Animal models of osteogenesis imperfecta and craniofacial development

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Abstract
Introduction
Osteogenesis imperfecta (OI) is a human genetic disorder of increased bone fragility and low bone mass. Severity varies widely, ranging from intratertiary fractures and perinatal lethality to very mild forms without fractures. There is variable association of typical extra skeletal manifestations with the disorder, including blue sclera, dentinogenesis imperfecta, hypermobility of ligaments and skin, hearing impairment and the presence of Wormian bones on skull radiography.

The most widely used classification of OI distinguished four clinical types. The most relevant clinical characteristic of all OI types is bone fragility, the severity of which increases in the order type I < type IV < type III < type II. It is now widely recognized that there may be many more types of OI than those classified by Silenè et al. Some forms of congenital brittle bones have been considered OI and have been added as types V, VI and VII. There is still no perfect consensus about the definition of OI. Plotkin recently proposed defining OI as syndromes resulting from mutations in either COL1A1 or COL1A2 genes, and to group all other syndromes with congenital brittle bones as ‘syndromes resembling OI (SROI)’, pending the identification of their causal mutations.

In the new Nosology and Classification of the Genetic Skeletal disorders, OI is defined in several forms depending on the severity of the phenotype, whatever the mode of the transmission or the gene involved. We first review the genetic mutations implicated in the eight different types of OI, then, the craniofacial consequences of OI mutations are summarized.

Discussion
The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institution guidelines.

Clinical forms of OI
OI type I includes patients with mild disease and absence of major bone deformities. Typical features of OI type I are normal height or mild short stature, blue sclera and no dentinogenesis imperfecta. However, vertebral fractures are typical and can lead to mild scoliosis. Type II is lethal in the perinatal period, usually because of respiratory failure resulting from multiple rib fractures. Typical features of OI type II are multiple fractures at birth, pronounced deformities, broad long bones, low density of skull bones on radiography and dark sclera. Type III is the most severe form in children surviving the neonatal period. Typical features of OI type III are very short stature, triangular face, severe scoliosis, greyish sclera and dentinogenesis imperfecta. Patients with mild-to-moderate bone deformities and a variable short stature are classified as OI type IV. This last group includes all individuals who are not clearly part of the first three types. Typical features of OI type IV are a moderately short stature, mild-to-moderate scoliosis, greyish or white sclera and dentinogenesis imperfecta.

Patients with OI type V have a history of moderate-to-severe increased fragility of long bones and vertebral bodies, and they experience at least
one episode of hyperplastic callus formation. None of the type V patients present blue sclera or dentinogenesis imperfecta, but ligamentous laxity is similar to that in patients with OI type IV. Patients with OI type VI sustain more frequent fractures than patients with OI type IV. Sclerae are white or faintly blue and dentinogenesis imperfecta is uniformly absent. All patients have vertebral compression fractures. Biopsy specimens of the patients show accumulation of osteoid due to a mineralization defect. OI type VII is a form of autosomal recessive OI. The phenotype is moderate to severe, characterized by fractures at birth, bluish sclera and early deformity of the lower extremities, coxa vara and osteopenia. Rhizomelia is a prominent clinical feature. Histomorphometric analyses of iliac crest bone samples reveal findings similar to OI type I. Another form of autosomal recessive OI was also described and designed as OI type VIII. This form is characterized by white sclera, severe growth deficiency, extreme skeletal hypomineralization and bulbous metaphysis.

Genetics forms of OI
DNA linkage studies have suggested that more than 90% of probands with OI have a heterozygous mutation in COL1A1 or COL1A2, encoding the pro-α1 and pro-α2 chains of type I collagen, respectively. The typical associated mutation for OI type I is a premature stop codon in the COL1A1 gene. Glycine substitutions in pro-α1 (I) or pro-α2 (I) collagen chains are the typical mutations associated with OI types II, III and IV. The mildest form of OI typically results from diminished synthesis of structurally normal type I procollagen, whereas moderately severe-to-lethal forms of OI usually result from structural defects in one of the type I procollagen chains. Rauch et al. show that compared with patients with helical mutations, patients with COL1A1 haploinsufficiency, on average, were taller and heavier and had higher bone densitometry. Correlations between genotype and phenotype could not be done in OI. Rules exist, but with many exceptions: severity of the phenotype increases with N-position of the substitution, with larger and electric charged and with COL1A1 mutation (vs. COL1A2).

Actually, for many years, only mutations in COL1A1 and COL1A2 have been reported. But autosomal recessive forms of OI were already identified. In 2006, Morello et al. reported homozygous mutation of cartilage-associated protein (CRTAP). Together with cyclophilin B (CyPB), CRTAP and prolyl-3-hydroxylase-1 (P3H1) comprise the collagen prolyl 3-hydroxylation complex, which catalyses a specific posttranslational modification of types I, II and V collagens, and may act as a general chaperone. The collagen produced by cells with an absence of Pro986 hydroxylation has helical overmodification by lysyl hydroxylase and prolyl 4-hydroxylase, indicating that the folding of the collagen helix has been substantially delayed. Recessive OI is caused by a dysfunctional P3H1/CRTAP/CyPB complex rather than the lack of 3-prolyl 3-hydroxylation in a single proline residue in the alpha1 chains of collagen type I.

The CyPB altered proband’s collagen has normal collagen folding and normal prolyl 3-hydroxylation, suggesting that CyPB is not the exclusive peptidylprolyl cis-trans isomerase that catalyses the rate-limiting step in collagen folding, as is currently thought.

Collagen fibrils in peptide-prolyl isomerase cytophilin B−/− mice (Ppib−/− mice) had abnormal morphology, further consistent with an OI phenotype. In vitro studies revealed that in CyPB-deficient fibroblasts, procollagen did not localize properly to the Golgi.

Recently, publications revealed new genes implicated in autosomal recessive forms of OI; they concern collagen I processing or transcription factors (Tables 1–3).

**OI and craniofacial development**

**Dental consequences of OI**
Genetic defects of collagen I lead to dentinogenesis imperfecta. Clinical prevalence of the affection in OI patients varies among authors. Some reports suggest that all OI patients have dentinogenesis imperfecta. Some patients have clinical forms, whereas other patients can be identified only from the examination of histological sections. Because of odontoblast–ameloblast interactions, dentine–enamel junction and even enamel could be affected.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>New genes implicated in human OI (prolyl 3-hydroxylation of collagen)</th>
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<tr>
<td>Publication</td>
<td>van Dijk et al. 2009</td>
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<tr>
<td>Phenotype</td>
<td>Severe, type IIB/III</td>
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<tr>
<td>Transmission</td>
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<td>Population</td>
<td>Senegalese family with consanguinity</td>
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<tr>
<td>Gene</td>
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<tr>
<td>Protein/function</td>
<td>Cyclophilin B (CyPB)</td>
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Review

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conditions are probably combined with the skeletal and dentoalveolar abnormality.

Ectopic eruption of maxillary molars may be related to maxillary hypodevelopment and globulous shape of the crowns in dentinogenesis imperfecta.

Craniofacial characteristics

Bone fragility and deformities can also affect the skull and spine. The weight of the brain exceeds the load-bearing capacity of the bones at the skull base, deforming then gradually with age and severity of the disease, leading to basilar abnormality. Protrusion of the uppermost vertebrae into the foramen magnum causes brain compression, ranging from asymptomatic to severe neurological symptoms or death.

OI patients are usually described with a triangular face and larger head perimeter, protrusive temporal and frontal bones and overhanging occiput. Many authors show higher incidence of skeletal class III and mandibular prognathism, ranging from asymptomatic to severe neurological symptoms or death.

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**Dentoalveolar disturbances**

Dental class III is confirmed by AoBo (distance between orthogonal projection of maxillary point A and mandibular point B on occlusal plane). Maxillary incisors are labially proclined—compensation of class III—and mandibular incisors inclination is extremely variable. Anterior and posterior alveolar bone is 10% reduced, for mandibular and maxillary incisors as well. Because of short radicular lengths for patients with dentinogenesis imperfecta, alveolar height is more reduced. Due to the loss of dental crown occurring in the dentinogenesis imperfecta, teeth, the lower part of the face appears reduced in height. A high incidence of malocclusions is found, including anterior and posterior cross-bite, and posterior open-bite. Hypodontia is seen by some authors but not reported by all of them. These conditions are probably combined with the skeletal and dentoalveolar abnormality.

**Craniofacial characteristics**

Bone fragility and deformities can also affect the skull and spine. The weight of the brain exceeds the load-bearing capacity of the bones at the skull base, deforming then gradually with age and severity of the disease, leading to basilar abnormality. Protrusion of the uppermost vertebrae into the foramen magnum causes brain compression, ranging from asymptomatic to severe neurological symptoms or death.

brain, which results in a downward bending of the skull base. The anterior cranial base is shorter. Due to normal brain size for these patients and soft calvaria, vertical compensations of skull take place, leading to larger head.\textsuperscript{29,31} Maxillary length is reduced at the same proportion as anterior cranial base. Class III was thought to be mandible related but, in fact, Harvold length of the mandible is smaller for OI patients than for controls. The growth of the ramus is more defective than the mandibular body, related to their differential developmental mechanisms. The latter forms through intramembranous ossification, whereas endochondral bone formation is an essential part of condylar process growth. So, mandibular protrusion is due to mandibular retrusion and a closing rotation growth pattern of the mandible.

Posture of these patients is often altered because of short neck, thorax deformities and basilar impression, with consequences on craniofacial development, such as an aggravation of the vertical facial underdevelopment.

OI affects the growth of all craniofacial bones, despite their various developmental mechanisms. The intramembranous bones in the face and jaws of the OI patients remained smaller than normal. Endochondral growth (skull base and condylar process) is affected by both a primary growth defect and alterations in skull base flexure, with adaptive downregulation in the condylar growth.

Jensen and Lund concluded that structural abnormalities of collagen I generally give higher severe alterations of the craniofacial features than a quantitative defect of collagen.\textsuperscript{31}

Recently, Cheung et al.\textsuperscript{22} concluded that clinical severity of OI, as expressed by the height Z-score, was the strongest predictor of skull base abnormalities.

Bisphosphonates affect osteoclast activity and bone remodelling and are given to young children. Consequences to craniofacial development will have to be explored.

**Craniofacial phenotype according to OI type**\textsuperscript{33}

**OI type I:** Angular measurements are close to those of controls; however sagittal and linear measurements of OI patients are smaller. Shorter anterior cranial base is compensated by longer posterior cranial base. Because of few osseous deformations for these patients, cranial base angulation is subnormal.

**OI type II:** This form is lethal; no craniofacial observation could have been performed.

**OI types III and IV:** The anterior cranial base is shorter with no compensation by posterior cranial base. The deformations are those described below.

**Animal models of OI/SROI**

Many animal models of OI have been described, and some are available for research in cellular, molecular or pharmacological therapy.

In Mov-13 mice, transcription of the pro-\(\alpha\)1 (I) gene is completely blocked as a result of Moloney leukaemia virus integration at the 5′-end of the gene.\textsuperscript{34} Only heterozygotes survived to young adulthood.\textsuperscript{35} According to its phenotype, the heterozygous Mov-13 mouse therefore serves as a model of human OI type I. Tooth rudiments from embryos of homogous Mov-13 mice produced a dentin layer containing normal amounts of type I collagen when grown as transplants either in the anterior chamber of the eye or under the kidney capsule of syngeneic hosts. There is evidence that odontoblasts can efficiently produce \(\alpha\)1 (I) mRNA despite stable integration of the retrovirus within the first intron of the \(\alpha\)1 (I) collagen gene.\textsuperscript{36}

Brittle II mouse is a model of OI type II, using the cre/lox recombination system to develop a lethal knock-in murine model of OI type II.\textsuperscript{37}

Chipman et al.\textsuperscript{38} described oim/oim mice, a strain of mice with a non-lethal recessively inherited mutation that resulted in phenotypic and biochemical features that simulate moderate-to-severe human OI type III. Nucleotide sequencing of cDNA encoding the COOH-propeptide revealed a G deletion at pro-\(\alpha\)2 (I) nucleotide 3983; this results in an alteration of the sequence of the last 48 amino acids. The dental phenotype in oim/oim is more severe in incisors than in molars and includes changes in pulp chamber size, tooth shape and dentin ultrastructure.\textsuperscript{39} Alendronate, a third-generation bisphosphonate drug, acts directly on the osteoclast, inhibiting its resorption capability. Its effects on linear bone growth were studied in oim/oim mice.\textsuperscript{40}

A moderately severe OI phenotype was obtained from a \(\alpha\)1 (I) 349 Gly→Cys substitution in type I collagen, which is the same mutation in a type IV OI child. These mice were designated Brittle IV (BritIV).\textsuperscript{41} In patients with OI, phenotypic variability has been reported in several instances of both related and unrelated probands with the same collagen mutation. Mice with variable phenotypes have equivalent expression of mutant \(\alpha\)1 (I) mRNA in several tissues, including bone and skin. There is a post-pubertal adaptation in which Britf femoral strength and stiffness increase through a mechanism independent of changes in whole bone geometry.\textsuperscript{42} Similarly, moderately severe OI patients experienced a dramatic decrease in fracture frequency following puberty.

There are other animal models of OI ranging from type I to IV; some of them are only clinically described.

Prolyl hydroxylation is a critical post-translational modification that affects structure, function and turnover of target proteins. Prolyl 3-hydroxylation occurs at only one position in the triple-helical domain of fibrillar collagen chains. Cartilage-associated protein (CRTAP) co-purifies with collagen types I, II and XI. CRTAP is the preferred target for prolyl hydroxylation.

protein fractions containing prolyl 3-hydroxylase type I activity and affects its enzymatic activity. CRTAP-null mice develop progressive and severe kyphoscoliosis over the first 6 months of age. Moreover, they exhibit prenatal and postnatal growth delay. CRTAP-null mice exhibit a severe osteoporosis characterized by low bone mass, normal osteoblast and osteoclast numbers, reduced bone formation rate and mineral apposition rate and decreased osteoid synthesis and mineralization lag time. The phenotype of the Crtap−/− mice also revealed multiple abnormalities of connective tissue, including in the lungs, kidneys and skin.

Fragilitas ossium, fro, is an often-lethal recessive mutation that was discovered in a random-bred stock of mice after treatment with a chemical mutagen. The fro/fro mutation has clinical, radiographic and morphological manifestations similar to those that arise in autosomal recessive forms of OI in humans. Approximately 90% of mutant offspring were perinatally lethal, with clinical and radiographic findings similar to those of OI type II subgroup A in humans. The 10% of mutant mice that survived followed a course very similar to severe progressively deforming OI type III. No defect in type I collagen could be detected in the fro/fro mouse. Positional cloning of the locus identified a deletion in the gene encoding Smpd3, the gene responsible for the fro mutation.

Across 24–30 weeks of age, nearly every rib in Zmpste 24−/− mice was broken in the vicinity of the costovertebral junction with hypertrophic calluses at the fracture sites.

The current standard of care includes a multidisciplinary approach with surgical intervention when necessary, proactive physiotherapy and consideration for the use of bisphosphonates—all in attempts to improve the quality of life. Animal models of OI are available for research in cellular, molecular or pharmacological therapy.

For example in their study, Panaroni et al. evaluated intrauterine transplantation of adult bone marrow into heterozygous BrtlIV mice. The transplantation eliminated the perinatal lethality of heterozygous BrtlIV mice. At 2 months of age, femora of treated Brtl mice had significant improvement in geometric parameters (P<0.05) versus untreated Brtl mice, and their mechanical properties attained wild-type values.

**Fro/fro mice and micro-CT**

In 1981, Guenet et al. have reported that after the injections to the male of the chemical mutagen tris(i-aziridinyl)phosphine-sulphine (Thiotepa®), a recessive mutation is observed in the offspring. The mice are smaller; they show bent long bones (deformities of the four limbs). The mice are osteoporotic and therefore display bone fragility. Therefore, this mutation was named fro. With about 90% of lethality and 10% of non-lethality, the fro/fro mice display similarities with non-collagenous forms of OI, despite the fact that there is no spontaneous fracture as is the case in many human forms. Guenet et al., Muriel et al. and Silence et al. further confirmed these findings. Muriel et al. also showed that osteonectin was decreased by 30–50%, with a 5% increase of BSP. Therefore, the question arises if this was a direct or indirect effect due to osteonectin reduction.

The physiopathology mechanisms were clarified later, when the identification of the mutation was made by Aubin et al. The deletion was located on chromosome 8 and was encompassing part of intron 8 and most of the exon 9 of Smpd3 gene.

Neutral sphingomyelinase cleaves sphingomyelin into ceramide, a
substrate for ceramidase resulting in the production of sphingosine. Modified by a specific kinase, sphingosine is converted into sphingosine 1-phosphate (SIP). SIP has a mitogenic action on osteoblasts. In close association with Smpd, defective molecules affect bone development and remodelling. Bone fragility and increased bone resorption lead to a form of OI. In addition, identification was made for the first time of a defective dentinogenesis, appearing either as dentinogenesis imperfecta or as dentin dysplasia. This pointed out a role of sphingomyelin in the mineralization process, since long suspected, but supported by this observation.

Proliferation of cells (as visualized by PCNA) in the forming part of the incisors is much lower in Fro−/− compared with Fro+/− mice. This may account for the difference in length of the Fro−/− incisor, about one half of the Fro+/− mandibular incisor. In molars, proliferation near the tip of the cusps was diminished in the Fro−/− compared with the heterozygote Fro+/−. In addition, the profile of the cusps was more scalloped in the homozygote, with deep recesses between the cusps. The von Kossa staining, revealing calcium phosphate, supported the reduction in number and thickness of the alveolar bone trabeculae, confirming the occurrence of bone osteopenia in the craniofacial skeleton, as was the case for the appendicular skeleton. As a consequence of the general hypomineralization, CS/DS GAGs located in non-mineralized areas are enhanced in the fro/fro−/− mouse.

During aging, a partial self-repair occurs. MicroCT analysis shows gradual recovery. The mandibular bone of day 7 mice is clearly hypomineralized in the Fro−/− compared with the age-matched Fro+/− (Figure 2a, b). Defective bone is still observed at day 21 (Figure 3a, b), but no difference is detectable in a 1-year-old mice (Figure 4a) Fro+/−; (b) Fro−/−.

**Figure 2:** MicroCT: day 7 Fro+/− (a) compared with Fro−/− (b).

**Figure 3:** MicroCT: day 21 Fro+/− (a) vs. Fro−/− (b).
This was not confirmed by three-dimensional reconstruction of the dental pulp. The pulps or the three mandibular molars were taller and larger for the Fro+/– at day 21 compared with the Fro–/– (Figure 5). The same difference was seen in the pulps of a 1-year-old first molar (Figure 6). Quantitative data revealed a 0.0681 mm³ pulp volume in the age-matched pulp of the Fro+/– vs. 0.0748 mm³ in the Fro–/– mice. The fact the pulp volume was larger in homozygote mice suggest that dentinogenesis was impaired and less dentin formation occurred in the Fro–/– mice.

Important differences were detected between the Fro mutation and the smpd3–/– mouse56, although the same gene was targeted. The smpd3–/– mouse might mimic a form of human disease associated with pituitary hormone deficiency. The smpd3–/– mouse shares its dwarf and chondrodysplasia phenotype, the most common form of human achondrodysplasia, linked to the fibroblast growth factor receptor 3 locus, and not linked to deficits in the hypothalamic–pituitary epiphyseal growth plate axis.

For years in our group, attempts were made to elucidate the role of phospholipids in dental and skeletal tissues. Although we obtained biochemical and histochemical evidence that phospholipids are present in the extracellular matrix of mineralized tissues, our experimental approaches failed to establish a firm link between the presence of acidic ECM components and bone and/or teeth mineralization57–63. This mutation provides the first experimental evidence that some lipids are involved in the formation and mineralization of bonny and dental tissues.

Conclusion
The interest for OI focuses on two different aspects. First, a number of studies have clarified the clinical aspects of this group of craniofacial pathologies. Second, all the information obtained from these mutations provide additional understanding on the mechanisms of normality and on the pathologic alterations of skeletal mineralization.

Abbreviations list
CRTAP, cartilage-associated protein; OI, osteogenesis imperfecta; SIP, sphingosine 1-phosphate; SROI, syndromes resembling OI.

References
Figure 5: Three-dimensional reconstruction of the dental pulps of the three mandibular molars of 21-day-old Fro mice.


Figure 6: Three-dimensional reconstruction of the dental pulp of 1-year-old Fro+/- vs. Fro-/- mice. The pulp volume is larger in the homozygote compared with the heterozygote, suggesting dentinogenesis impairment.