Amelioration of osteomalacia in late-onset HPP mice via pharmacological inhibition of ENPP1

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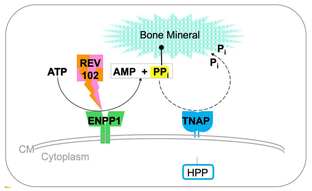
INTRODUCTION

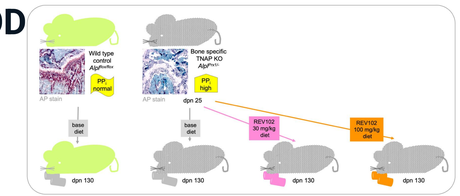
Hypophosphatasia (HPP) is caused by loss-of-function mutations in the human *ALPL* gene that encodes tissue-nonspecific alkaline phosphatase (TNAP), whose deficiency results in the accumulation of the calcification inhibitor, inorganic pyrophosphate (PP_i), resulting in skeletal and dental hypomineralization(1). Enzyme replacement with mineral-targeted TNAP (asfotase alfa) improves skeletal mineralization but frequent injections of this biologic can lead to injection site reactions and discontinuation of treatment. PPi is produced by ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) from ATP. We reported earlier that a mouse model of HPP (TNAP KO) in the background of ENPP1 KO showed normalized PP_i levels and improved bone phenotype, indicating that both ENPP1 and TNAP are antagonistic regulators of PPi metabolism(2).

AIM

We aimed to test if ENPP1 could be a druggable target for the development of an alternative treatment for HPP, particularly for the non-lethal later-onset forms of HPP where enzyme replacement is not currently approved.



METHOD



Alpl$^{flox/flox}$ (Alpl$^{tm3.1Jlm}$) mice were crossed to a Prx1-Cre transgenic mouse (B6.Cg-Tg(Prrx1-cre)1Cjt/J) to generate Prx1-Cre; *Alpl*$^{flox/flox}$ (*Alpl*$^{Prx1/Prx1}$)(3). Among the offspring animals, we identified *Alpl*$^{Prx1/-}$ mice that carry one allele of *Alpl* gene conditionally inactivated and the second *Alpl* allele constitutively deleted, since the Prx1-Cre gene is often expressed in the germ cells producing offspring with a constitutively inactivated *Alpl* allele. The bone phenotype of *Alpl*$^{Prx1/-}$ is slightly more pronounced than that of *Alpl*$^{Prx1/Prx1}$ mice as their plasma ALP levels are approximately 50% of the *Alpl*$^{Prx1/Prx1}$. Thus, we used *Alpl*$^{Prx1/-}$ mice as a model of later-onset HPP in this study.

Pellet diets (#2918, Teklad Global 18% Protein-6% fat Rodent Diet, irradiated) containing REV102 (aka REC-102)at concentrations of 0, 0.188 and 0.625 g/kg were used for dosing the HPP mice, corresponding to 0, 30, 100 mg/kg body weight under the assumptions that each mouse weighs approximately 25 grams and consumes 4 g pellet diet per day. The three diets were prepared by Inotiv (https://www.inotiv.com). The diet was given *ad libitum* and renewed once a week starting at postnatal day (dpn) 25 until collection day, at dpn 130. Body weight was monitored every 20th day. Wild-type control mice (*Alpl*$^{flox/flox}$) were given the same base diet, #2918, containing 0 mg/kg REV102 and collected on dpn 130.

RESULTS

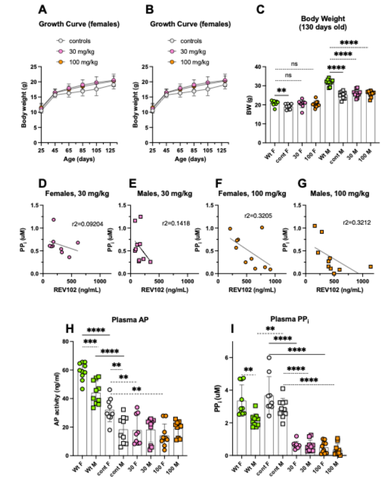


Fig. 1
Growth curves of females (A) and males (B) during 105-day-dosing. Body weight comparison at collection time, PD130 (C). Correlation of REV102 drug levels and PPi in the terminal plasma from females with 30 mg/kg dose (D), males with 30 mg/kg dose (E), females with 100 mg/kg dose (F) and males with 100 mg/kg dose (G). Alkaline phosphatase levels in the plasma at PD130 (H). PPi levels in the plasma at PD130 (I). F: female, M: male, Wt: wild type under base diet, cont: aHPP mice under base diet, 30: aHPP mice under 30 mg/kg dose, 100: aHPP mice under 100 mg/kg dose. Females: Wt n=10, control n=9, 30 mg/kg n=9, 100 mg/kg n=10. All male groups: n=10.

Animals dosed with 30 and 100 mg/kg REV102 grown normally and did not show abnormality in the appearance as well as plasma chemistry analysis(4) .
Plasma levels of REV102 and PP_i showed negative correlation.

Plasma PP_i levels were significantly reduced in the dosed *Alpl*$^{Prx1/-}$ mice.

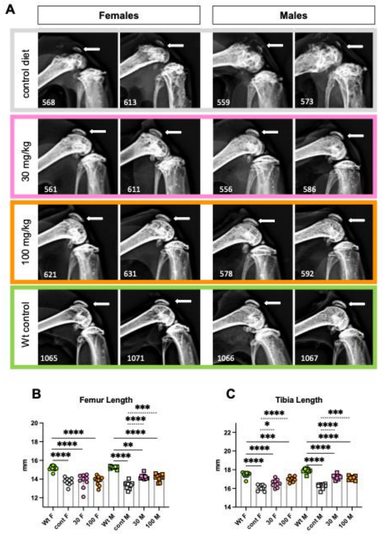


Fig. 2
Representative X-ray images of the left hind limb joints (A). Comparison of length of femur (B) and tibia (C) obtained using ImageJ software from the X-ray images. Images were saved with automatic exposure in Trident X-ray device, and no modification was added. Numbers at the bottom left corner in each picture are mouse ID numbers. The HPP mice show diminished patella structure but all the treated mice present patella as same as wild type controls. (white arrows). Numbers at the bottom left corner in each picture are mouse ID numbers.

X-ray views reveal that knee joints of *Alpl*$^{Prx1/-}$ mice have widened femoral epiphysis with highly abnormal/poor patella structure; however, the treated *Alpl*$^{Prx1/-}$ mice show improved appearance of the epiphysis with a well-defined patella structure, undistinguishable from Wt control mice in X-ray views.

Length of the femur and tibia are improved with REV102 treatment, while it did not reach the levels of wild type mice.

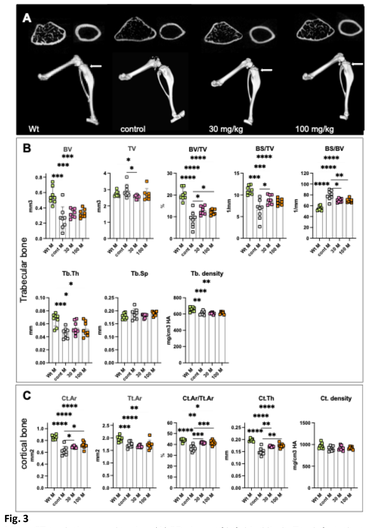


Fig. 3
Micro CT analysis on male mice. (A) 3D views of left hind limb. Top left: trabecular bone. Top right: cortical bone. (B) mCT analysis of trabecular bone. Parameters analyzed include bone volume (BV), tissue volume (TV), bone volume fraction (BV/TV), bone surface (BS), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), Trabecular density (Tb. density), cortical area (Ct.Ar), tissue area (Tt.Ar), cortical thickness (Ct.Th), cortical density (Ct.density).

In male *Alpl*$^{Prx1/-}$ mice, BV values were widely scattered in control mice, while those in REV102 treated males showed smaller SD. BV/TV values indicate increased mineralization in the dosed males. Ct.Ar/Tt.Ar and Ct.Th values indicate increased mineralization in the cortical bone of the *Alpl*$^{Prx1/-}$ mice with the treatment, while all those factors in treated animals did not match to the levels of Wt mice. In female *Alpl*$^{Prx1/-}$ mice, improvement in the trabecular bones was less significant than males, while cortical bones of the treated females also showed increased mineralization(4).

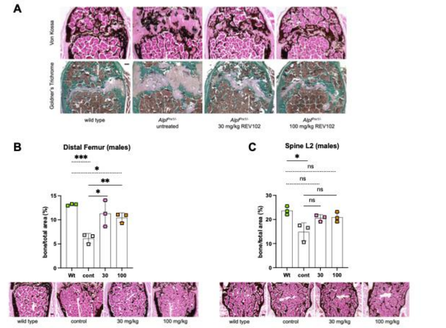


Fig. 4 (A) Growth plate views of distal femur from male mice stained with Von Kossa and Goldner's trichrome. (B) Bone morphometry analysis on distal femur and representing sections. (C) Bone morphometry analysis on L2 vertebrate bone and representing sections. Black bars 200 μm.

Alpl$^{Prx1/-}$ mice show disorganized growth plates with unmineralized zone, while treated *Alpl*$^{Prx1/-}$ mice exhibited reduced unmineralized area. Bone morphometry analysis on femurs indicates increased mineralization in the treated *Alpl*$^{Prx1/-}$ - mice. In vertebrate bone L2 samples, a statistically non-significant small improvement was seen in the treated groups.

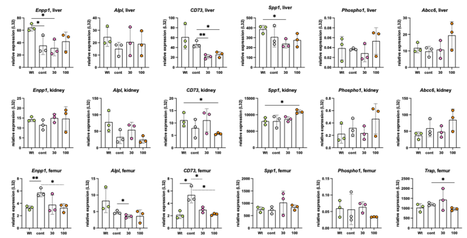


Fig. 5
qRTPCR analysis. Mouse ribosomal protein L32 was used for normalization. Biological replictes were three female mice, and technical replicates were done with quadruplets.

Enpp1 expression in the *Alpl*$^{Prx1/-}$ mice was low in the liver but high in the bone comparing to Wt mice, and ENPP1 inhibition reduced Enpp1 expression in their bones. CD73 (Nt5e) is upregulated in the bone of *Alpl*$^{Prx1/-}$ mice and the levels are lowered with REV102 treatment. OPN (*Spp1*) induction was not observed in *Alpl*$^{Prx1/-}$ mice.

CONCLUSIONS

- *Alpl*$^{Prx1/-}$ mice consumed REV102 diet for 105 days grown normally without showing any detectable changes in the appearance and blood chemistry measurements.
- Plasma PPi levels were significantly reduced in the dosed animals.
- Mineralization of the appendicular bones in *Alpl*$^{Prx1/-}$mice was improved with REV102 administration according to analyses by X-ray, micro-CT and bone morphometry.
- This study suggests that oral administration of an ENPP1 inhibitor has a potential to be used for the treatment of HPP patients.

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