helix with a larger amino acid (80%) or mutations that affect splice sites (20%) leading to the skipping of exons or the appearance of cryptic splice sites, are the most common qualitative abnormalities¹¹. Glycine substitution by any other amino acid, causes the delay of helix formation, resulting to chain over modifications^{11,15}, abnormal extracellular matrix structure¹⁶⁻¹⁷, impaired cell-matrix¹⁸ and cell-cell interactions¹⁹, and intracellular stress²⁰. Mineralization is influenced by primary organic matrix pathology and OI is linked to alterations in the mineral phase of bone, such as abnormally shaped, sized, composed, and aligned mineral crystals²¹⁻²². Today the large number of causative genes of OI, led to a new genetic classification system that is now based on genetic data⁴.

Sillence classification (type I-IV)

Approximately 80-85% of OI cases are attributed to the Sillence types (types I-IV) with autosomal dominant inheritance, and they are associated with mutations in the *COL1A1* and *COL1A2* genes⁷.

Type I

Osteogenesis imperfecta type I (OI1) is the most common and usually the mildest form of Osteogenesis imperfecta type I (OI1) is the most common and usually the mildest form of OI including minimal skeletal deformity, late-onset hearing loss and not evident dentinogenesis imperfecta. Fracture incidence is often reduced in adulthood but may increase again at older stages of life, especially in women. A feature that is mainly found in type I is blue sclera, which causes a bluish discoloration of the eyes. The null allele mutation that was described above in *COL1A1*, is the most common cause of this form and there is an argument whether it should be a prerequisite for type I. However, a moderate helical glycine mutation can give the same clinical manifestations²³.

Type II

Osteogenesis imperfecta type II (OI2) is the most severe, commonly fatal, form of OI, accounting for about 5-10% of cases^{7,24-25}. Infants with OI type II are stillborn or die early by either a small thorax or fractures that cause respiratory insufficiency or pneumonia. Their arms and legs are abnormally short, their tissues are extremely fragile, they have low muscle tone and present severe deformity and multiple fractures²⁶. Qualitative mutations in collagen genes are generally the cause²⁷.

Type III

Osteogenesis imperfecta type III (OI3) accounts for 10% of OI cases²⁴⁻²⁵. Individuals with OI type III have a progressive

deforming variety, including scoliosis, short stature, bowing of long bones and white sclera, with reduced life expectancy as a result of pulmonary and cardiovascular mortality. OI III is caused by qualitative, mainly heterozygous mutations, in one type I collagen genes, *COL1A1* or *COL1A2*²⁸.

Type IV

Osteogenesis imperfecta type IV (OI4) severity ranges between types I and III and represents 10% of OI cases²⁴⁻²⁵. Individuals are moderately affected and have white sclera, abnormalities in bone structure, reduced height and severe dentinogenesis imperfecta⁷. Life expectancy is generally normal^{7,29}. OI IV is caused by heterozygous mutations in one of the genes for type I collagen, *COL1A1* or *COL1A2*.

Additional classification (type V-XXI)

The non-collagen cases taken together as a whole comprise approximately 10% of OI. The categorization of OI subtypes has been extended up to OI type XXI due to the large number of genes associated with OI (Table 1; ³⁰⁻³¹). Different phenotypes are reported even between patients with the same genetic alterations, making it challenging to match the molecular genetic categorization with the oversimplified Sillence classification (Table 2; ³²).

Type V

The severity of osteogenesis imperfecta type V (OI5) is moderate, and the clinical manifestation is comparable to type IV. Patients may develop an abundance of healing bone (hypertrophic callus) at sites where the bone is fractured. They also present improper bone connection between the two long bones of their forearms, limiting their hand motion. The most prevalent dominant mutation has been found in the 5'-untranslated region of the *IFITM5* gene (OMIM #614757), that codes for interferon-induced transmembrane protein 5 (IFITM5)³³. IFITM5 is involved in the differentiation of osteoblasts and the mineralization of bone. In addition, mutations in the coding area of *IFITM5* cause decreased mineralization of the bone³⁴.

Type VI

Osteogenesis imperfecta type VI (OI6) is of moderate severity, with clinical symptoms similar to type IV. Fractures occur later in life in patients. Scoliosis, spinal compression fractures and progressive bending of the arms and legs are all widespread among patients. In most cases, the sclerae and teeth are unaffected. There is a moderate to severe effect on height³⁵. Recessive mutations in the *SERPINF1* gene (OMIM #172860) are responsible for OI VI. The protein pigment epithelium-derived factor (PEDF) encoded by *SERPINF1* upregulates osteoprotegerin, a natural suppressor of osteoclastogenesis, by inhibiting RANKL. Although a homozygous loss-of-function mutation in the *SERPINF1* gene has no effect on collagen production or osteoblast differentiation, it causes a mineralization

Table 2. OI clinical characteristics and associated defective genes.

Type	Prenatal	Skeletal deformity	Fractures	Short stature	Scoliosis	Dentinogenesis imperfecta	Hearing loss	Sclera	Respiratory insufficiency	Gene
OI1	-	Moderate	+	-	-	-	+	Blue	-	<i>COL1A1</i>
OI2	+	Severe	+	+	-	-	-	-	+	<i>COL1A1/</i> <i>COL1A2</i>
OI3	-	Progressive	+	+	+	+	+	White	+	<i>COL1A1/</i> <i>COL1A2</i>
OI4	-	Moderate	-	+	-	+	+	White	-	<i>COL1A1/</i> <i>COL1A2</i>
OI5	-	Moderate	+	+	-	-	+	-	-	<i>IFITM5</i>
OI6	-	Progressive	+	+	+	-	+	-	-	<i>SERPINF1</i>
OI7	+	Moderate	+	+	-	-	-	White or blue	-	<i>CRTAP</i>
OI8	+	Severe	+	+	-	-	-	White	-	<i>P3H1 OR</i> <i>LEPRE1</i>
OI9	-	Moderate	+	-	-	-	-	-	-	<i>PPIB</i>
OI10	-	Severe	+	-	-	+	-	Blue	+	<i>SERPINH1</i>
OI11	-	Progressive	-	-	Progressive	-	-	-	-	<i>FKBP10</i>
OI12	-	Moderate	+	+	-	-	Progressive	White	-	<i>SP7</i>
OI13	-	Severe	+	+	-	-	-	Blue	-	<i>BMP1</i>
OI14	+	Moderate to Severe	+	-	-	-	-	-	-	<i>TMEM38B</i>
OI15	-	Progressive	-	+	-	-	-	Blue	-	<i>WNT1</i>
OI16	+	Moderate	+	-	-	-	-	Blue	-	<i>CREB3L1</i>
OI17	-	Moderate	+	+	+	-	-	-	-	<i>SPARC</i>
OI18	-	Severe	+	-	+	-	-	White or blue	-	<i>TENT5A</i>
OI19	+	Variable	+	+	+	-	-	-	-	<i>MBTPS2</i>
OI20	-	Progressive	+	-	-	-	-	-	+	<i>MESD</i>
OI21	In some cases	Progressive	+	+	+	-	-	-	-	<i>KDELR2</i>
UNCLASSIFIED	In some cases	+	+	+	+	-	-	Gray-white or blue	+	<i>PLOD2</i>
UNCLASSIFIED	-	n/a	+	n/a	n/a	In some cases	In some cases	Blue	n/a	<i>PLS3</i>

n/a: not available

deficiency. An imbalance in the RANKL/osteoprotegerin system causes osteoclasts to differentiate and activate more frequently, leading to an increased rate of bone loss³⁶⁻³⁷. The observation of overactive osteoclasts led to the development of a new therapeutic approach that allows for more precise treatment. Denosumab, a RANKL antibody, has been shown to benefit children with OI caused by *SERPINF1* mutations and is licensed for use in adults with osteoporosis³⁸.

Type VII

Osteogenesis imperfecta type VII (OI7) is a severe to fatal recessive OI type, phenotypically similar to types II-III. This type is associated with mutations in *CRTAP* gene (OMIM # 605497) encoding the cartilage associated protein (CRTAP). This protein belongs to a collagen-modifying complex that hydroxylates specific proline residues, namely Pro986 in the $\alpha 1$ -chain. The long bones of the majority of children are fractured before or shortly after birth. The majority of individuals rely on a wheelchair or other mobility aids³⁹⁻⁴⁰.

Type VIII

Osteogenesis imperfecta Type VIII (OI8) is frequently reported to cause severe phenotypes and cannot be distinguished from type VII. White sclera, significant growth insufficiency and a clinical history comparable to types II or III, characterize those who are affected. However, some patients are mildly harmed, have no fetal fractures, and are able to walk as they become older. Type VIII is the result of recessive mutations in the *P3H1* (OMIM # 610339) gene which encodes the LERPE protein, part of the same complex as *CRTAP*⁴¹.

Type IX

Osteogenesis imperfecta type IX (OI9) commonly causes multiple long-bone fractures, and is caused by recessive homozygous mutations in the *PPIB* (OMIM # 123841) gene encoding cyclophilin B (CyPB)⁴²⁻⁴³. Despite the fact that CyPB is part of the same collagen-modifying complex as *CRTAP* and *P3H1*, type IX includes a less severe spectrum than types VII and VIII⁴⁴.

The three abovementioned genes *CRTAP*, *P3H1* and *PPIB* (responsible for OI type VII, VIII and IX respectively) are three different prolyl-3-hydroxylase isoforms, members of a complex responsible for the proline-986 hydroxylation in the alpha 1 collagen chain. As mentioned previously, repetitive proline residues are abundant in the helical region of the collagen molecule. Alterations in the members of this complex lead to reduced proline-986 hydroxylation which in turn causes a delay in the folding of collagen, as well as in excessive modification⁴². Subsequently, aggregation of overmodified collagen and intracellular retention may induce ER stress and cell death. Furthermore, cyclophilin B secures the cis-trans isomerization of the collagen-prolyl-peptide bond during hydroxylation and, in combination with the molecular chaperone FKBP65 (or FKBP10 below), prevents the procollagen chains from forming premature fibrils.

Additionally, lysyl hydroxylase 1 (LH1) and cyclophilin B may interact, affecting collagen chains lysyl hydroxylation and intermolecular crosslinking⁴⁵.

Type X

Osteogenesis imperfecta type X (OI10) phenotypes range from severe to fatal and is caused by mutations in the *SERPINH1* (OMIM # 600943) gene, which codes for the collagen chaperone heat shock protein 47 (HSP47) and has been linked to lung illness and kidney stones⁴⁶. Heat shock proteins act as molecular chaperones that prevent improperly folded proteins from clumping together. Additionally, they are involved in the process of collagen chain interaction with larger fibrillar structures. Mutations in the *SERPINH1* gene cause protein misfolding and/or destabilization, leading to a delay in collagen secretion and abnormal collagen structure⁴⁷.

Type XI

Osteogenesis imperfecta type XI (OI11) includes brittle bones and abnormal joint mobility with variable severity. Common features include progressive scoliosis and kyphosis, deformed hips and normal hearing. Recessive mutations in *FKBP10* (OMIM #607063), which encodes FKBP65 (65-kDa FK506-binding protein 10), a collagen chaperone important for telopeptide lysyl hydroxylation, is found in people with a progressively deforming disorder⁴⁸. Additionally, mutations of this gene can result in a severe OI phenotype with no contractures. Despite appearing structurally normal, collagen may have stability abnormalities as suggested by some reports, leading to the build-up of procollagen aggregates in the ER⁴⁸.

Type XII

Osteogenesis imperfecta type XII (OI12) is an autosomal recessive disorder whose clinical characteristics include repeated fractures, modest bone abnormalities, teeth eruption occurs later than expected, and hearing is within the normal range. Type XII is caused by mutations in the *SP7/SOX* (OMIM #606633) gene. *SP7/SOX* gene encodes the osteoblast-specific transcription factor SP7 (or osterix), a factor that promotes the transition of pre-osteoblasts to osteoblasts and osteocytes. Early hearing loss can occur in patients with mutations in the SP7 gene⁴⁹. Enhanced bone porosity has been seen in these patients, which may be attributable to increased trabecular bone remodeling as a result of an imbalance between bone synthesis by osteoblasts and bone resorption by osteoclasts⁵⁰.

Type XIII

Osteogenesis imperfecta type XIII (OI13) is caused by recessive mutations in bone morphogenetic protein- 1, encoded by *BMP1* (OMIM #112264) that cleaves the C-terminal globular end of procollagen in the pericellular space. This type causes severe defects. These patients, demonstrate decreased procollagen processing and

formation of mature collagen fibrils, leading to enhanced collagen matrix mineralization and increased bone mass⁵¹. Additionally, a moderate form of OI with increased bone mass is caused by mutations in both collagen type I α chains that impact the BMP1 cleavage site⁵².

Type XIV

Osteogenesis imperfecta type XIV (OI14) is a moderate to severe OI type and individuals with osteopenia and fractures have normal sclerae and teeth, no gradual hearing loss and no other organ dysfunction⁵³⁻⁵⁴. Type XIV is caused by recessive mutations in the *TMEM38B* gene (OMIM #611236), which encodes for the monovalent cation channel TRIC-B (trimeric intracellular cation channel type B). This is an ER membrane integral channel which is involved in cell differentiation and is required for emptying intracellular calcium stores. Collagen modification in the ER is abnormally regulated, as a result of defective intracellular calcium release. Recessive mutations in the *TMEM38B* gene lead to ER stress and reduced collagen production.

Type XV

OI type XV (OI15) is progressively deforming and characterized by visible blue sclerae with normal teeth and hearing. Recessive mutations in *WNT1* (OMIM #164820), which encodes the secreted glycoprotein (wingless-type MMTV integration site family 1) WNT1, a crucial factor in the primary pathway of bone development and maintenance, cause OI type XV⁵⁵. When Frizzled and LRP5/6 (low-density lipoprotein receptor related protein 5/6) form a dual receptor complex and bind with WNT1, β -catenin is stabilized and transported to the nucleus, where it stimulates the expression of genes that govern osteoblast growth and function. *WNT1* mutations lead to an impairment in signal transduction, reduced bone cell homeostasis and thus to an imbalance between bone resorption and formation. Patients with these mutations have normal bone mineralization but impaired bone remodeling⁵⁶. Additionally, as *WNT1* is also expressed in the brain, individuals are frequently simultaneously impacted by central nervous system developmental problems and have variable range of cognitive impairment⁵⁶. Early onset osteoporosis has been observed in heterozygous *WNT1*-mutation carriers⁵⁷.

Type XVI

OI type XVI (OI16) is a severe form of OI in which fractures are evident at birth, and the long bones of the upper arms and legs bow inward. Severe OI has been linked to homozygous genomic deletion of the ER-stress transducer CR3L1 (encoded by *CREB3L1* (OMIM #616215), cyclic AMP-responsive element-binding protein 3-like protein 1). During ER stress, the N-terminal portion of CR3L1, which includes a transcription factor, is released by two metalloproteases (S1P and S2P), that function together in a sequential manner. This event initiates the unfolded protein response gene expression. The CR3L1 UPR element-like

sequence activates the *COL1A1* promoter region specific to osteoblasts, which is absent from the skin-specific *COL1A1* promoter area. As a result, mutations cause decreased collagen formation in bone cells but not in skin cells, partly accompanied by changes in the bone matrix's composition and hypermineralization. Prenatal fractures and protracted bone shortening are two clinical consequences of moderate to severe biallelic abnormalities. Patients with a heterozygous genotype have a milder form of the disease, with fractures occurring only after birth, and the majority can walk on their own, while some others develop no symptoms of skeletal illness⁵⁸.

Type XVII

Osteogenesis imperfecta type XVII (OI17) is caused by homozygous mutation in the *SPARC* gene (OMIM #182120). SPARC protein, is a secreted acidic and cysteine-rich protein, also known as osteonectin/BM-40 which acts as a molecular chaperone within cells during collagen formation. It is a key protein in the preservation of the bone mass and quality. So far, two missense mutations in the *SPARC* gene have been reported leading to delayed collagen secretion. Extracellularly, SPARC binds to collagen and hydroxyapatite, inducing extracellular matrix-cell interactions and promoting extracellular matrix mineralization⁵⁹.

Type XVIII

This type of OI (OI18) causes severe bone abnormalities, scoliosis, chest wall deformity and blue or white sclera. Mutations in the *TENT5A* gene (OMIM #611357; Otherwise: Family with sequence similarity 46 member A, *FAM46A*), which codes for the terminal nucleotidyltransferase 5A, have recently been linked with this OI type. *TENT5A* expression in osteoblasts shows a potential role in bone homeostasis and mineralization⁶⁰.

Type XIX

A mutation on the X chromosome causes osteogenesis imperfecta type XIX (OI19), which is a severe type. Prenatal fractures, growth deficit, variable scoliosis, and significant angulation of the lower leg bone are the symptoms. Site-2 protease (S2P) is a membrane bound zinc metalloprotease that is coded by *MBTPS2*, an X-linked gene (OMIM #300294). Missense mutations in *MBTPS2* at highly conserved S2P residues have been observed in two distinct kindreds with moderate/severe OI. When S2P is mutated, the regulated intramembrane proteolysis (RIP) of the transcription factors OASIS, ATF6, and SREBP is impaired, which is associated with decreased type I collagen secretion in the proband. Furthermore, the presence of SP2 mutations is linked to decreased hydroxylation of the lysine residue (K87) that is essential for crosslinking collagen in the proband bone tissue, likely due to reduced lysyl hydroxylase 1 levels in osteoblasts. The reduction in collagen crosslinks are thought to be detrimental to bone strength⁶¹.

Type XX

Osteogenesis imperfecta type XX (OI20) is a bone disorder that worsens over time and its main clinical features are osteopenia, skeletal deformities, and radiographic evidence of both new and previously healed fractures. A few patients have died as a result of respiratory failure. This type is linked to *MESD* gene (OMIM #607783; mesoderm development gene, formerly *MESDC2*) which encodes an ER chaperone for the LRP5 and LRP6 canonical WNT signaling receptors. A type of OI that results in increasing abnormalities has been reported in five independent families, with four frameshift mutations identified in *MESD*. Because of the hypomorphic alleles that are produced by these mutations, LRP5 and LRP6 trafficking is reduced but not entirely eliminated. Unlike patients who are fully deficient in LRP5, the affected individuals have normal eye development, and they do not experience limb or brain patterning abnormalities as mice without LRP6 do. However, they are more sensitive to reduced WNT signaling, which impacts bone mass accrual and dental patterning. In individuals suffering from *MESD*-related OI, biological treatments that boost WNT signaling via LRP5 and LRP6 may be beneficial⁶².

Type XXI

Osteogenesis imperfecta type XXI (OI21) is described by multiple fractures that frequently occur following minor trauma. Fractures in some affected individuals may be present at birth. Patients have disproportionately short stature and scoliosis and by adulthood, they are frequently wheelchair-bound⁶³. Bi-allelic pathogenic mutations in the *KDEL2* gene (OMIM #609024), which codes for a receptor involved in recycling proteins between the Golgi and ER, via COPI retrograde transport using a KDEL-like peptide, were shown to be the cause of OI in four families. *KDEL2*-mediated Golgi-ER transport is hampered by the inactive receptor caused by the *KDEL2* variations. In patient's primary fibroblasts HSP47, FKBP65 and procollagen type I levels were reduced and secreted collagen fibrils were abnormal, simultaneously with increased HSP47 binding to monomeric and multimeric collagen molecules. It is hypothesized that OI21 is caused by HSP47's inability to interact with *KDEL2* and to separate from collagen type I, resulting in impaired fiber production⁶³.

Unclassified Type

Recent cases of OI have been linked to pathogenic mutations in the *PLS3* gene (OMIM 300131) on the X chromosome. *PLS3* mutations were discovered in 2013 in five families with X-linked osteoporosis⁶⁴. *PLS3* contains 16 exons, is located on Xq23 chromosome and is widely expressed in solid tissues. The molecular mechanism of its role in the control of the skeletal development is still unknown. Since F-actin binding is the primary function of *PLS3*, it plays a role in all processes relevant to F-actin binding, including cell motility, cell division, endocytosis,

neurotransmission, focal adhesion, vesicle trafficking, axonal local translation, and intracellular calcium regulation⁶⁵⁻⁶⁶. Recently, it has been proposed that *PLS3* plays a part in the mineralization process of the bone. Recurrent fractures are a common presenting symptom in patients with *PLS3* mutations, although extraskeletal OI symptoms are infrequent. Males with *PLS3*-induced OI experience more severe symptoms than females with the same condition, caused by its X-linked inheritance. However, the exceedingly low prevalence, genotypic variability, and phenotypic variation of the skeletal disease linked to the *PLS3* mutation prevented a thorough understanding of the condition⁶⁷. *PLS3* is crucial for the formation of neuromuscular connections, and its aberrant expression may also be the cause of the altered gait pattern⁶⁸. Low bone mineral density, frequent fractures, and sometimes additional symptoms outside the skeletal system, including joint hypermobility, blue sclerae, hearing impairment, and dentinogenesis imperfecta, were indicated to make up the *PLS3* mutation's phenotype in recent studies. Peripheral fractures and vertebral compression fractures appeared to be the clinical hallmarks and the initial manifestation of the condition in patients with *PLS3* mutations. In conclusion, X-linked OI caused by *PLS3* mutations was clinically distinguished by repeated fractures. Treatments for high blood pressure may assist these patients' bone mineral density increase⁶⁹. However, more research is required to determine whether *PLS3* mutations and phenotypes are related. *PLS3*'s functions in maintaining bone homeostasis are still unclear.

Discussion

Over the last decade, there has been a major progress in identifying novel genes and comprehending the molecular mechanisms responsible for OI. To develop targeted therapies for various OI symptoms, a thorough understanding of the mechanisms underlying OI development and pathogenesis is necessary. The "traditional" approach for OI diagnosis was based on cultured skin fibroblasts to identify reduced or aberrant synthesis of abnormal type I (pro)collagen molecules, which was assessed by gel electrophoresis¹². In addition, cultured fibroblasts could be used to study RNA splicing and unclassified variations of OI. Nowadays, next-generation sequencing (NGS) has come to the forefront in the laboratory diagnosis of OI, as the advantages that the information offers and the lower costs, make easier the identification of novel genetic factors responsible for OI.

The current therapeutic options for OI are limited. OI patients have bones that are shorter than usual, thin cortices that might exhibit bending and flaring. As a result, the patients with more severe cases are more likely to undergo physical therapy and surgical treatment that involves repairing bone fractures to increase their self-sufficiency. Osteotomies and stabilization with intramedullary implants or plates are the most common surgical corrections for the aforementioned abnormalities. Unfortunately, due to the exceedingly

weak bone, osteosynthesis is vulnerable to failure, and complications are highly common in these individuals. Fragility fractures can be treated surgically or conservatively, although there is little evidence to suggest which is the best choice. Casts, braces, splints, and bandages are used for conservative therapy⁷⁰. Scoliosis is seldom treated with bracing and corrective surgery is required, however the best procedure is yet unknown⁷¹. For some people, stabilizing the basilar impression is required, although it hardly leads to clinically substantial compression⁷². Bisphosphonate therapy aiming at reducing bone turnover is the major pharmaceutical therapy for dominant and possibly also recessive OI. However, it is unclear if the observed rise in bone mineral density leads to a decrease in fractures and an improvement in function⁷³. Reduced height in OI type III and IV is being studied with growth hormone therapy⁷⁴.

The ideal approach for treating OI, would be to combine stem cells with gene therapy⁷⁵. In a few people with severe dominant OI, clinical studies with bone marrow transplantation and mesenchymal stem cell transplantation have been done. During the first six months after infusion, in five of six patients', engraftment in one or more locations, including bone, skin, and marrow stroma, increased growth velocity was reported. The median of improvement was 70% and no significant toxicity was observed besides an urticarial rash in one patient shortly after the second infusion⁷⁶. Recent findings suggest that it is possible to directly reprogram a somatic cell into a specific type of differentiated cell, without the need for it to first transform into a stem or progenitor cell⁷⁷. The most promising therapeutic method for dominant and recessive OI is to transplant patient fibroblasts that have undergone a process of dedifferentiation and subsequent redifferentiation into osteoblasts, resulting in cells that lack the genetic abnormality. The removal of mutant alleles in the cells appears to be the most difficult obstacle to overcome in dominant OI. However, gene therapy may be more effective in treating recessive OI as defective molecules are not present in the bone matrix⁷⁵.

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