Noonan syndrome-causing SHP2 mutant inhibits murine growth plate chondrogenesis and longitudinal bone growth

Mylène Tajan [1], Isabelle Gennero [2], Jean-Luc Davignon [2], Florence Capilla [3], Benjamin G. Neel [4], Philippe Valet [1], Maithé Tauber [2,5], Jean-Pierre Salles [2,5], Armelle Yart [1], Thomas Edouard [2,5]

[1] INSERM UMR 1048, Institute of Cardiovascular and Metabolic Diseases (I2MC), University of Toulouse Paul Sabatier, Toulouse, France, [2] INSERM UMR 1043, Centre of Pathophysiology of Toulouse Purpan (CPTP), University of Toulouse Paul Sabatier, Toulouse, France, [3] INSERM, US006, ANEXPLO/CREFRE, Histopathology Unit, Purpan Hospital, Toulouse, France, [4] Campbell Family Cancer Research Institute, Ontario Cancer Institute, University of Toronto, Toronto, ON, Canada, [5] Endocrine, Bone Diseases, and Genetics Unit, Children's Hospital, Toulouse University Hospital, Toulouse, France

Background:
Growth retardation affects more than 80% of patients with Noonan syndrome (NS; MIM#163950), one of the most common developmental disorders, but its origin remains poorly understood. We have recently demonstrated that mutations of the tyrosine phosphatase SHP2, that are responsible for half the cases of NS, impair the systemic production of Insulin-like Growth Factor-I (IGF-I), the biological mediator of growth hormone (GH) acting on growth plate, through a hyperactivation of the Ras/Mitogen-Activated Protein Kinase (MAPK) signalling pathway [1]. This is in accordance with clinical data suggesting partial GH insensitivity in NS patients [2]. Besides this, evidences have been recently accumulated for an important role of SHP2 in bone/cartilage development [3-4]. However, the direct impact of NS-causing mutations on growth plate and bone has never been explored.

Objectives: To evaluate the impact of NS-causing mutants on growth plate and bone development.

Materials and methods: *In vivo* and *in vitro* analyses were performed in a mouse model of NS (SHP2 $^{D61G/+}$ mice) and in chondrogenic ATDC5 cells expressing NS-causing SHP2 mutants, respectively.

Results:

NS mice display abnormal bone development with homogeneous postnatal growth retardation, and osteoporosis
NS mice exhibited decreased weight and body length by 2 wk of age, which continues for at least 4 wk after birth (Fig. 1A). X-ray images showed smaller skull length and homogenous growth retardation affecting the axial as well the appendicular skeleton, without obvious bone deformity (Fig.1B).

µCT analysis revealed a significant decrease in bone volume per tissue volume in the trabecular bone of the vertebrae of NS mice (-25%, P = 0.0130), consistent with an osteoporosis (Fig. 2A-B). This was due to a significant reduction in the trabecular number and thickness (P = 0.0153 and 0.0135 respectively) (Fib 2C-D). Similar tendencies were observed in the trabecular bone of the femurs of NS mice, although they did not reach statistical significance (Fig. 2A-F).

Figure 1

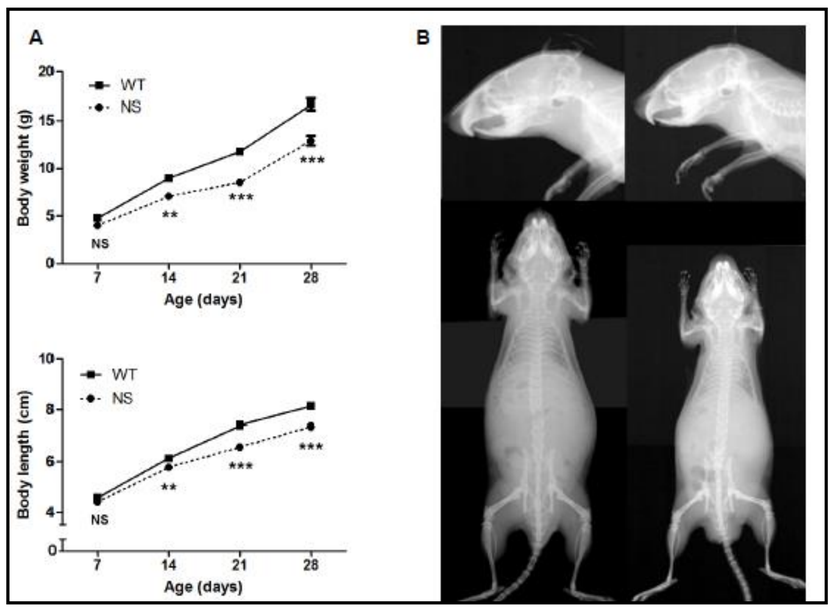
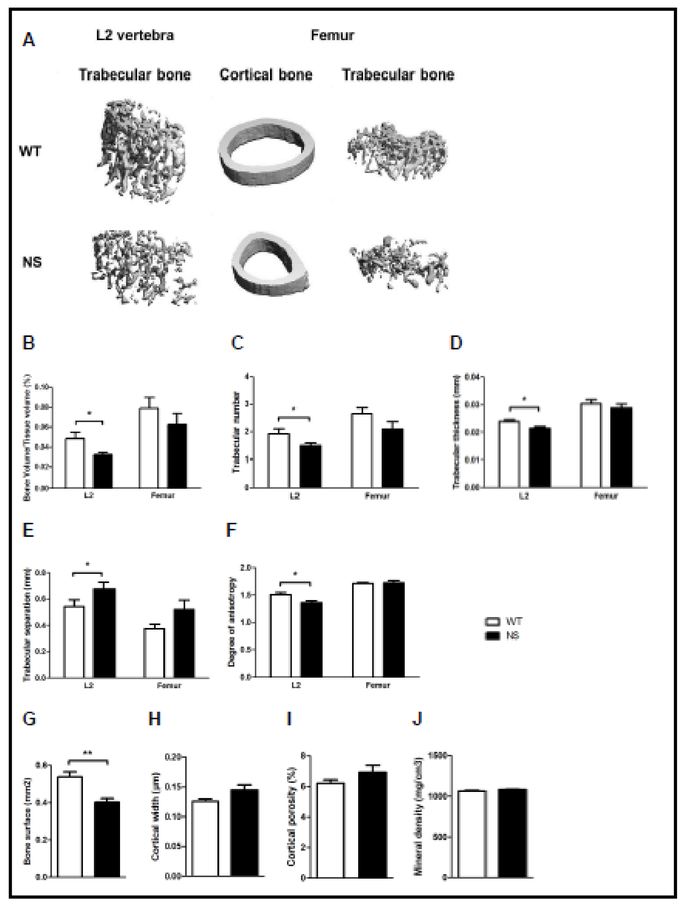
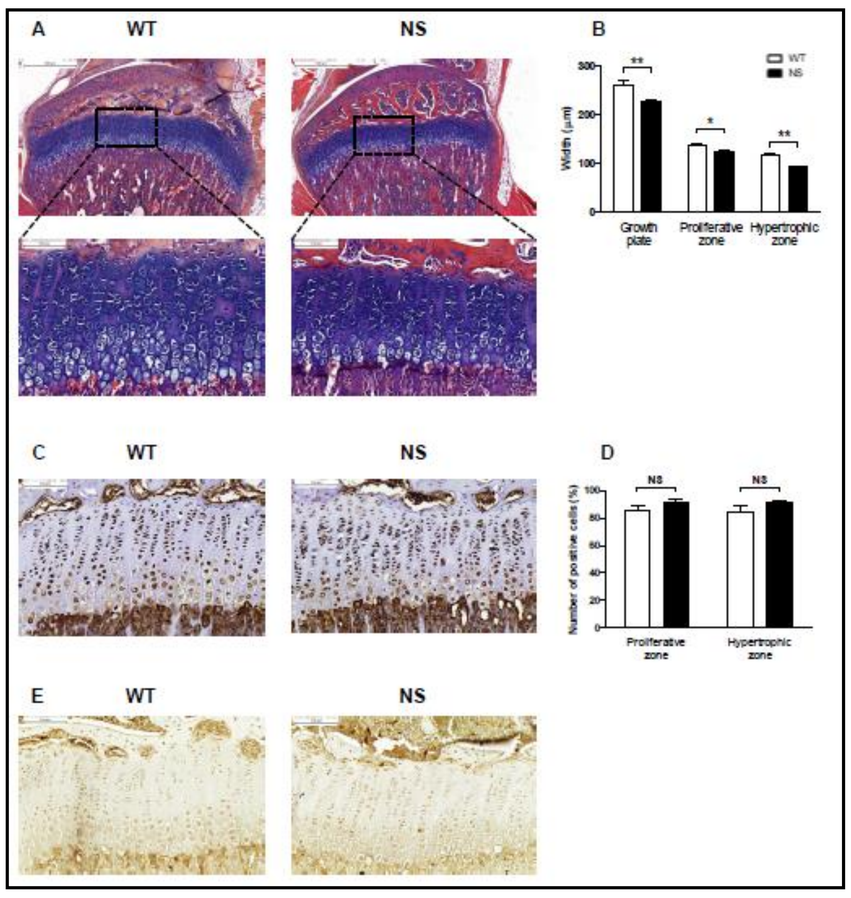


Figure 2



Growth plate length is reduced in NS mice due to a shortening of the hypertrophic zone
The histology of growth plates of proximal tibias from 4-wk-old WT and NS mice was examined. Alcian blue staining revealed a normal organization of the growth plate with columnar structures in the proliferating zone and enlarged chondrocytes in the hypertrophic zone (Fig. 3A). However, measurements revealed that the length of the growth plate was significantly shorter in NS mice due to a decrease in the length of the hypertrophic zone, whereas the length of the proliferating zone was unaffected (Fig. 3B).
Chondrocyte proliferation, assessed by immunohistochemistry using an antibody against PCNA, was similar in WT and NS mice (Fig 3C-D).
Chondrocyte apoptosis, assessed by TUNEL approach, was extremely limited and did not differ between WT and NS mice (Fig. 3E).

Figure 3



NS mutants enhance Erk1/2 activation in chondrocytes in vivo and in vitro
We first assessed Ras/MAPK activation in vivo on histologic sections by immunohistochemistry using an antibody against phosphoErk1/2. Interestingly, increased Erk1/2 phosphorylation was observed in the hypertrophic chondrocytes of NS mice compared to their WT littermates (Fig. 4A-B).

We then monitored Erk1/2 phosphorylation in ATDC5 cells, a standard model for chondrocytes, transfected with the D61G NS-causing SHP2 mutant. These cells were stimulated by the fibroblast growth factor (FGF), a paracrine factor which plays an important role in chondrogenesis, and GH. We found that NS-causing SHP2 mutants significantly enhanced FGF- and GH-induced Erk1/2 phosphorylation in comparison to WT SHP2 (Fig. 5).

Figure 4

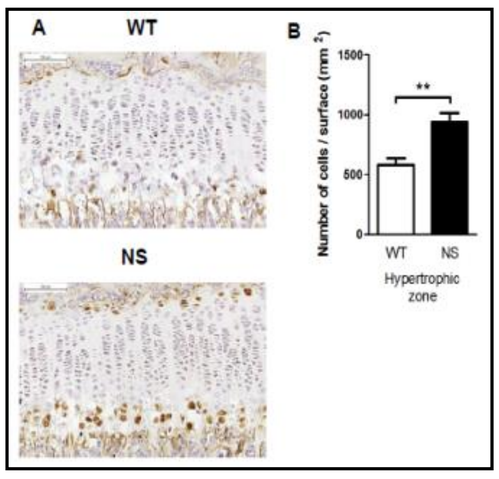
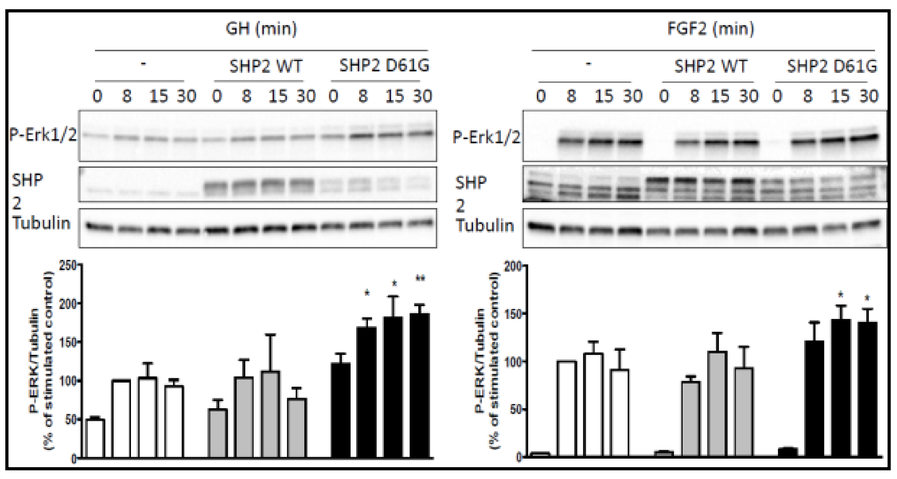


Figure 5



Conclusion:
In this study, we demonstrated that NS-causing SHP2 mutants result in impaired endochondral ossification, through a hyperactivation of the Ras/MAPK signalling pathway, that contributes to growth retardation.

References:
1. De Rocca Serra-Nedelec, A., et al., Noonan syndrome-causing SHP2 mutants inhibit insulin-like growth factor 1 release via growth hormone-induced ERK hyperactivation, which contributes to short stature. Proc Natl Acad Sci U S A, 2012. 109(11): 4257-62.
2. Limal, J.M., et al., Noonan syndrome: relationships between genotype, growth, and growth factors. J Clin Endocrinol Metab, 2006. 91(1): 300-6.
3. Bauler, T.J., et al., Development of severe skeletal defects in induced SHP-2-deficient adult mice: a model of skeletal malformation in humans with SHP-2 mutations. Dis Model Mech, 2011. 4(2): 228-39.
4. Bowen, M.E., et al., SHP2 regulates chondrocyte terminal differentiation, growth plate architecture and skeletal cell fates. PLoS Genet, 2014. 10(5): p. e1004364.

Acknowledgments:
T. Edouard was the recipient of a grant (ASPIRE Young Investigator Research Awards in Endocrinology) supported by Pfizer.