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Animal models of Osteogenesis Imperfecta and craniofacial development

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Abstract

Firstly, we review the genetic mutations implicated in the VIII different types of Osteogenesis Imperfecta (OI). Secondly, the craniofacial consequences of OI mutations are summarized. Depending on the OI type, dentoalveolar disturbances and variations in the craniofacial phenotype are reported. Some OI or SROI animal models ranging from type I to IV are analyzed. They included the oim/oim mice, Brittle IV, cartilage associated protein null mice, and fragilitas ossium [the fro/fro mice resulting from the mutation of a gene encoding neutral sphingomyelin phosphodiesterase 3 (SMPD3)]. The dental consequences of the mutated fro/fro mice reported here shed some lights on the role of phospholipids in skeletal mineralization.

Introduction

Osteogenesis imperfecta (OI) is a human genetic disorder of increased bone fragility and low bone mass. Severity varies widely, ranging from intrauterine fractures and perinatal lethality to very mild forms without fractures. There is variable association of typical extraskeletal manifestations with the disorder, including blue sclera, dentinogenesis imperfecta, hyperlaxity of ligaments and skin, hearing impairment, and the presence of Wormian bones on skull radiography[1]. Even though the range of clinical severity in OI is a continuum, categorization of patients into separate types can be useful to assess prognosis and to evaluate the effects of therapeutic interventions. The most widely used classification of OI distinguished four clinical type[2]. 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 much more types of OI than those classified by Sillence et al. Some forms of congenital brittle bones have been considered OI and have been added as types V, VI and VII[3-5].There is still no perfect consensus about the definition of OI. Plotkin recently proposed to define 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 [6]. In the new Nosology and Classification of the Genetic Skeletal disorders[7], OI is declined in several forms depending of the severity of the phenotype whatever the mode of the transmission or the gene involved.

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. Deformities secondary to multiple fractures can lead to respiratory difficulties, identified as a leading cause of death in this patient group[8,9]. Patients with mild to moderate bone deformities and 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 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 sclerae 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[10]. This form is characterized by white sclera, severe growth deficiency, extreme skeletal hypo mineralization and bulbous metaphysis.

Genetics forms of OI

DNA linkage studies have suggested that more than 90% of probands with OI have an heterozygous mutation in COL1A1 or COL1A2, respectively encoding the pro-α1 and pro-α2 chains of type I collagen[11]. The typical associated mutation for OI type I is a premature stop codon in the COL1A1 gene. Glycine substitutions in proα1 (I) or proα2 (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[12]. Rauch et al[13] 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 been identified. In 2006, Morello et al. reported homozygous mutation of CRTAP[14]. Together with cyclophilin B (PPIB), CRTAP and P3H1 comprise the collagen prolyl 3-hydroxylation complex, which catalyzes a specific posttranslational modification of types I, II and V collagens, and may act as a general chaperone[15]. 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 by the lack of 3-prolyl hydroxylation of a single proline residue in the alpha1 chains of collagen type I[16].

The CyPB altered proband's collagen has normal collagen folding and normal prolyl 3-hydroxylation, suggesting that CyPB is not the exclusive peptidyl-prolyl cis-trans isomerase that catalyzes the rate-limiting step in collagen folding, as is currently thought[17].
Collagen fibrils in 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[18].

Table 1: New genes implicated in human OI (prolyl-3 hydroxylation of collagen)

Publication	Van Dijk 2009[16]	Morello 2006[14]	Barnes 2010[17]
Phenotype	Severe, type IIB/III	Lethal to severe	Moderate without rhizomelia
Transmission	Autosomal recessive	Autosomal recessive	Autosomal recessive
Population			Senegalese family with consanguinity
Gene	PPIB	CRTAP or P3H1/LEPRE1	Start codon mutation in PPIB
Protein/function	CyPB	Cartilage-associated protein or prolyl 3-hydroxylase 1(loss of function)	Lack of cyclophilin B (CyPB)

Recently, publications revealed new genes implicated in autosomal recessive forms of OI; they concern collagen I processing or transcription factors (Tab 2)

Table 2: New gene implicated in human OI (P3H1/CRTAP/CyPB complex excluded)

Publication	Becker et al. 2011[19]	Alanay et al. 2010[20]	Christiansen et al. 2010[21]	Lapunzina et al. 2010 [22]
Phenotype	Severe, with vertebral compression fractures and resulting deformities	Moderately severe	Severe	Recurrent fractures, mild bone deformities,
Transmission	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive
Population		Five consanguineous Turkish families and a Mexican-American family		Egyptian child
Gene	Truncating mutation, affecting SERPINF1	Mutations in FKBP10	Missense mutation (c.233T>C, p. Leu78Pro) in SERPINH1	Homozygous single base pair deletion (c.1052del) in SP7/Oster (OSX)
Protein/function	Pigment epithelium-derived factor (PEDF), multifunctional glycoprotein, inhibitor of angiogenesis	FKBP65, a chaperone that participates in type I procollagen folding	Collagen chaperone-like protein HSP47 *	Transcription factor with three Cys2-His2 zinc-finger DNA-binding domains, the third removed by deletion

*: The mutation results in degradation of the endoplasmic reticulum resident HSP47 via the proteasome. Type I procollagen accumulates in the Golgi apparatus of fibroblasts. These findings suggest that HSP47 monitors the integrity of the triple helix of type I procollagen at the ER/cis-Golgi boundary. When HSP47 is absent, the rate of transit from the ER to the Golgi is increased and the helical structure formation is compromised. The role of HSP47 is downstream from the CRTAP/P3H1/CyPB complex.

OI and craniofacial development

Dental consequences of OI

Genetic defects of collagen I leads to dentinogenesis imperfecta. Clinical prevalence of the affection in OI patients varies among authors. Some reports[23] suggest that all OI patients have dentinogenesis imperfecta. Some patients have clinical forms, whereas others patients can be identified only from the examination of histological sections. Because of odontoblasts-ameloblasts interactions, dentine-enamel junction and even enamel could be affected[24].

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 as well [25]. Because of short radicular lengths for patients with dentinogenesis imperfecta, alveolar height is more reduced. With loss of dental crown height of dentinogenesis imperfecta affected teeth, lower face heights are particularly small. 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.

Ectopic eruption of maxillary molars may be related to maxillary hypodevelopment and globulous shape of the crowns in dentinogenesis imperfecta[26].

Craniofacial characteristics

Bone fragility and deformities can affect also skull and spine[27]. 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, and 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 triangular face and larger head perimeter, protrusive temporal and frontal bones, and overhanging occiput[28]. Many authors show higher incidence of skeletal class III[25, 28-30]. For Waltimo-Siren[25], the sella region is depressed by the weight of the brain, which results a downward bending of the skull base. The anterior cranial base is shorter. Because of normal brain size for these patients and soft calvaria, vertical compensations of skull take place, leading to larger head [29, 31]. Maxillary length is reduced is 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 essential part of condylar process growth. So, mandibular protrusion is due to maxillary 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[31].

Recently, Cheung et al.[32] 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 remodeling, and are given to young children. Consequences to craniofacial development will have to be explored.

Craniofacial phenotype according to OI type [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 type 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 proα1 (I) gene is completely blocked as a result of Moloney leukemia virus integration at the 5' end of the gene[34]. Only heterozygotes survived to young adulthood[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 homozygous 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 α1 (I) mRNA despite stable integration of the retrovirus within the first intron of the α1(I) collagen gene[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 [37].

Chipman et al.[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 proα2 (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[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[40].

A moderately severe OI phenotype was obtained from a α1 (I) 349 Gly → Cys substitution in type I collagen, which is the same mutation in a type IV OI child. These mice were designated as Brtl IV (Brittle IV)[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 phenotype have equivalent expression of mutant α1 (I) mRNA in several tissues, including bone and skin. There is a post pubertal adaptation in which Brtl femoral strength and stiffness increase through a mechanism independent of changes in whole bone geometry[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) copurifies with 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[14]. The phenotype of the Crtap-/- mice also revealed multiple abnormalities of connective tissue, including in the lungs, kidneys, and skin [43].

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[44, 45]. 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 neutral sphingomyelin phosphodiesterase 3 (Smpd3) that led to complete loss of enzymatic activity. The smpd3 promoter is activated by BMP2 and is directly regulated by the Runx2 transcription factor[46]. The precise relationship between impairment of the gene encoding Smpd3 and bone fragility have to be explored.

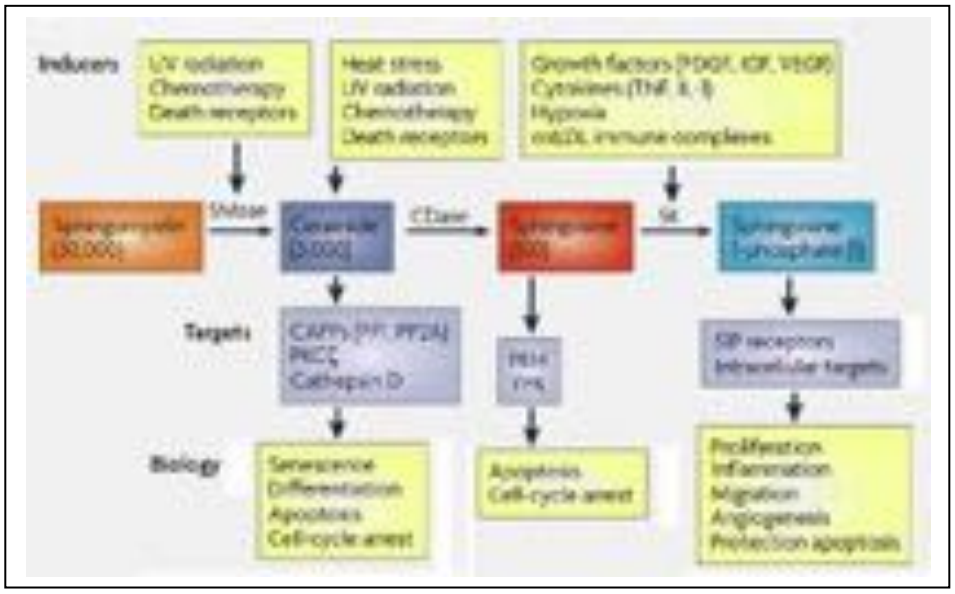

Figure 1: Metabolism of sphingolipids with indication of some molecular targets and subsequent biological effects (modified from Hannun & Obeid 2008)[47]
http://www.nature.com/nrm/journal/v9/n2/abs/ - a2 Nat Rev Mol Cell Biol *2008, **9**, 139-150).*

Another gene-alteration of a protease seems also to play a role in a form of OI. Zmpste-24 acts as a CAAX endoprotease, clipping off the C-terminal three amino acids from the protein (i.e., the –AAX of the CAAX motif). By 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[48].

Table 3: New genes implicated in animal OI or SROI with no human equivalent yet

Publication	Aubin et al. 2005 [49]	Bergo et al. 2002 [48]
Phenotype	Lethal to severe	Moderate (-/+) and severe (-/-)
Transmission	Recessive	
Population	Fro/fro mice	Mice homozygous and heterozygous
Gene	Deletion in SMPD3	Zmpste-24
Protein/function	Loss of enzyme activity of neutral sphingomyelinases cleaving sphingomyelin into ceramide	Integral membrane zinc metalloproteinase of the endoplasmic reticulum
Human	none	mandibuloacral dysplasia lethal restrictive dermopathy

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 quality of life[50]. Animal models of OI are available for research in cellular, molecular or pharmacological therapy.

For example in their study, Panaroni et al[51] evaluated intra-uterine 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 ($P < .05$) 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[52]. The mice are smaller; they show bended long bones (deformities of the four limbs). The mice are osteoporotic, and therefore display bone fragility. Therefore, this mutation was named Fragilitas Ossium (Fro). With about 90% of lethality and 10% of non-

lethality, the Fro/fro mice display similarities with non-collagenous forms of Ostegenesis Imperfecta, despite there is no spontaneous fracture as it is the case in many human forms. Guenet[52], Muriel et al.[53] and Silence et al.[45] further confirmed these findings. Muriel et al.[53] also evidenced that osteonectin was decreased by 30-50%, with a 5% increase of BSP. Therefore the question arises if it 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.[49.] 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 sphingosine1-phosphate (SIP) [see for review: Hannun & Obeid,[47]]. SIP has a mitogenic action on osteoblasts. In close association with Smpd, defective molecules affect bone development and remodeling. Bone fragility and increased bone resorption leads to a form of osteogenesis imperfecta. In addition, identification was made for the first time of a defective dentinogenesis, appearing either as Dentinogenesis imperfecta, or Dentin Dysplasia [49]. This pointed out on 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 [54,55]. The von Kossa staining, revealing calcium phosphate supported the reduction in number and thickness of alveolar bone trabeculae, confirming the occurrence of bone osteopenia in the cranio-facial skeleton, as it was the case for the appendicular skeleton. As the 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+/- (**Fig. 1a,b**). Defective bone is still observed at day 21 (**Fig. 2a,b**), but no difference is detectable in 1year-old mice (**Fig. 3a,b**)[a: Fro+/-; b: Fro-/-]

This was not confirmed by 3D reconstruction of the dental pulp. The pulps or the 3 mandibular molars were taller and larger for the Fro+/- at day 21 compared with the Fro-/- (**Fig. 4**). The same difference was seen in the pulps of 1 year-old first molar (**Fig. 5**).

Quantitative data revealed a 0.0681mm^3 pulp volume in the age-matched pulp of the Fro+/- vs. 0.0748mm^3 in the -/- Fro mice. The fact the pulp volume was larger in the homozygote mice suggests that dentinogenesis was impaired and less dentin formation occurred in the Fro -/- mice.

Important differences were detected between the Fro mutation and the smpd3 -/- mouse[56], although the same gene was targeted. The smpd3 -/- mouse might mimic a form of human disease associated to 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 get 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 mineralization[57-59]. This mutation provides the first experimental evidence that some lipids are involved in the formation and mineralization of bonny and dental tissues.

VII Conclusion

The interest for Osteogenesis Imperfecta focus on two different aspects. Firstly, a number of studies have clarified the clinical aspects of this group of craniofacial pathologies. Our aim is to identify and get a better knowledge on the different types of mutations involved in the disease, especially within the frame of a therapeutic prospect[60-63, 33]. Secondly, all the information get from these mutations provide additional understanding on the mechanisms of normality and on the pathologic alterations of skeletal mineralization.

List of references:

1. Rauch F, Glorieux FH. Osteogenesis imperfecta. Lancet 2004; 363:1377-1385.
2. Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet 1979; 16 : 101-116
3. Glorieux FH, Rauch F, Plotkin H, Ward L, Travers R, Roughley P, Lalic L, Glorieux DF, Fassier F, Bishop NJ. Type V osteogenesis imperfecta: a new form of brittle bone disease J Bone Miner Res 2000; 15 : 1650-1658.
4. Glorieux FH, Ward LM, Rauch F, Lalic L, Roughley PJ, Travers R. Osteogenesis imperfecta type VI: a form of brittle bone disease with a mineralization defect. J Bone Miner Res 2002; 17 : 30-38.

5. Ward LM, Rauch F, Travers R, Chabot G, Azouz EM, Lalic L, Roughley PJ, Glorieux FH. Osteogenesis imperfecta type VII: an autosomal recessive form of brittle bone disease. Bone 2002; 31 : 12-18.
6. Plotkin H. Syndromes with congenital brittle bones. BMC Pediatr 2004; 4 : 16.
7. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, Lemerrer M, Mortier G, Mundlos S, Nishimura G, Rimoin DL, Robertson S, Savarirayan R, Sillence D, Spranger J, Unger S, Zabel B, Superti-Furga A. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A. 2011; 155(5): 943-68.
8. McAllion SJ, Paterson CR. Causes of death in osteogenesis imperfecta. J Clin Pathol 1996; 49 : 627-630.
9. Paterson CR, Ogston SA, Henry RM. Life expectancy in osteogenesis imperfecta. BMJ 1996; 312 : 351.
10. Cabral WA, Chang W, Barnes AM, Weis MA, Scott MA, Leikin S, Makareeva E, Kuznetsova NV, Rosenbaum KN, Tifft CJ, Bulas DI, Kozma C, Smith PA, Eyre DR, Marini JC. Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. Nature Genetics 2007; 39 : 359 – 365.
11. Sykes B, Ogilvie D, Wordsworth P, Wallis G, Mathew C,Beighton P, Nicholls A, Pope FM, Thompson E, Tsipouras P,Schwartz R, Jensson D, Aznason A, Borresen AL, HeibergA, Frey D, Steinmann B. Consistent linkage of dominantly inherited osteogenesis imperfecta to the type I collagen loci: COL1A1 and COL1 A2. Am J Hum Genet 1990; 46 : 293–307.
12. Roughley PJ, Rauch F, Glorieux FH. Osteogenesis imperfecta-clinical and molecular diversity. Eur Cell Mater 2003; 5 : 41–47.
13. Rauch F, Lalic L, Roughley P, Glorieux FH. Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. J Bone Min Res 2010; 25 : 1367-1374.
14. Morello R, Bertin T, Chen Y, et al. CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. Cell 2006; 127: 291-304.
15. Marini JC, Cabral WA, Barnes AM. Null mutations in LEPRE1 and CRTAP cause severe recessive osteogenesis imperfecta. Cell Tissue Res 2010 ; 339 : 59-70.
16. van Dijk FS, Nesbitt IM, Zwikstra EH, Nikkels PG, Piersma SR, Fratantoni SA, Jimenez CR, Huizer M, Morsman AC, Cobben JM, van Roij MH, Elting MW, Verbeke JI, Wijnaendts LC, Shaw NJ, Högler W, McKeown C, Sistermans EA, Dalton A, Meijers-Heijboer H, Pals G. PPIB mutations cause severe osteogenesis imperfecta. Am J Hum Genet 2009;85: 521-527.
17. Barnes AM, Carter EM, Cabral WA, Weis M, Chang W, Makareeva E, Leikin S, Rotimi CN, Eyre DR, Raggio CL, Marini JC. Lack of cyclophilin B in osteogenesis imperfecta with normal collagen folding. N Eng J Med 2010 362: 521-528.
18. Choi JW, Sutor SL, Lindquist L, Evans GL, Madden BJ, Bergen HR 3rd, Hefferan TE, Yaszemski MJ, Bram RJ. Severe osteogenesis imperfecta in cyclophilin B-deficient mice. PloS Genet 2009 Dec;5(12):e1000750. Epub 2009 Dec 4.
19. Becker J, Semler O, Gilissen C, Li Y, Bolz HJ, Giunta C, Bergmann C, Rohrbach M, Koerber F, Zimmermann K, de Vries P, Wirth B, Schoenau E, Wollnik B, Veltman JA, Hoischen A, Netzer C. Exome sequencing identifies truncating mutations in human

SERPINF1 in autosomal-recessive osteogenesis imperfecta. Am J Hum Genet. 2011 Mar 11;88 (3):362-71.

20. Alanay Y, Avaygan H, Camacho N, Utine GE, Boduroglu K, Aktas D, Alikasifoglu M, Tuncbilek E, Orhan D, Bakar FT, Zabel B, Superti-Furga A, Bruckner-Tuderman L, Curry CJ, Pyott S, Byers PH, Eyre DR, Baldridge D, Lee B, Merrill AE, Davis EC, Cohn DH, Akarsu N, Krakow D. Mutations in the gene encoding the RER protein FKBP65 cause autosomal-recessive osteogenesis imperfecta. Am J Hum Genet 2010; 86(4) : 551-559.
21. Christiansen HE, Schwarze U, Pyott SM, AlSwaid A, Al Balwi M, Alrasheed S, Pepin MG, Weis MA, Eyre DR, Byers PH. Homozygosity for a missense mutation in SERPINH1, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. Am J Hum Genet 2010; 86(3): 389-398.
22. Lapunzina P, Aglan M, Temtamy S, Caparrós-Martín JA, Valencia M, Letón R, Martínez-Glez V, Elhossini R, Amr K, Vilaboa N, Ruiz-Perez VL.Identification of a frameshift mutation in Osterix in a patient with recessive osteogenesis imperfecta. Am J Hum Genet 2010; 87:110-114.
23. Lindau BM, Dietz W, Hoyer I, Lundgren T, Storhaug K, Norén JG. Morphology of dental enamel and dentine-enamel junction in osteogenesis imperfecta. Int J Paediatr Dent. 1999 ; 9(1):13-21.
24. Lindau B, Dietz W, Lundgren T, Storhaug K, Norén JG. Discrimination of morphological findings in dentine from osteogenesis imperfecta patients using combinations of polarized light microscopy, microradiography and scanning electron microscopy. Int J Paediatr Dent 1999; 9 (4):253-61.
25. Waltimo-Sirén J, Kolkka M, Pynnönen S, Kuurila K, Kaitila I, Kovero O. Craniofacial features in osteogenesis imperfecta: a cephalometric study. Am J Med Genet A 2005 Mar 1;133A(2):142-50.
26. Kamoun-Goldrat AS. Genetic collagen disorders and the impact on craniofacial development. Orthod Fr 2007 Mar; 78(1):49-62.
27. Kovero O, Pynnönen S, Kuurila-Svahn K, Kaitila I, Waltimo-Sirén J. Skull base abnormalities in osteogenesis imperfecta: a cephalometric evaluation of 54 patients and 108 control volunteers. J Neurosurg 2006 Sep;105(3):361-70.
28. Chang PC, Lin SY, Hsu KH. The craniofacial characteristics of osteogenesis imperfecta patients. Eur J Orthod 2007 Jun; 29(3):232-7. Epub 2006 Sep 13.
29. O'Connell AC, Marini JC. Evaluation of oral problems in an osteogenesis imperfecta population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999 Feb; 87(2):189-96.
30. Ormiston IW, Tideman H. Orthognathic surgery in osteogenesis imperfecta: a case report with management considerations. J Craniomaxillofac Surg 1995 Aug; 23(4):261-5.
31. Jensen BL, Lund AM. Osteogenesis imperfecta: clinical, cephalometric, and biochemical investigations of OI types I, III, and IV. J Craniofac Genet Dev Biol 1997 Jul-Sep; 17(3): 121-32.
32. Cheung MS, Arponen H, Roughley P, Azouz ME, Glorieux FH, Waltimo-Sirén J, Rauch F. Cranial base abnormalities in osteogenesis imperfecta: phenotypic and genotypic determinants. J Bone Miner Res 2011 Feb;26(2):405-13. doi: 10.1002/jbmr.220.

33. Kamoun-Goldrat AS, Le Merrer MF. Osteogenesis imperfecta and dentinogenesis imperfecta: diagnostic frontiers and importance in dentofacial orthopedics. Orthod Fr 2007 Jun;78(2):89-99.
34. Harbers K, Kuehn M, Delius H, Jaenisch R. Insertion of retrovirus into the first intron of alpha 1(I) collagen gene to embryonic lethal mutation in mice. Proc Natl Acad Sci U S A 1984 ; 81:1504-1508
35. Jaenisch R, Harbers K, Schnieke A, Lohler J, Chumakov I, Jahner D, Grotkopp D, Hoffmann E. Germline integration of moloney murine leukemia virus at the Mov13 locus leads to recessive lethal mutation and early embryonic death. Cell 1983; 32:209-216.
36. Kratochwil K, von der Mark K, Kollar EJ, Jaenisch R, Mooslehner K, Schwarz M, Haase K, Gmachl I, Harbers K. Retrovirus-induced insertional mutation in Mov13 mice affects collagen I expression in a tissue-specific manner. Cell 1989; 57:807-816.
37. Forlino A, Porter FD, Lee EJ, Westphal H, Marini JC. Use of the Cre/lox recombination system to develop a non-lethal knock-in murine model for osteogenesis imperfecta with an alpha1(I) G349C substitution. Variability in phenotype in BrtlIV mice. J Biol Chem 1999; 274:37923-37931.
38. Chipman SD, Sweet HO, McBride DJ Jr, Davisson MT, Marks SC Jr, Shuldiner AR, Wenstrup RJ, Rowe DW, Shapiro JR. Defective pro alpha 2(I) collagen synthesis in a recessive mutation in mice: a model of human osteogenesis imperfecta. Proc Natl Acad Sci U S A 1993 ; 90:1701-1705.
39. Lopez Franco GE, Huang A, Pleshko Camacho N, Blank RD. Dental phenotype of the col1a2(oim) mutation: DI is present in both homozygotes and heterozygotes. Bone 2005; 36:1039-1046.
40. Evans KD, Lau ST, Oberbauer AM, Martin RB. Alendronate affects long bone length and growth plate morphology in the oim mouse model for Osteogenesis Imperfecta Bone 2003 ; 32:268-274.
41. Forlino A, Porter FD, Lee EJ, Westphal H, Marini JC. Use of the Cre/lox recombination system to develop a non-lethal knock-in murine model for osteogenesis imperfecta with an alpha1(I) G349C substitution. Variability in phenotype in BrtlIV mice. J Biol Chem 1999 ; 274:37923-37931.
42. Kozloff K M, Carden A, Bergwitz C, Forlino A, Uvegess TE, Morris MD, Marini JC, Goldstein SA. Brittle IV mouse model for Osteogenesis Imperfecta IV demonstrates postpubertal adaptation to improve whole bone strength. J Bone Miner Res 2004. 19: 614-622.
43. Baldridge D, Lennington J, Weis M, Homan EP, Jiang MM, Munivez E, Keene DR, Hogue WR, Pyott S, Byers PH, Krakow D, Cohn DH, Eyre DR, Lee B, Morello R.Generalized connective tissue disease in Crtap-/- mouse. PloS One 2010 May 11;5(5):e10560.
44. Guenet J-L., Stanescu R, Maroteaux P, Stanescu V. Fragilitas ossium: a new autosomal recessive mutation in the mouse. Journal of Heredity 1981; 72: 440-441.
45. Sillence DO, Ritchie HE, Dibbayawan T, Eteson D, Brown K. Fragilitas ossium (fro/fro) in the mouse: a model for a recessively inherited type of osteogenesis imperfecta. Am J Med Genet 1993 ; 45:276-83.

46. Chae YM, Heo SH, Kim JY, Lee JM, Ryoo HM, Cho JY. Upregulation of smpd3 via BMP2 stimulation and Runx2. BMB Rep 2009; 42:86-90.
47. Hannun YA, Obeid LM. Principes of bioactive lipid signalling: lessons from sphingolipids. Nature Reviews | Molecular Cell Biology 2008 ; 9 : 139- 150.
48. Bergo MO, Gavino B, Ross J, Schmidt WK, Hong C, Kendall LV, Mohr A, Meta M, Genant H, Jiang Y, Wisner ER, Van Bruggen N, Carano RA, Michaelis S, Griffey SM, Young SG. Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A processing defect. Proc Natl Acad Sci U S A 2002; 99:13049-54.
49. Aubin I, Adams CP, Opsahl S, Septier D, Bishop CE, Auge N, Salvayre R, Negre-Salvayre A, Goldberg M, Guenet JL, Poirier C. A deletion in the gene encoding sphingomyelin phosphodiesterase 3 (Smpd3) results in osteogenesis and dentinogenesis imperfecta in the mouse. Nat Genet 2005 ; 37:803-805.
50. Basel D, Steiner RD. Osteogenesis imperfecta: recent findings shed new light on this once well-understood condition. Genet Med 2009 Jun;11(6):375-85.
51. Panaroni C, Gioia R, Lupi A, Besio R, Goldstein SA, Kreider J, Leikin S, Vera JC, Mertz EL, Perilli E, Baruffaldi F, Villa I, Farina A, Casasco M, Cetta G, Rossi A, Frattini A, Marini JC, Vezzoni P, Forlino A. In utero transplantation of adult bone marrow decreases perinatal lethality and rescues the bone phenotype in the knockin murine model for classical, dominant osteogenesis imperfecta. Blood 2009; 114 : 459-468.
52. Guenet J-L. Fragilitas ossium (fro): an autosomal recessive mutation in the mouse. Prog Clin Biol Res. 1982;94: 265-267.
53. Muriel MP, Bonaventure J, Stanescu R, Maroteaux P, Guénet J-L, Stanescu V. Morphological and biochemical studies of a mouse mutant (fro/fro) with bone fragility *Bone* 1991; 12: 241-248.
54. Opsahl S, Septier D, Aubin I, Guenet J-L, Sreenath T, Kulkarni A, Vermelin L, Goldberg M.. Is the lingual forming part of the incisor a structural entity? Evidences from the *fragilitas ossium* (*fro/fro*) mouse mutation and the TGFβ1 overexpressing transgenic strain. Arch Oral Biol. 2005 ; 50 : 279-286.
55. Goldberg M, Opsahl S, Aubin I, Septier D, Chaussain-Miller C, Boskey A, Guenet J-L. Sphingomyelin Degradation is a Key Factor in Dentin and Bone Mineralization: Lessons from the *fro/fro* Mouse. J Dent Res 87(1):9-13, 2008.
56. Stoffel W, Jenke B, Blöck B, Zumbansen M, Koebke J. Neutral sphingomyelinase 2 (smpd3) in the control of postnatal growth and development PNAS, 2005 ; 102 : 4554–4559.
57. Goldberg M, Boskey AL. Lipids and biomineralizations. Progress in Histochem Cytochem. 1996; 31 (N°2): 1- 187.
58. Goldberg M, Septier D. Phospholipids in amelogenesis and dentinogenesis. Critical Review in Oral Biology and Medicine. 2002. 13 : 276-290.
59. Khavandgar Z, Poirier C, Clarke CJ, Li J, Wang N, McKee MD, Hannun YA, Murshed M. A cell-autonomous requirement for neutral sphingomyelinases 2 in bone mineralization. J Cell Biol. 2011, 194: 277-289.
60. Kawakami M, Yamamura K. Cranial bone morphometric study among mouse strains. BMC Evol Biol. 2008 ; 29(8) : 73.

61. Nishimura I, Drake TA, Lusis AJ, Lyons KM, Nadeau JH, Zernik J. ENU large-scale mutagenesis and quantitative trait linkage (QTL) analysis in mice: novel technologies for searching polygenetic determinants of craniofacial abnormalities. Crit Rev Oral Biol Med. 2003 ; 14(5) : 320-330.
62. Drögemüller C, Becker D, Brunner A, Haase B, Kircher P, Seeliger F, Fehr M, Baumann U, Lindblad-Toh K, Leeb T. A missense mutation in the SERPINH1 gene in Dachshunds with osteogenesis imperfecta. PloS Genet 2009 Jul;5(7):e1000579. Epub 2009 Jul 24.
63. Kovero O, Pynnönen S, Kuurila-Svahn K, Kaitila I, Waltimo-Sirén J. Skull base abnormalities in osteogenesis imperfecta: a cephalometric evaluation of 54 patients and 108 control volunteers. J Neurosurg 2006 Sep; 105(3):361-70.

Legends of the figures

Figure 1: MicroCT Day 7 Fro +/- (a) compared with Fro-/- (b).

Figure 2: MicroCT Day 21 Fro +/- (a) vs Fro-/- (b).

Figure 3: MicroCT 1 year old mandible of Fro+/- mice vs. Fro-/- .

Figure 4: 3D reconstruction of the dental pulps of the 3 mandibular molars of 21 day old Fro mice

Figure 5: 3D reconstruction of the dental pulp of 1year-old Fro +/- vs -/- . The pulp volume is larger in the homozygote compared with the heterozygote, suggesting dentinogenesis impairment.