

# Animal models of osteogenesis imperfecta: applications in clinical research

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**Abstract:** Osteogenesis imperfecta (OI), commonly known as brittle bone disease, is a genetic disease characterized by extreme bone fragility and consequent skeletal deformities. This connective tissue disorder is caused by mutations in the quality and quantity of the collagen that in turn affect the overall mechanical integrity of the bone, increasing its vulnerability to fracture. Animal models of the disease have played a critical role in the understanding of the pathology and causes of OI and in the investigation of a broad range of clinical therapies for the disease. Currently, at least 20 animal models have been officially recognized to represent the phenotype and biochemistry of the 17 different types of OI in humans. These include mice, dogs, and fish. Here, we describe each of the animal models and the type of OI they represent, and present their application in clinical research for treatments of OI, such as drug therapies (ie, bisphosphonates and sclerostin) and mechanical (ie, vibrational) loading. In the future, different dosages and lengths of treatment need to be further investigated on different animal models of OI using potentially promising treatments, such as cellular and chaperone therapies. A combination of therapies may also offer a viable treatment regime to improve bone quality and reduce fragility in animals before being introduced into clinical trials for OI patients.

**Keywords:** OI, brittle bone, clinical research, mouse, dog, zebrafish

## Introduction

Osteogenesis imperfecta (OI), commonly known as brittle bone disease, is a genetic disorder characterized by extreme bone fragility, even if only exposed to mild trauma. The earliest known case of OI to occur in a human was determined by Peter Gray in 1969 in his analysis of bones of an approximately 3,000-year-old mummified infant.<sup>1</sup> However, it was only in 1788 that OI became recognized as a human disease, when it was given its first true pathological description.<sup>1</sup> By 1979, researchers had classified OI into four different types (I–IV), and identified OI I and IV to be caused by an autosomal-dominant gene and OI II and III to be caused by an autosomal-recessive gene, but the exact gene sequences responsible had not yet been determined.<sup>2</sup> Advances in the field of genetics have made it easier to characterize genetic sequences, and the genes linked to the development of OI have since been identified for all of the four originally defined types of OI, in addition to several of the more recently classified types.<sup>3</sup> This has been a major step in the advancement of clinical treatments for OI, as it is easier to identify and generate appropriate animal models for OI research by targeting specific genetic defects.

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## OI types I–IV Characteristics in humans

When OI was first categorized into types I–IV, the description that was given to type I was “dominantly inherited OI with blue sclerae”, and included such characteristics as having a history of broken bones from a very young age, bowing of long bones in lower extremities, bruising easily, and hearing impairment.<sup>2</sup> Additional features observed in persons with OI I include being of normal height or slightly short in stature and not suffering from dentinogenesis imperfecta (DI).<sup>4</sup> OI II was labeled “lethal perinatal OI with radiographically crumpled femora and beaded ribs”, and was characterized as being a fatal condition either in utero or shortly after birth.<sup>2</sup> Features observed in individuals with OI II include multiple fractures at birth, obvious deformities, flattened long bones, and dark sclerae.<sup>3</sup> OI III, referred to as “progressively deforming OI with normal sclerae”, was characterized as having normal sclerae, distorted and fragile bones that progressively degenerated over time, ligament hypermobility, and a predisposition to DI.<sup>2</sup> Also, individuals with OI III tend to be very short in stature and have a triangular face and severe scoliosis.<sup>3</sup> Finally, OI IV was originally classified as “dominantly inherited OI with normal sclerae”, and had similar defining characteristics to OI I, but with normal sclerae, reduced likelihood of spontaneous bone fractures, and no hearing loss.<sup>2</sup>

Therefore, in terms of severity, OI I–IV are ordered II>III>IV>I. Although OI I–IV are vastly different in how they affect humans, they all have a similar genetic basis.<sup>4</sup> Specifically, OI I is caused by a premature stop codon in COL1A1 and OI II–IV are caused by glycine substitutions in either the pro- $\alpha_1$ (I) or pro- $\alpha_2$ (I) collagen chains of COL1A1 or COL1A2.<sup>3</sup> It has been determined that the phenotypes for OI I–IV depend on the location of the mutation in the COL1A1 and COL1A2 genes; however, several different mutations occurring at different points in the sequence can lead to the same phenotype of OI.<sup>5</sup>

## Animal models

### Mov-13 mouse: OI I and II

The Mov-13 mouse was first created by blocking transcription of the COL1A1 gene by integrating the Moloney leukemia virus at the 5' end of the gene in what was referred to as the Mov-13 locus,<sup>6</sup> later shown to be the murine equivalent to human Col1a1.<sup>7</sup> The Mov-13 mutation in mice results in a failure to produce type I collagen.<sup>8–10</sup> The homozygous (Mov-13<sup>-/-</sup>) form of this mutation is lethal to mice.<sup>6,7,9</sup>

Therefore, the Mov-13<sup>-/-</sup> mouse is considered a model for OI II,<sup>11</sup> but unfortunately it is unable to duplicate properly the development seen in humans.<sup>12</sup>

The heterozygous Mov-13 mutation (Mov-13<sup>+/-</sup>) is associated with a reduced amount of collagen in soft connective tissue, increased hearing loss over time, and a substantial reduction in both mechanical and material properties of long bones similar to that seen in humans affected by OI I.<sup>13</sup> A disorganized cortical lamellar structure in Mov-13<sup>+/-</sup> mice has been observed,<sup>12</sup> with a 22%–25% reduction in tissue-bending strength due to disrupted damage accumulation mechanisms in Mov-13<sup>+/-</sup> cortical bone.<sup>14</sup> While Mov-13<sup>+/-</sup> exhibits a 50% decrease in type I collagen production, the collagen that is produced has normal amounts of both pro- $\alpha_1$ (I) and pro- $\alpha_2$ (I) chains,<sup>13,15</sup> making Mov-13<sup>+/-</sup> mice a proper model for OI I in humans.<sup>16</sup>

### *oim* mouse: OI I and III

One animal model of OI not created through the process of genetic engineering is the OI murine (*oim*) model. (The other five naturally occurring mutations are the G208A Col1a1 Golden Retriever, Col1a2 frameshift Beagle, SerpinH1 Dachshund, Chi zebrafish, and Frf<sup>-/-</sup> zebrafish.) It was produced in the Jackson Laboratory (Bar Harbor, ME, USA) as a result of breeding C3H/HeJ and C57BL/6JLe mice.<sup>12</sup> The mice present a spontaneous mutation in type I collagen structure with a deficiency of the pro- $\alpha_2$ (I) chain, and exhibit phenotypic and biochemical features typical of the most debilitating, nonlethal forms of OI.<sup>12</sup> Characteristics observed in the homozygous *oim* mice (*oim/oim*) include susceptibility to fractures from a very young age, severe osteopenia, cortical thinning, bowing of long bones, bone deformities, joint laxity, small body size, and kyphosis.<sup>12</sup> Their bones have altered collagen cross-links,<sup>17</sup> reduced mineral size and heterogeneity,<sup>18</sup> reduced lamellar structure,<sup>19</sup> and increased vascular and osteocyte lacunar density.<sup>20</sup> *oim/oim* bones have inferior mechanical properties compared to wild-type (+/+) counterparts.<sup>17,20–22</sup> Reduced fracture toughness, with a flat crack path and quick propagation, has been observed in these bones.<sup>17</sup> Similar characteristics have also been observed in OI III in humans,<sup>23–26</sup> for which the *oim/oim* mouse is a model.<sup>12</sup> Compared to *oim/oim*, the heterozygous *oim* mouse (*oim*<sup>+/-</sup>) does not experience spontaneous fractures, but does have bone material properties that lie in between those found in *oim/oim* and wild-type mice, and is thus considered a model of OI I in humans.<sup>27</sup>

Not only bones but also tendons are weaker in the *oim* mouse, as they are composed of collagen type I. Tensile

testing performed on the tail tendons of *oim/oim* and *oim*<sup>+/+</sup> mice revealed a twofold reduction in the ultimate tensile strain compared to wild-type mice.<sup>28</sup>

### G610C mouse: OI I and IV

In 2004, the Cre/Lox strategy, a method for genetically engineering animals for biomedical research, was combined with embryonic stem cells to mutate a section of murine *Colla2* in the stem cells, resulting in G610C-positive stem cells that were then injected into the mouse strain C57BL/6J (B6) blastocysts.<sup>29</sup> Mice in the founder generation that retained the neomycin-targeting vector were termed Neo<sup>+</sup> mice, and offspring from founder-generation males with Cre recombinase-expressing females were called Neo<sup>-</sup> mice.<sup>29</sup> The founder line of G610C mice was homozygous, and is now commonly known as G610C/G610C<sup>Neo+</sup>, whereas heterozygous mice are known as +/G610C<sup>Neo+</sup>.<sup>11</sup> The common name for the Neo<sup>-</sup> mouse (+/G610C<sup>Neo-</sup>) is the Amish mouse, because it models a large human Amish population consisting of 64 individuals exhibiting OI I/IV in both genetic code and phenotypic expression.<sup>11,29</sup> Phenotypic characteristics of the mutant mice include decreased body weight, bone mineral density, and bone volume.<sup>29</sup> Additionally, a four-point bending test of femora indicated a decrease in mechanical properties with reduced stiffness, postyield ultimate displacement, failure load, and energy to failure compared to wild type.<sup>29</sup> Therefore, G610C/G610C<sup>Neo+</sup>, +/G610C<sup>Neo+</sup>, and +/G610C<sup>Neo-</sup> are all acceptable models of OI IV, and +/G610C<sup>Neo+</sup> is also considered a model for OI I.<sup>11</sup>

### G859C *Colla1* mouse: OI II

In 1988, a site-directed mutagenesis approach was used to prepare transgenic mice expressing a mutated *COL1A1* gene that resulted in perinatal death.<sup>11,30</sup> The mutation was introduced to *COL1A1* at residue 859 in the triple helical domain of the gene.<sup>11,30</sup> Inspection of fetuses' radiographs showed short and wavy ribs, poor bone mineralization, underdeveloped skeletons, and pliable limbs.<sup>11,30</sup> There were also large cavities noted in the bones of the mice, indicating that a high level of resorption had occurred.<sup>11,31</sup> Due to the disease characteristics expressed in the G859C mutagenic mice, they have been accepted as a model for OI II.<sup>11</sup>

### Aga2 mouse: OI II and III

A strain of mutagenic mice produced according to the standards of the German Mouse Clinic (<http://www.mouseclinic.de>), a system for the phenotyping of mutant mouse lines,<sup>32</sup> was created using the Munich *N*-ethyl-*N*-nitrosourea

mutagenesis protocol.<sup>11,33</sup> This new line was called the Aga2 (abnormal gait 2) mouse, as the Aga2<sup>+/+</sup> mice that were first produced exhibited an abnormal gait as a result of excessive hind-limb deformity.<sup>33</sup> The heterozygous (Aga2<sup>+/+</sup>) mice expressed a range of phenotypic variation from skeletal deformities, fractures, fragility, disorganized trabecular bone and collagen structure, and osteopenia to perinatal death.<sup>33</sup> Aga2<sup>+/+</sup> mice that did not survive were classified as phenotypically similar to OI II.<sup>33</sup> Homozygous (Aga2<sup>-/-</sup>) mice that were produced from inter se breeding of Aga2<sup>+/+</sup> males and females all died in utero around embryonic stage E9.5.<sup>33</sup> Although the original research published on the Aga2 mice did not say which types of OI the Aga2<sup>+/+</sup> mice that survived to adulthood and the Aga2<sup>-/-</sup> mice were similar to, they have since been considered to be appropriate models for OI III and II, respectively.<sup>11</sup> However, it needs to be noted that although the mutation affects *Colla1*, there has not yet been an equivalent genetic mutation identified in humans.<sup>11</sup>

### BrtIII and BrtIV mice: OI II and IV

In 1999, two phenotypes of mice were created using the Cre/Lox system.<sup>34</sup> Initially, the researchers' intent was to use a recombination strategy to substitute a cysteine for glycine at position 349 in type I collagen and create a mouse model of OI I, but the floxed stop-cassette placement instead led to the expression of an allele that was supposed to be suppressed.<sup>34</sup> Two chimeric males were obtained from this process, and after being mated with wild-type females, the resulting offspring that expressed the heterozygous mutation were described as having similar weight and size as healthy littermates, but they all died within hours of birth, and X-rays and skeletal staining revealed that they had multiple rib fractures, short vertebral bodies, and poor skull mineralization.<sup>34</sup> Due to the fact that the phenotype expressed in these mice was similar to OI II in humans, this initial strain of mice was named Brittle II (BrtII).<sup>34</sup>

To remove the stop cassette that led to the BrtII phenotype, the two chimeric males were mated with EIIa-Cre mice, a strain of mice carrying a transgene mediated by the adenovirus EIIa promoter to target Cre-recombinase expression, and the resulting male founder-generation offspring that expressed the heterozygous mutation (F1-mosaic) were mated with wild-type CD1 and C3H/HeJ female mice to produce an F2 generation.<sup>34</sup> There was large variability in phenotype noted in the mutant F2 mice, ranging from perinatal death to long-term survival, and their bones exhibited deformities, fragility, osteopenia, and a disorganized trabecular structure.<sup>34</sup> These heterozygous mutant mice expressed the

mutation that had been desired in the original recombination strategy, and were termed *BrtlIV* mice.<sup>34</sup> *BrtlIV*<sup>+/+</sup> mice have geometrically smaller bones with reduced cortical width and lower trabecular thickness and bone-volume density compared to wild-type mice, increasing their vulnerability to fracture.<sup>35</sup> Femoral elastic modulus, strength, and stiffness increase after puberty independently of the whole-bone geometry in *BrtlIV*<sup>+/+</sup> mice with values comparable with wild-type mice.<sup>16</sup> This strengthening is due to an increase in the material properties of the extracellular matrix without any typical corresponding structural adaptations, which means that *BrtlIV*<sup>+/+</sup> mouse femora still have a geometric organization that is less resistant to applied loads.<sup>16</sup> Mice also showed decreased molar volume and mineralized tissue volume in the teeth, although enamel properties were normal.<sup>36</sup> The *BrtlIV* mice are heterozygous mice (*BrtlIV*<sup>+/+</sup>);<sup>34</sup> however, there is also a homozygous (*BrtlIV/BrtlIV*) type that exhibits a much milder form of OI than *BrtlIV*<sup>+/+</sup>.<sup>11,37</sup>

#### Human *COL1A1*-minigene mouse: OI II–IV

A transgenic animal model of OI was produced by generating a minigene construct composed of sequences extracted from human *COL1A1* that were missing a central region containing 41 exons,<sup>38,39</sup> encompassing exons 6–46.<sup>40</sup> The construct resulted in shortened pro- $\alpha_1$ (I) chains that ultimately prevented folding of the protein into a triple helix, leading to a lethal form of OI.<sup>38,39</sup> Without proper folding, degradation of the three chains through procollagen suicide occurs, and leads to the depletion of normal pro- $\alpha$ (I) chains.<sup>39,41</sup> Mice expressing high levels of the minigene exhibit a perinatal mortality rate up to 90%.<sup>41</sup> However, mice that only express a moderate minigene level tend to have a less severe phenotype, including low bone mineral density and collagen content, and a susceptibility to fractures.<sup>38</sup> Therefore, human *COL1A1*-minigene mice can be considered a model for OI II–IV.<sup>11,38</sup>

#### G208A *Col1a1* Golden Retriever: OI III

Another naturally occurring animal model of OI is the G208A *Col1a1* Golden Retriever, which was observed in a 12-week-old puppy that presented with DI and multiple bone fractures, including ribs and long bones.<sup>11,42,43</sup> Samples of collagen type I taken from the puppy's skin showed that the collagen was not functioning normally.<sup>42,43</sup> After it was determined that the puppy had naturally inherited a canine form of OI, samples of *COL1A1* from both a healthy canine and the puppy with OI were sequenced and compared, and it was determined that the puppy's OI was due to a substitution of alanine for glycine at amino acid position 208.<sup>43</sup> The location of the mutation

is the namesake for this model, and because it is a naturally occurring mutation it is likely that the mutation is a heritable trait and could be reproduced in other dogs. However, the puppy used in these studies remains the only published case of this mutation having occurred in a canine.

#### *Col1a2* frameshift Beagle: OI III

The second naturally occurring canine model of OI was identified with the discovery of a heterozygous *COL1A2* frameshift mutation in the complementary DNA (cDNA) of a proband Beagle (CU3) exhibiting a history of multiple long-bone fractures and overhydroxylated type I collagen.<sup>42,44</sup> Four nucleotides (3991–3994) were deleted and replaced, with nine in the *COL1A2* region responsible for coding for the C-propeptide of the pro- $\alpha_2$ (I) chain.<sup>44</sup> C-propeptides are essential for proper triple-helix formation, and mutations can lead to altered *COL1A1* structure.<sup>45</sup> Also, the 30-amino acid code for human and canine pro- $\alpha_2$ (I) sequences normally has 100% identity, which suggests the CU3 Beagle is a good model for human OI III.<sup>46</sup>

#### *Med*<sup>-/-</sup> zebrafish: OI I–IV

In 1996 a homozygous mutant zebrafish was characterized as having curtain-like waves in its fin epithelium and was termed “microwaved” (*Med*<sup>-/-</sup>).<sup>47</sup> Fish with this mutation also exhibit delayed bone ossification, undulation of the larval fin, and severe reduction in bone density.<sup>48</sup> One of the constituents of zebrafish bone, actinotrichia, is partially formed by type I collagen, and *Col1a1* has been identified in actinotrichia-forming cells.<sup>49</sup> Additionally, gene sequencing has been confirmed that *Med*<sup>-/-</sup> zebrafish exhibit an alanine-for-glycine substitution at position 888 in their cDNA, which is predicted to result in the substitution of a glutamic acid for a lysine.<sup>48</sup> This illustrates the validity of *Med* zebrafish as a model for OI I–IV.<sup>48</sup>

#### Chi zebrafish: OI I–IV

Another zebrafish that serves as a model for OI is the Chihuahua (Chi) mutant. This mutation can occur either in heterozygous (*Chi*<sup>+/+</sup>) or homozygous (*Chi/Chi*) form, but there has not been a phenotypic difference noted between the two.<sup>50</sup> It has, however, been determined that the mutation causes defective bone growth, with characteristics similar to those found in humans with OI.<sup>3,50</sup> Radiographs show distorted bones with irregular radiodensity as early as 1 month of age.<sup>50</sup> The Chi mutation occurs at position 2207 in the cDNA, where the wild-type sequence is unaltered, but there is a base-pair change that causes a missense mutation and

changes the encoded amino acid from glycine to aspartate.<sup>50</sup> The sequence of zebrafish *COL1A1* is approximately 76% identical to the human gene, making it a good model for OI caused by *COL1A1* mutations.<sup>3,50</sup>

## OI types V–VIII Characteristics in humans

In 2004, it was suggested that three new phenotypes be added to the classification of OI and referred to as OI V–VII.<sup>4</sup> A few years later, another phenotype was proposed and classified as OI VIII.<sup>51</sup> The features of the disorder classified as OI V include being slightly to moderately short in stature, with a dislocated head of the radius, a mineralized interosseous membrane, and normal sclerae.<sup>4</sup> Characteristics of OI VI include moderately short stature, presence of scoliosis, accumulation of osteoid in bone as well as a fish-scale bone-lamellation pattern, normal sclerae, and absence of DI.<sup>4</sup> Traits described for OI VII include slightly short stature with shortened humeri and femora, decreased angle between the head and shaft of the femora, normal sclerae, and no presence of DI.<sup>4</sup> Finally, the characteristics of the severe-to-fatal disease known as OI VIII include normal sclerae, bulbous metaphyses, and extremely short stature with very low bone mineralization.<sup>51</sup>

Genetic causes of OI V–VII were unknown at the time it was proposed that they be added under the classification of OI, but have since been identified. In 2013, it was suggested that the expression of OI V is caused by a mutation in the *IFITM5* gene,<sup>52</sup> which encodes a transmembrane protein important to osteoblast expression.<sup>53</sup> A mutation in the *SERPINF1* gene was identified as the cause of OI VI.<sup>54</sup> A genetic link between the decreased expression of CRTAP and the presence of OI VII had been discovered.<sup>55</sup> A genetic mutation caused by defects in the *P3H1/LEPRE1* gene resulted in an osteopathic disease with OI-type characteristics,<sup>51</sup> later classified as OI VIII.<sup>56</sup>

## Animal models

### Ifitm5 transgenic mouse: OI V

A mouse model with *IFITM5* mutation was very recently created by first placing murine *Ifitm5* cDNA under a *Col1a1* 2.3 kB promoter, and then using mutagenesis to achieve the correct mutation (c.-14C>T) that occurs in humans.<sup>53</sup> Mice that inherited mutant *Ifitm5* had severe skeletal deformities, as well as perinatal death.<sup>53</sup> Additionally, the limbs of the mice lacked growth abnormalities, but they were facing downward at birth, and skeletal samples taken at E15.5 and E18.5 showed a consistent delay in mineralization. At E18.5

there was also the presence of forelimb and hind-limb fractures in utero, as well as extreme deformities of the rib cage.<sup>53</sup>

### Pedf<sup>-/-</sup> mouse: OI VI

Mice genetically modified to be deficient in *Pedf* were first created by replacing exons 3–6 of *SERPINF1* with a cassette comprised of an internal ribosomal entry site,  $\beta$ -galactosidase genes, and neomycin-resistance genes.<sup>57</sup> After it was discovered that OI VI in humans is caused by mutations in *SERPINF1*, this procedure was replicated to produce *Pedf<sup>-/-</sup>* mice for the purpose of studying the role of *PEDF* in bone and other tissues.<sup>58</sup> Bending tests performed on femora from wild-type and *Pedf<sup>-/-</sup>* mice indicated that while the ultimate force applied to both groups was about the same, the ultimate displacement in the *Pedf<sup>-/-</sup>* bone was approximately half of that in wild-type bone, with the energy of failure being 25% lower in the *Pedf<sup>-/-</sup>* mice.<sup>58</sup> Through this study, it was determined that *Pedf<sup>-/-</sup>* mice are a suitable model for OI VI.<sup>58</sup>

### Crtap<sup>-/-</sup> mouse: OI VII

Around the same time that the genetic cause of OI VII in humans was linked to a deficiency in *CRTAP* expression, a strain of homozygous *Crtap*-deficient (*Crtap<sup>-/-</sup>*) mice was produced using a homologous recombination approach.<sup>59,60</sup> It has been found that *Crtap<sup>-/-</sup>* mice properly model OI VII in humans, as they have an abnormally high mineral content and increased mineral densities in their bones compared to wild-type mice.<sup>60</sup> Other characteristics noted in *Crtap<sup>-/-</sup>* mice include a progressive severe kyphoscoliosis, low bone density, and cartilage dysplasia.<sup>11</sup> However, *Crtap<sup>-/-</sup>* mice tend to have a less severe form of the disease than is found in most humans.<sup>11,56</sup> Tensile testing done on the skin of *Crtap<sup>-/-</sup>* mice showed that the peak load and stiffness of the tissue were 60% lower than the skin of wild-type mice.<sup>61</sup>

### P3h1<sup>-/-</sup> mouse: OI VIII

In 2010, a method of homologous recombination was used to create a *P3H1*-knockout mouse by targeting exons 1–3 of *P3H1*.<sup>62</sup> The heterozygous (*P3h1<sup>+/+</sup>*) mice did not express an OI phenotype, but homozygous (*P3h1<sup>-/-</sup>*) mice exhibited significantly smaller body size, lower mineral density in the skull and long bones, progressive kyphoscoliosis, and shorter femora that had decreased stiffness and failure load compared to wild-type littermates.<sup>62</sup> *P3h1<sup>-/-</sup>* mice also demonstrate elevated auditory thresholds and altered morphology of the middle-ear bone joints, which make them an ideal model for investigating the mechanisms that cause hearing loss in OI patients.<sup>63</sup>

## OI types IX and X Characteristics in humans

Recently, there have been several new genetic mutations identified that lead to a disorder similar to OI, and a few have been subsequently classified as OI types IX and X. *PPIB* was identified as the gene responsible for OI IX after patients initially diagnosed with OI II–IV were not found to have mutations present in *COL1A1* or *COL1A2*.<sup>64</sup> In fact, multiple studies have shown that although the mutation occurs in *PPIB*, the phenotype expressed is similar to classical OI type II, III, or IV.<sup>64–67</sup> OI X, by comparison, is far less broad in its phenotype: the available data were obtained from a study of a single patient who exhibited small bone size, blue sclerae, multiple fractures, malformed bones, and DI.<sup>68</sup> The gene identified as causing the OI in this patient was *HSP47/SERPINH1*,<sup>68</sup> which is a molecular chaperone required for the maturation of collagen types I and IV in the endoplasmic reticulum (ER).<sup>69,70</sup>

### Animal models

#### Ppib<sup>-/-</sup> mouse: OI X

Between 2006 and 2007, several research articles were published indicating the roles of P3h1 and Crtap in the development of OI in humans and mice.<sup>51,55,59,71</sup> There was also a relationship established between P3h1, Crtap, and Ppib,<sup>59,62,71,72</sup> and it was found that they interact to form a complex in the rough ER that is believed to function as a collagen chaperone.<sup>72</sup> To investigate this relationship further, the Cre/Lox system was utilized to create a *PPIB*-knockout mouse by targeting exon 3 through homologous recombination.<sup>11,71</sup> The heterozygous Ppib<sup>+/-</sup> mice that were produced using this technique did not exhibit any symptoms of OI, and were mated to produce Ppib<sup>-/-</sup> offspring,<sup>71</sup> which instead exhibit symptoms of OI, including decreased body size and weight, low bone mineral density and volume, progressive kyphosis, decreased skin stiffness, and increased laxity.<sup>11,71</sup> Although the phenotype observed in the Ppib<sup>-/-</sup> mice is less severe than in humans, it is considered an appropriate model of OI IX.<sup>11</sup>

#### Serpinh1 (Hsp47<sup>-/-</sup>) mouse: OI X

An *HSP47*-knockout mouse was created in 2000 as the result of a gene-targeting approach.<sup>73</sup> While first-generation heterozygous (Hsp47<sup>+/-</sup>) mice displayed no apparent OI phenotype, second-generation homozygous (Hsp47<sup>-/-</sup>) embryos did not survive past E11.5, and displayed ruptured blood vessels and abnormally oriented epithelial tissues.<sup>73</sup> Mutant embryos were more translucent than wild-type mice, and appeared

shortened.<sup>73</sup> Although Northern blot analysis showed that Hsp47 messenger RNA was lower in Hsp47<sup>+/-</sup>, it was not expressed at all in Hsp47<sup>-/-</sup> mice.<sup>73</sup> Electron microscopy observations of collagen fibrils secreted by Hsp47<sup>-/-</sup> cells showed thin fibrils forming abnormal branches, thus indicating that the collagen type I secreted from the Hsp47<sup>-/-</sup> mouse cells could not form normal supramolecular fibrillar structures.<sup>70</sup> It is believed that Hsp47 plays an important role either in preventing the collagen triple helix from unfolding and providing stabilization during the progression of procollagen folding, or in preventing lateral association of procollagen triple helices in the ER.<sup>73,74</sup>

#### Serpinh1 (L326P) Dachshund: OI X

Dachshunds exhibiting multiple fractures and history of lameness were found to have different long bones, ribs, mandibles, and teeth from normal. Their long bone exhibited thin cortical bone lacking an organized Haversian system and secondary cancellous bone, and their teeth had a thin dentine layer.<sup>75</sup> A homozygosity-mapping approach determined the position of the mutation responsible for these abnormalities to be located at 24.66–29.4 Mb interval where the *SERPINH1* gene maps.<sup>76</sup> The Serpinh1 Dachshund does not share an equivalent mutation noted in humans, but its phenotype makes it a suitable model for OI X.<sup>11</sup> Drögemüller et al<sup>76</sup> suggested the *SERPINH1* canine mutation could be a valuable model for the identification of a fifth gene, in addition to *COL1A1*, *COL1A2*, *CRTAP*, and *LEPRE1*, that generates OI in humans.

## OI types XI–XVII Characteristics in humans

Since 2010, there have been an additional seven new OI types with similar phenotypes to but different genetic bases from the original four types of OI added to the OI-classification system. It was proposed that the syndrome resembling OI known as Bruck syndrome 1 be reclassified as OI<sup>77,78</sup> following research that identified a mutation in the *FKBP10* gene as the cause of a recessive form of OI with characteristics similar to OI III.<sup>79</sup> This phenotype is now known as OI XI, and the suspected mechanism of the *FKBP10* mutation is that it causes a decrease of collagen type I deposition in the extracellular matrix.<sup>66</sup>

OI XII has been identified in a single proband with a phenotype similar to OI IV, but the cause of this OI type was linked to a frameshift mutation occurring in the *SP7* gene.<sup>80</sup> OI XIII was identified as being caused by a homozygous missense mutation in the gene that encodes the BMP1/Toll-like proteinases,

and results in a phenotype similar to OI III.<sup>81</sup> OI XIV was first described when a homozygous mutation in the *TMEM38B* gene was found to lead to a phenotype with characteristics in line with an autosomal-recessive type of OI, including osteopenia and fractures of varying severity, but normal teeth, sclerae, and hearing.<sup>82</sup>

What is now known as OI XV was described in several publications when multiple mutations in the *WNT1* gene were found in individuals displaying phenotypes characteristic of OI III–IV.<sup>83–86</sup> OI XVI has been documented in one proband family where two siblings were affected by severe OI characterized by frequent fractures starting in utero, varus deformities of the legs, soft skull bones with large fontanelles, and beaded ribs.<sup>87</sup> Analysis of DNA from one sibling indicated a homozygous deletion in the *CREB3L1* gene.<sup>87</sup> OI XVII was classified after two individuals diagnosed with OI IV were found to have a mutation in the *SPARC* gene.<sup>88</sup>

## Animal models

### Fkbp10<sup>-/-</sup> mouse: OI XI

Mice containing the European conditional mutagenesis allele were created to study the function of Fkbp10.<sup>89</sup> In the first generation of heterozygotes (Fkbp10<sup>+/+</sup>), no OI phenotype was noted.<sup>89</sup> However, homozygotes (Fkbp10<sup>-/-</sup>) obtained from breeding Fkbp10<sup>+/+</sup> mice exhibited delayed growth from E13.5 on, as well as downward-facing forelimbs, fragile tissue, flattened facial features, ER dilation caused by accumulation of abnormal procollagen chains in the ER, and perinatal death.<sup>89</sup> While humans with OI XI do not exhibit perinatal death, the effect the mutation has on the ER and collagen formation is phenotypically similar to Fkbp10<sup>-/-</sup> mice.<sup>89</sup>

### Osterix-null Sp7<sup>-/-</sup> mouse: OI XII

One initial method to create an Sp7-null mouse involved using a homologous recombination of embryonic stem cells to create nonphenotypic heterozygous (Sp7<sup>+/-</sup>) mice.<sup>90</sup> However, Sp7-null offspring obtained from Sp7<sup>+/-</sup> pairings all died within 15 minutes of birth following difficulty breathing, and exhibited severe limb deformities.<sup>90</sup> A second method used the Cre/Lox system to create conditional *SP7*-knockout mice with *Coll1a1-Cre* (*Osx<sup>Flox</sup>;*Coll1a1-Cre), which were designed to inactivate *SP7* without perinatal lethality.<sup>91</sup> *Osx<sup>Flox</sup>;*Coll1a1-Cre mice exhibited decreased trabecular bone mineralization, as well as osteopenia and cortical bone thinning.<sup>91</sup> Although both models existed before *SP7* was linked to OI in humans, *Osx<sup>Flox</sup>;*Coll1a1-Cre may possibly be considered a model for OI XII, but further studies are needed in this regard.

### Frfr<sup>-/-</sup> zebrafish: OI XIII

It has been shown that mutations in *BMP1* cause high bone mineral density and multiple fractures in human OI XIII.<sup>48,81</sup> This mutation in zebrafish, termed frilly fins (Frfr<sup>-/-</sup>), causes a ruffled larval fin, short body axis, malformed craniofacial bones and vertebrae, and reduced ossification and bone density.<sup>48</sup> Frfr<sup>-/-</sup> was created by missense substitutions in *BMP1*, and led to osteoblasts exhibiting a more cuboidal structure than wild-type fish.<sup>48</sup> Bmp1 has been found to cleave and inactivate the Bmp2/4 inhibitor chordin, which aids in generating mature collagen type I, and thus it may disrupt osteogenesis when present at elevated levels.<sup>92</sup>

### Swaying (Wnt1<sup>Sw/Sw</sup>) mouse: OI XV

The Swaying (Sw) mouse was first described as having poor coordination, with a rotational movement pattern and cerebellar deficit.<sup>93</sup> The Sw mouse was later found to be phenotypically similar to mice with a targeted *WNT1* mutation causing osteopenia and fragile bones,<sup>94</sup> and was subsequently determined to be caused by a frameshift mutation in *WNT1* (Wnt1<sup>Sw</sup>), with homozygous (Wnt1<sup>Sw/Sw</sup>) mice expressing the Sw phenotype.<sup>95</sup> Following the classification of *WNT1* mutations as a cause of documented OI in humans, Wnt1<sup>Sw/Sw</sup> was proposed to be a suitable model for OI XV.<sup>96</sup>

Currently, there are no animal models for OI XIV, XVI, or XVII. However, the genes that were discovered to be the cause of OI XVI and XVII were selected for investigation based on genetic mutations in mice that cause similar phenotypes to what human cases presented with.<sup>87,88</sup>

## Animal models in clinical research

Bone fracture and fragility are the primary symptoms of OI, and thus clinical research on treatments has been focused mainly on understanding the bone properties of the animal models for OI and on regaining mechanical integrity in these bones. Most of the research dedicated to finding treatments for OI has primarily utilized mouse models of classical OI (Table 1).

## Pharmacological treatments

Many studies have tested drug treatment as a clinical therapy for OI. In particular, bisphosphonates, a class of antiresorptive drugs, have been widely used to treat OI in an effort to reduce fracture incidence and improve bone density in OI patients. Between the different bisphosphonates, alendronate and pamidronate have been the most used in clinical studies run on animals. Short-term therapy studies conducted on *oim/oim* and *BrtlIV<sup>+</sup>* mice for both alendronate and pamidronate

**Table I** Current animal models for human osteogenesis imperfecta (OI), with their genetic and phenotypic characteristics and clinical therapy applications

Common name	Human OI type	Gene	Characteristics	Clinical therapy
<b>Mouse models</b>				
Mov-13 <sup>-/-</sup>	OI II	COL1A1	Perinatal mortality	Gene replacement
Mov-13 <sup>+/-</sup>	OI I	COL1A1	Hearing loss, reduced mechanical properties in bone, and reduced collagen production	
<i>oim/oim</i>	OI III	COL1A2	Fragile bones, osteopenia, cortical thinning, bone deformities, joint laxity, kyphosis	Bisphosphonate therapy, stem cell transplantation, bone marrow stromal cells, gene supplementation, whole-body vibration
<i>oim</i> <sup>+</sup>	OI I	COL1A2	Decreased material properties of bone	
G610C/G610C <sup>Neo+</sup>	OI IV	COL1A2	Decreased body weight, bone mineral density, bone volume, and mechanical properties of bone	
+/G610C <sup>Neo+</sup>	OI I/IV	COL1A2	Decreased body weight, bone mineral density, bone volume, and mechanical properties of bone	
+/G610C <sup>Neo-</sup> (Amish)	OI IV	COL1A2	Decreased body weight, bone mineral density, bone volume, and mechanical properties of bone	
G859C	OI II	COL1A1	Short and wavy ribs, poor bone mineralization, underdeveloped skeleton, pliable limbs, perinatal death	
Aga2 <sup>+</sup>	OI II/III	COL1A1	Skeletal deformities, fragile bones, osteopenia, perinatal death (in some cases)	
Aga2 <sup>-/-</sup>	OI II	COL1A1	Embryonic mortality	
BrtIII	OI II	COL1A1	Rib fractures, short vertebral bodies, poor skull mineralization, perinatal mortality	
BrtIV <sup>+</sup>	OI IV	COL1A1	Perinatal death to long-term survival, bone deformity, fragile bones, osteopenia	Bisphosphonate therapy, sclerostin antibody therapy, bone marrow stromal cells
BrtIV/BrtIV	OI IV (mild)	COL1A1	Bone deformity, bone fragility, osteopenia	
Human COL1A1 minigene	OI II-IV	COL1A1	Fragile bones, low bone mineral density, ~90% perinatal mortality rate (when high levels of minigene expressed)	Bone marrow stromal cells, oligonucleotide antisense gene therapy
Ifitm5 transgenic	OI V	IFITM5	Skeletal deformities, fragile bones, poor bone mineralization, perinatal death	
Pedf <sup>-/-</sup>	OI VI	SERPINF1	Decreased material properties of bone	
Crtap <sup>-/-</sup>	OI VII	CRTAP	High bone mineralization and mineral density in bone (but low bone density), progressive kyphoscoliosis, cartilage dysplasia, decreased material properties in skin	
P3h1 <sup>-/-</sup>	OI VIII	LEPRE1	Small body size, low mineral density in calvarial and long bones, decreased material properties of bone, impaired hearing	
Ppib <sup>-/-</sup>	OI IX	PPIB	Decreased body size and weight, low bone mineral density and volume, progressive kyphosis, decreased material properties in skin	
Hsp47 <sup>-/-</sup>	OI X	SERPINH1	Embryonic mortality	
Fkbp10 <sup>-/-</sup>	OI XI	FKBP10	Delayed growth beginning at E13.5, fragile tissue, skeletal deformities, perinatal mortality	
Sp7 <sup>-/-</sup>	Possibly OI XII	SP7	Decreased trabecular bone mineralization, osteopenia, cortical bone thinning	
Wnt1 <sup>Sw/Sw</sup> (Swaying)	OI XV	WNT1	Poor coordination, osteopenia, fragile bones, cerebellar deficit	
<b>Canine models</b>				
G208A Col1a1 Golden Retriever	OI III	COL1A1	Fragile bones, dentinogenesis imperfecta	
Col1a2 frameshift Beagle	OI III	COL1A2	Fragile bones	
SerpinH1 (L326P) Dachshund	OI X	SERPINH1	Fragile bones, lameness, thin cortical bone and dentine	



Common name	Human OI type	Gene	Characteristics	Clinical therapy
<b>Zebrafish models</b>				
Med <sup>-/-</sup>	OI I-IV	COL1A1	Delayed bone ossification and low bone mineral density, larval fin undulation, curtain-like waves in fin epithelium	
Chi <sup>+/+</sup>	OI I-IV	COL1A1	Generalized defective bone growth	
Chi/Chi	OI I-IV	COL1A1	Generalized defective bone growth	
Frfr <sup>-/-</sup>	OI XIII	BMP1	Ruffled larval fin, short body axis, deformed craniofacial bones and vertebrae, reduced ossification and bone density	

injections showed a reduction in the fracture rate,<sup>97,98</sup> with thicker and stiffer bones,<sup>99-101</sup> enhanced density,<sup>97,99-101</sup> but no mineral maturity,<sup>102</sup> and variable biomechanical properties.<sup>97-99,101</sup> In both models, bisphosphonate treatment altered the growth plate in mice by increasing their height, mainly at the proliferation and hypertrophic zones, reducing vascular invasion<sup>103</sup> and growth-plate cell turnover,<sup>98</sup> and with detrimental effects on osteoblasts and bone formation.<sup>99</sup> In trabecular *oim/oim* bone, alendronate increased trabecular number, decreasing thickness and separation and increasing bone volume density,<sup>97,104</sup> and produced no improvement in strength or mechanical properties. This shows that the reduction in fracture rate is primarily attributable to an increase in bone mass.<sup>104</sup> High doses of alendronate were more effective in short-term therapy.<sup>98</sup>

Long-term alendronate therapy in growing *oim/oim* mice also appeared to be effective in increasing metaphyseal density. The increased length and bowing of tibia metaphysis seen in short-term therapy was not observed in long-term treatment, suggesting a dissipation of these effects over time.<sup>105</sup> Sex was a factor contributing to the success of the treatment, as alendronate increased compositional heterogeneity in male mice, improving bone quality.<sup>106</sup>

More recently, alendronate treatment was proved to affect the fracture-healing process of bone in *BrtlIV<sup>+</sup>* mice positively.<sup>107</sup> Mice treated with alendronate before and after fracture exhibited increased callus volume, with decreased mineralization quality and quantity, and increased bone volume and torque at failure. However, no effect was observed if the drug treatment was used only before fracture.<sup>107</sup>

RANKL inhibitors have shown promise in both human<sup>108,109</sup> and animal<sup>97,110-113</sup> trials for the treatment of several different osteopathologies. When RANKL binds to RANK, it activates the pathway that leads to osteoclastogenesis,<sup>109,111-114</sup> so the pharmacological application of RANKL inhibitors aims to prevent bone resorption by blocking the RANK pathway from being activated.<sup>97,108-114</sup> The inhibitor

denosumab, a fully human monoclonal antibody,<sup>97,111-114</sup> has a higher specificity and affinity for RANKL than osteoprotegerin,<sup>112-114</sup> a naturally occurring decoy RANKL receptor.<sup>110-114</sup> Unfortunately, denosumab is not recognized by murine RANKL,<sup>97,111-114</sup> although preliminary studies done in primates<sup>97,112</sup> and knock-in mice expressing murine/human RANK<sup>97,113</sup> have yielded positive results, including increased mineral content, density, and strength in primate bone,<sup>97,112</sup> as well as suppressed resorption and increased density in human RANKL mouse bone<sup>97,113</sup> following denosumab treatment.

The successful application of RANKL inhibitors in OI treatment was demonstrated in two children with OI VI who exhibited decreased bone resorption following administration of denosumab.<sup>109</sup> Animal research of RANKL inhibitors in the treatment of OI has so far been limited to the use of RANK-Fc,<sup>97,110,111</sup> a recombinant RANKL inhibitor with properties similar to osteoprotegerin.<sup>97,110,114</sup> The results of treating *oim/oim* mice with RANK-Fc show that it increases bone density,<sup>97,111</sup> improves some mechanical and geometric properties of bone,<sup>111</sup> delays callus remodeling following fracture,<sup>110</sup> and reduces fracture incidence.<sup>97</sup> Inspired by the success of the teriparatide (human parathyroid hormone) as an anabolic drug for osteoporosis,<sup>115-120</sup> and as adjuvant for bone<sup>121,122</sup> and allograft<sup>123-125</sup> healing, scientists have recently tested teriparatide in adults with OI I-IV.<sup>126</sup> Results showed an enhancement in vertebral bone mineral density and strength, and thus there is a need for animal research on the use of teriparatide for treatment of OI.

Finally, a study performed on *BrtlIV<sup>+</sup>* mice determined the effect of sclerostin antibody (SA), an anabolic bone therapeutic, on OI bone formation.<sup>127</sup> It was thought that SA could neutralize sclerostin, ultimately increasing bone formation by preventing the inhibition of anabolic canonical Wnt signaling.<sup>127</sup> While previous antiresorptive bisphosphonate-treatment studies using *BrtlIV<sup>+</sup>* and *oim* mice resulted in increased trabecular number but not thickness, the treatment study using SA instead found an increase in trabecular

thickness but not number.<sup>127</sup> After 2 weeks of SA therapy, *BrtlIV<sup>+</sup>* mice had increased cortical and trabecular bone mass, reduced fragility in long bones, including improved stiffness, and the ability to withstand higher loads before breaking.<sup>127</sup> SA therapy increased the rate of bone formation, increasing both cortical and cancellous bone volume, and also improved the strength of cortical bone without affecting bone mineralization.<sup>127</sup> Although more research is needed, SA treatments could eventually prove to be an effective therapy for patients with OI IV in the future.

## Stem cell transplantation

The *oim/oim* mouse has recently undergone stem cell therapies to alter developing OI bone pathology. Mesenchymal stem cell (MSC) therapy for tissue regeneration can improve damaged or diseased tissue when delivered to relevant locations.<sup>128</sup> MSCs injected into *oim/oim* mice from adult wild-type mice migrate to sites of new bone formation.<sup>129</sup> As an expansion on this study, human fetal blood MSCs, collected by cardiocentesis under ultrasound guidance, were considered as a therapy for *oim/oim* mice.<sup>130,131</sup> Virgin *oim/oim* females were mated with *oim/oim* males and then underwent intrauterine transplantation at E13.5–E15 so that each fetus could be injected intraperitoneally with human fetal blood MSCs.<sup>130</sup> Offspring were analyzed at E18, week 1 after birth, and weeks 2, 4, 8, and 12 thereafter. At each point measured, findings showed a 70% reduction in the number of fractures with only 5% of cells ingrafted.<sup>130</sup> The fetal blood stem/stromal cells engrafted into *oim/oim* bone differentiated into osteoblasts, producing osteocalcin and *Col1a2*, which is absent in *oim/oim* mice.<sup>132</sup> As a result, these bones showed a clinically relevant increase in cortical bone strength, length, and thickness, and clustered cells around sites of bone formation and repair, suggesting osteogenic differentiation.<sup>130,132</sup>

## Bone marrow stromal cells

Studies involving the transplant of bone marrow stromal cells (BMSCs) from wild-type mice into human *COL1A1*-minigene mice demonstrated that BMSCs could renew cells in multiple nonhematopoietic tissues, and may be a prospective treatment for OI.<sup>133</sup> Transplanted BMSCs have been shown to contribute to bone formation in vivo and to improve the mechanical properties of the recipient bone.<sup>134</sup> In a study conducted to determine the effect of transplanting BMSCs alone or suspended in a collagen matrix, BMSCs were harvested from the marrow of the femora and tibiae of wild-type mice syngeneic to *oim* mice, then cultured and

injected into the femora of *oim* mice.<sup>134</sup> Mechanical testing of the bone conducted 6 weeks after implantation found that femora injected with BMSCs suspended in collagen matrix were stronger than those injected with BMSCs alone, and that in both BMSC test groups treated bone was stronger when compared to *oim/oim*.<sup>134</sup> Donor cells, tracked using green fluorescent proteins, differentiated into osteoblasts in vivo and actively participated in new bone formation.<sup>134</sup> Histological analysis of the femora showed that those injected with BMSCs suspended in collagen matrix had more bone deposition than those injected with BMSCs alone.<sup>134</sup> Comparison of the BMSC-treated samples with *oim/oim* samples indicated that all of the new bone deposition was a result of the combination of endogenous and exogenous cells.<sup>134</sup>

BMSCs may not only be transplanted between adult mice but also in utero and in developing animals.<sup>134,135</sup> In another study, BMSCs were transplanted from long bones of adult cytomegalovirus/eGFP CD1 transgenic mice into embryos of wild-type females mated with *BrtlIV<sup>+</sup>* males.<sup>135</sup> Of the pups that reached weaning age, 51.4% were wild-type and 48.5% *BrtlIV<sup>+</sup>* mice, demonstrating a nearly equal survival rate between the two genotypes.<sup>135</sup> Comparing these mice with a control group in which no treatments were administered, it was seen that BMSC treatment improved mechanical properties of bone in *BrtlIV<sup>+</sup>* mice,<sup>135</sup> with BMSC-treated *BrtlIV<sup>+</sup>* mice having significantly higher bone mineral density in both trabecular and cortical bone compared to untreated *BrtlIV<sup>+</sup>* mice.<sup>135</sup>

## Gene therapies

Several methods have been proposed for implementing gene therapies in the treatment of OI, including gene replacement, antisense approaches, gene supplementation, and gene delivery to bone.<sup>136</sup> One of the first studies in using animal models for gene replacement to treat OI was accomplished by transfecting a *Col1a1*-null allele taken from a *Mov-13<sup>-/-</sup>* embryo with human *COL1A1*.<sup>137,138</sup> In this case, functional collagen type I was formed in the mouse through transgene expression.<sup>137,138</sup> This collagen was composed of two human  $\alpha_1(I)$  and one mouse  $\alpha_2(I)$  chains, suggesting that this could be a means for using gene therapy in treating OI in humans.<sup>137,138</sup>

Another approach of gene therapy is gene supplementation, which in OI research could consist of the delivery of *Col1a1* from healthy mice to a mouse with OI caused by a *COL1A1* mutation. However, the challenge with this therapy is that most types of OI mutations are dominant-negative, meaning that the mutant allele disrupts the function of the normally functioning allele, so supplementation would not be

effective without first blocking the expression of the mutant gene.<sup>136</sup> Delivery of the healthy gene is further complicated by the fact that *COL1A1* and *COL1A2* are large genes and difficult to manipulate.<sup>138</sup> The combination of gene supplementation with antisense gene therapy, a method of using antisense oligodeoxynucleotides, antisense RNA, or ribozymes to block transcription of certain gene sequences that are thought to cause the disease,<sup>139</sup> could make gene therapy more feasible.<sup>136</sup> One study involving linear oligonucleotide antisense therapy was done using human *COL1A1*-minigene mice.<sup>41</sup> Mating mice where both parents expressed antisense minigene produced offspring that inherited both genes, and the rate of occurrence of the lethal fragile-bone phenotype was reduced from 92% to 27%, demonstrating the effects of the therapy.<sup>41,140</sup> One potential application for studying gene supplementation in an animal model is the use of the *oim/oim* mouse, as the deletion of the *COL1A2* in these mice stops incorporation of pro- $\alpha_2$ (I) chains into heterotrimers and leads to a buildup of pro- $\alpha_1$ (I) heterotrimers in tissue.<sup>136</sup>

One application of gene therapy that appears to hold a great deal of promise is gene delivery to bone. One way this can be done is through the use of noncoding RNA or a protein, which can be delivered using a vector, such as a virus.<sup>141</sup> However, the use of viral therapy is not ideal in the clinical setting, due to the risks involved with it.<sup>142</sup> Other studies have been performed to test the efficacy of gene delivery without a vector, as in one case using the delivery of naked plasmid DNA to the gastrocnemius of a wild-type ICR mouse.<sup>143</sup> Unfortunately, although this method did lead to bone formation, it was determined that this was not an efficient method of gene delivery.<sup>142,143</sup> Other methods, such as RNA interfacing and using synthetic carriers for delivery, have also been attempted, with varying degrees of success,<sup>142</sup> and gene-delivery therapy still needs to be thoroughly tested before being safely implemented.

## Mechanical therapies

Bone adapts both its mass and architecture to the mechanical loading applied upon it.<sup>144</sup> Therefore, mechanical therapies that do not result in fracture and do not involve drugs are very attractive for application in the OI population. One potential mechanical therapy, whole-body vibration (WBV), has been studied in young *oim/oim* mice, and resulted in the improvement of trabecular and cortical bone morphologies during hind-limb growth.<sup>144</sup> WBV involves placing mice into a container on a vertically oscillating plate, which effectively introduces a vibrational mechanical stimulus to normal activities.<sup>145</sup> Previous numerical studies have shown that increasing

the frequency of the loading stimulus up to 30 Hz results in improved bone formation.<sup>146</sup> Vanleene and Shefelbine utilized high-frequency, low-amplitude WBV in order to determine its effect on the volume and mechanical properties of young *oim/oim* bone.<sup>144</sup> The study found that WBV caused an increase in *oim/oim* femoral and tibial cortical thickness and cross-sectional area, as well as an increase in trabecular bone volume in *oim/oim* tibiae.<sup>144</sup> WBV thus appears to be a potentially effective and safe treatment for increasing bone formation and possibly bone strength in OI. This therapy is particularly attractive for treating the growing skeletons of children with OI without the disadvantages of current long-term pharmacological therapies.

## Discussion and conclusion

This review presented existing animal models for OI I–XVII and clinical therapies, some still emerging, that these models serve as a platform for OI research. Most of the animal models used for clinical trials were mice, representing moderate-to-severe conditions of the classic OI disease. In fact, mutagenesis studies that have led to the development of new models of OI have only been conducted using mice. The canine models of OI mentioned in this article all occurred spontaneously in animals that had not been bred for research purposes. It is probable that the bone structure of canines would more adequately model the disease physiology in humans than the bone structure of mice, but there are limiting factors that would make the development of canine OI models difficult.<sup>11</sup>

Currently, treatments for human OI rely largely on bisphosphonates and exercise management, which can increase the amount of bone but not its quality.<sup>37</sup> More animal studies are needed to understand the actual efficacy and long-term effects of these treatments on bone, and also more research is envisaged to explore other therapies for the management and cure of OI. In this sense, animal models, and in particular mouse models of OI, offer an extremely valuable resource for the understanding of the disease and for the advancement of therapies for reducing fracture risk.

Mutagenic mouse models are relatively easy and quick to produce, and genetically assimilate mutations typical of human cases of OI. On the other hand, mouse bones are very small in size and do not allow for typical sample preparation as used in human bones (ie, milling out exact perfect beam samples from human bone for mechanical testing but not from a mouse bone, where the entire bone with its geometrical imperfections needs to be used). Also, the structure of mouse cortical bone is different from human cortical bone in that it is missing the

Haversian system, as the whole mouse bone is about the size of a large osteon in human bone. This may have implications in terms of mechanics, because the lamellar osteonal structure is thought to provide toughness to human bone,<sup>147</sup> although recent research on *oim/oim* mouse bone has shown that possibly the lamellar structure can yield a similar toughness to mouse bone.<sup>17</sup> Apart from these two differences, mouse bone represents an indispensable resource to understand bone pathology and to anticipate the effects of potential therapies on humans, as mouse skeletal growth is accelerated, allowing for long-term investigation in a 6- to 12-month period.

In the future, there should be a much wider breadth of studies utilizing OI animal models for further understanding clinical applications and their translation to human therapies. It would be interesting to study methodological factors, such as drug dosage or mechanical loading, affecting treatment, and to investigate further long-term effects of treatment in each type of OI. Also, the combination of different therapies, such as using gene therapy with mechanical loading, may be a viable approach that needs further investigation. Therefore, more clinical studies should be conducted on the animal models presented here to determine which therapy holds the most promise for human OI. Finally, more studies allowing the direct translation of therapies from animal models to human cases should be put in place to facilitate implementation of new therapies.

## Disclosure

The authors report no conflicts of interest in this work.

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