ENPP1 Inhibition as a Therapeutic Approach for Later-onset Hypophosphatasia

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INTRODUCTION

Hypophosphatasia (HPP) is caused by loss-of-function mutations in the gene (*ALPL* in humans; *Alpl* in mice) that encodes tissue-nonspecific alkaline phosphatase (TNAP), whose deficiency results in the accumulation of extracellular PP_i.

HPP features skeletal and dental hypomineralization, with disease severity varying from the life-threatening perinatal and infantile forms to the milder later-onset forms that manifest in adulthood or only affect dentition[1].

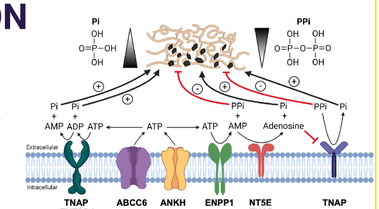
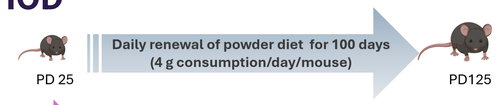


Fig 1: Molecules that control the extracellular concentration of pyrophosphate[2]

AIM

Earlier genetic studies by our group demonstrated that the double ablation of *Alpl* and *Enpp1* led to normalizing plasma PP_i concentrations and improvements in skeletal mineralization[3], particularly in the axial skeleton[4]. Here, we tested if ENPP1 could be a druggable target to treat the non-lethal forms of HPP, using the *Alpl*$^{-/Prx1}$ mouse as a model[5] and an early lead ENPP1 inhibitor.

METHOD



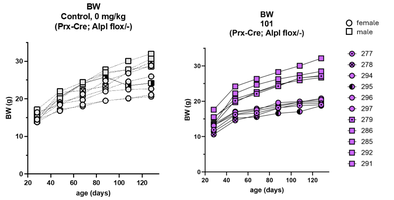


Fig 2: Diet schedule and body weight of the mice as they age.

CONCLUSIONS

- Oral (in food) dosing of an early lead ENPP1 inhibitor, REV101, to adult HPP mice lowers PP_i by 30%, leading to improvements in mineralization of long and vertebrate bones.
- Here we show for the first time that ENPP1 is a druggable target for late-onset HPP.
- No clinical signs and no obvious impacts on plasma chemistry and gene expression suggest good tolerability and no-off target effects after 100 days of dosing.
- An ENPP1 inhibitor with improved properties compared with REV101 is being developed as a differentiated therapy to address unmet need in HPP patients.

RESULTS

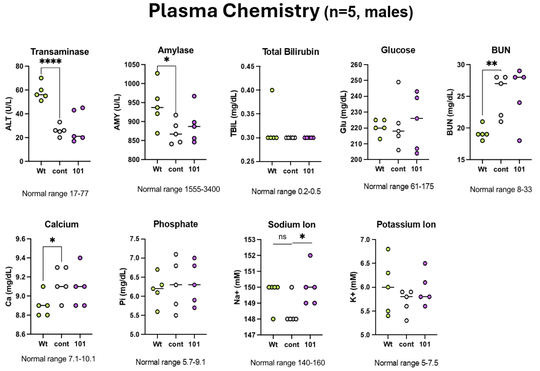


Fig 3. Plasma chemistry panel of WT, control, and REV101 treated male mice, n=5. Significant changes were only observed between WT and HPP mice (transaminase, amylase, BUN, and Ca).

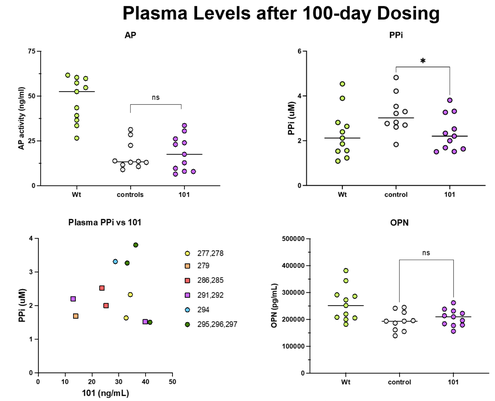


Fig 4. Plasma levels of AP and PP_i after 100 days of dosing, plasma PP_i vs.101, and OPN concentrations. Administration of REV101 did not affect plasma AP nor OPN levels. PP_i levels in the dosed animals were reduced to the levels of WT mice.

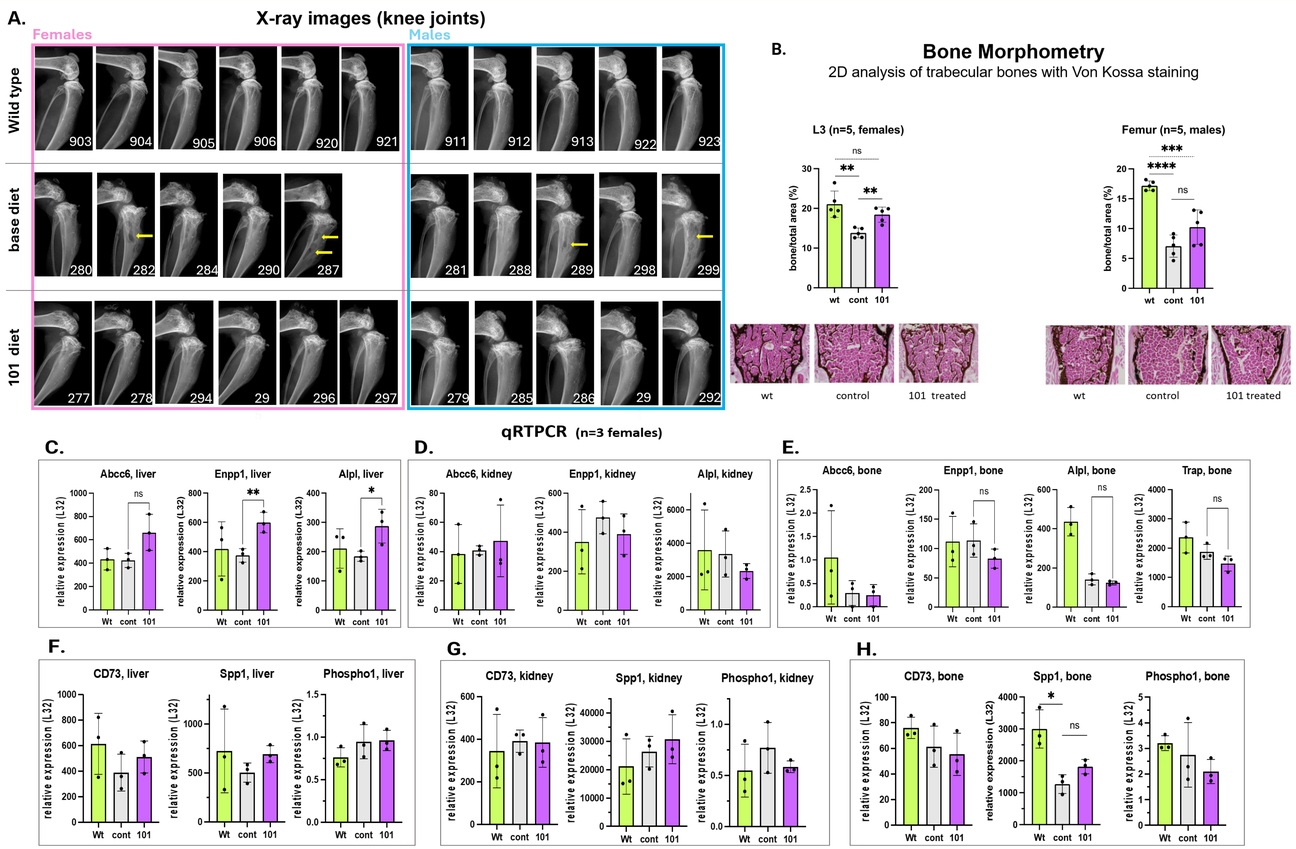


Fig 5. (A) X-ray views show darker areas in the tibia of untreated HPP mice, indicating lesser mineralization. The incidence of those darker areas was reduced in treated animals. This suggests that treatment with REV101 increased bone mineralization of the bone cortex. (B) Bone morphometric analysis was performed on Von Kossa-stained sections using the Kawamoto's film method. In L3 vertebrate bone of dosed HPP animals, mineralization was increased to the levels of wild-type mice. In the trabecular areas of distal femur, HPP mice with REV101 treatment showed slight improvement in mineralization. (C-H) *Abcc6* mRNA in the liver of treated mice was slightly upregulated. Expression of *Enpp1* was increased in the treated liver implying that compensation may occur. *Alpl* expression was increased in the liver of treated HPP mice. *CD73* expression was not significantly affected in liver, kidney or bone, despite the prediction that ENPP1 inhibition would lower AMP levels.

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