SMAD7, a new gene associated with osteosclerosis

ANAÏK PREVIDI[1] ET CORRINE COLLET[1,2]
[1]Laboratoire Bases moléculaires et physiopathologiques des ostéochondrodysplasies, INSERM U1163, Institut Imagine, 24 bd du Montparnasse, Paris.
[2]APHP.Centre-Université Paris Cité, Site Hôpital Necker Enfants-Malades, Service de génétique moléculaire, Paris, France

ABSTRACT

The project aims is to characterize the bone phenotype of a mice with a *SMAD7* homozygous variant identified by WES in a patient with severe facio-mandibular hyperostosis and long bones diaphyseal sclerosis that appeared during childhood and resembling to any known syndrome. It is a bone dysplasia characterised by excessive bone formation belonging to a vast family of bone diseases in which no treatment is available. *SMAD7* gene encodes one of the major intracellular inhibitors of TGF-ß/BMP pathways and has never been associated with a rare bone disease although a role in fibrosis, cancer, and inflammatory diseases has been described. Transcriptional analysis obtained from the patient's fibroblast seem to confirm the variants pathogenicity and suggest both an impaired osteoclast and osteoblast function. To understand the pathophysiology, a Smad7 KI mouse model was chosen as the most suitable system, as Smad7 KO mice are lethal. *In vivo* and *ex vivo* experiments, will be held to study the effects of this variant on bone formation and resorption and to compare the ability of mutated and wild-type bone cells to differentiate into osteoclasts and osteoblasts. This original project is the first study on *SMAD7* associated rare bone dysplasia. Results will help to unravel SMAD7 role during bone formation and to identify potential new therapeutic targets, essential for further drug testing, to reduce bone osteocondensation but also for other bone dysplasia where no therapeutics are available.

BACKGROUND

We report the case of a women aged 30 years old affected by severe atypical osteocondensation that had started at 5 years old. Radiographs revealed osteosclerosis of all long bones, the cranial vault, and the face, including the maxillae (Figure 1). She also presented a congenital cataract and has ventilatory disorders requiring night ventilation. The extensive hyperostosis worsened with age, and inability to move elbows appeared. She has maxilla-mandibular and knee pain but no muscle or neurologic impairment. Apart from maxillary surgeries, no therapeutic options exist to reduced or blocked osteocondensation. Serum markers of bone formation (bone-specific alkaline phosphatase [ALP], P1NP) and resorption (Trap5b, beta-Cross-laps [CTx]) were within normal ranges except an elevated bone-specific ALP activity. The overall phenotype did not suggest a known syndrome such as Camurati-Engelmann disease or craniodiaphyseal dysostosis. Initial molecular genetic investigations including NGS was negative. The proband and her two unaffected parents underwent whole-exome sequencing (WES). Analysis revealed two compounds heterozygous variants in exons 1 and 4 of *SMAD7*, a gene associated with signalling pathways involved in bone; inherited from the mother and father, respectively. According to the ACMG criteria, the variants were classified as variants of uncertain significance.

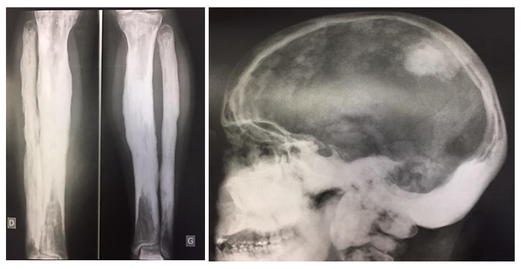

Figure 1. The patient's radiographs showing diaphyseal and facio-mandibular hyperostosis.

HYPOTHESIS, AIMS AND DESIGN

SMAD7 gene encodes one of the major intracellular inhibitors of TGF-ß/BMP pathways. TGF-ß pathway is involved in many biological processes and its dysregulation is associated with various skeletal human diseases : osteoarthritis, osteoporosis but also Camurati-Engelman disease or Loeys-Dietz syndrome. To date, *SMAD7* gene has never been described in human diseases although many studies have found a physiological role in fibrosis, cancer, and inflammatory diseases. During bone formation, SMAD7 inhibits proliferation, differentiation, and mineralization of osteoblasts via TGFβ/BMP pathways. Therefore, we suggest that *SMAD7* variants could explain our patient's phenotype with an inhibitory mechanism that remains to be elucidated. Preliminary functional studies of the patient's primary fibroblast cells showed decreased *SMAD7* expression level and revealed impaired osteoblast and osteoclast functions supporting a pathogenic role of *SMAD7* variants. To understand the pathophysiology, a Smad7 KI mouse model was chosen as the most suitable system with multiple cons. As lack of expression of the entire Smad7 protein or MH2 domains are lethal, we generated the Smad7 heterozygous missense variant by CRISPR-Cas9 technology. *In vivo* and *ex vivo* experiments, will be held to study the effects of this variant on bone formation and resorption at different age in wild-type and KI mice (microCT-scan, radiography and histology), and to compare the ability of mutated and wild-type bone cells to differentiate into osteoclasts and osteoblasts (transcriptional analysis). This model will allow a better knowledge of the role of SMAD7 in bone In addition, it will be a model to test drugs to reduce the osteocondensation of the patient and other sclerosing bone dysplasias.

METHODOLOGY

In vivo analysis : 0-2-4-6-8-10 weeks

- Generation of *smad7* KI mouse model : introduction of a missense heterozygous variant by CRISPR-Cas9 technology (Illkirch institute, IGBMC)
- Morphology and imaging (CT scan, radiography)
- Bone marker and histology

In vitro and *ex vivo* analysis :

- Primary human fibroblast culture : qRT-PCR (*SMAD7, ALPL, RANKL*)
- Mice model : histochemical staining, qRT-PCR and RNAseq, Trap coloration, Bone resorption test

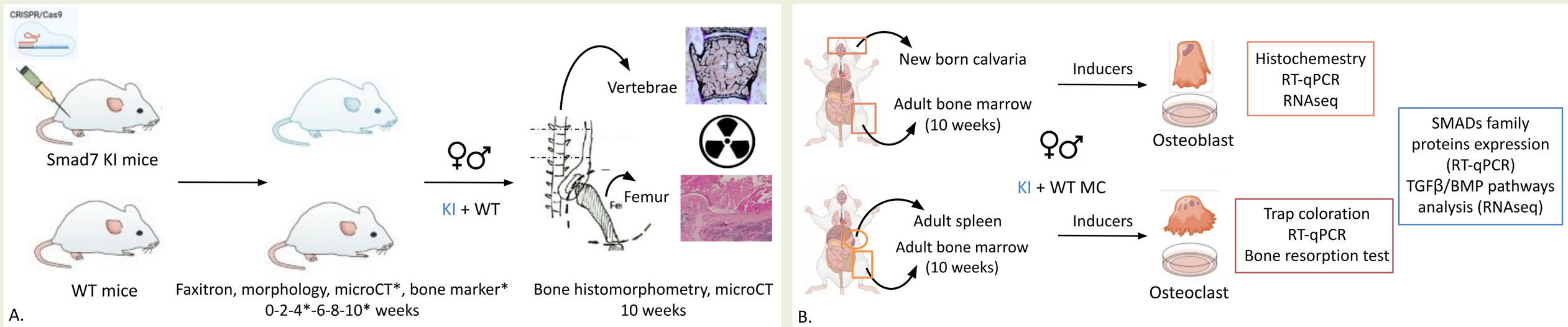

Fig 2. Further explorations : mice (KI) model. **A. *In vivo* study** : characterization of the Knock-In (KI) mice phenotype compared to Wild-type (WT) mice (height, weight, malformations). MicroCT, radiography, densitometry and histology will be performed in female and male mice for both genotypes. Femurs and vertebrae will be harvested and subjected to digital radiography to compare their size and shape, micro-CT to detect morphometric parameters, and bone histomorphometry to observe tissue morphology . **B. *Ex vivo* study** : The Smad7 variant probably affects osteoblastogenesis and osteoclastogenesis based on results obtained from primary fibroblast cells. Objectives will be to compare the ability of mutated and wild-type bone cells to differentiate into osteoclasts and osteoblasts. Osteoblasts culture will be obtained from mesenchymal cells (MC) of 10 weeks old mice and from calvaria of newborn mice after differentiation in the presence of inducers (ascorbic acid and beta-glycerophosphate). Kinetics of differentiation will be followed with histochemical techniques, RT-qPCR and RNAseq. Osteoclastic differentiation will be studied from mice bone marrow and spleen cells treated with M-CSF and RANKL. Effect of the variant on osteoclastogenesis will be held by TRAP coloration, RT-qPCR and bone resorption test. To confirm results obtained from the patient's fibroblasts, SMADs family proteins (phosphor-R-Smad and co-Smad) expression will be studied in both osteogenesis and osteoclastogenesis processes. In a second time and to precise effect of SMAD7 on TGFβ/BMP pathways and cross-talk with other pathways, proteomic and transcriptomic analysis will be done on osteoblasts and osteoclasts. According to the results, co-immunoprecipitation experiments will be planned.

CONCLUSION

The aim of this project is to reproduce the novel sclerosing bone dysplasia thanks to a knock-in (KI) *SMAD7* mouse model. This mouse model will confirm that a specific homozygous variant of *SMAD7* is responsible for the novel bone phenotype. In addition, this approach will allow us to study the pathophysiologic mechanism that leads to this atypical bone phenotype. This *Smad7* KI mouse model could also be used to test drugs to treat the patient and possibly other sclerosing bone dysplasias.

CONTACT INFORMATION

Anaïk Previdi (anaik.previdi@aphp.fr)
Bases moléculaires et physiopathologiques des ostéochondrodysplasies, INSERM U1163
Institut Imagine, 24 bd du Montparnasse, Paris