# Therapeutic effect of hard tissue by lentiviral gene therapy for hypophosphatasia

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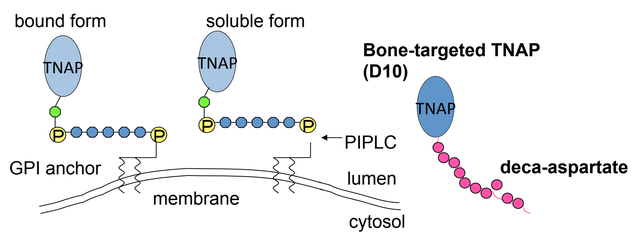
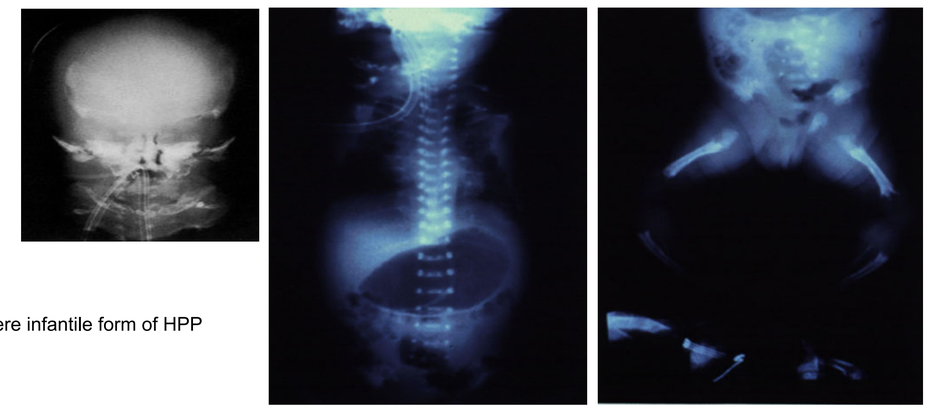
## Introduction

<Alkaline phosphatase (ALP)>
ALPs are ectoplasmic proteins and attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. ALP can be cleaved from the membrane by phospholipase and released into the blood stream where it can be measured and is currently utilise as a maker of mineral diseases. Four types of ALP have been identified in human. Tissue non-specific ALP (TNAP) is expressed at highest levels in liver, bone and kidney. Natural substrates of TNAP are pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP).




Hypophosphatasia (HPP) is an inherited skeletal disease caused by mutations of the gene encoding tissue-nonspecific alkaline phosphatase (TNAP). These mutations result in deficient activity of TNAP leading to rickets, often causing death in the first year of life. Although TNAP is expressed in various tissues, the forms of HPP is varies widely; from the most severe (perinatal and infantile) to the mildest forms (odont-hypophosphatasia). There is no established treatment. We reported that enzyme replacement therapy (ERT) using daily injections of bone-targeted TNAP was effective in preventing all the skeletal and dental manifestations of HPP in TNAP knockout mice (Akp2$^{-/-}$) (Millan et al., 2008; Manisha et al., 2012). Recently, we also showed that a single injection of either a lentiviral vector (Yamamoto et al., 2011) or an adeno-associated viral vector (Matsumoto et al., 2011) harbouring bone-targeted TNAP (TNAP D10) is equally effective in preventing all the manifestations of HPP.



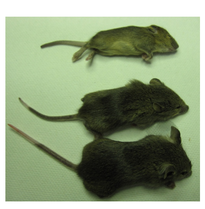
Severe infantile form of HPP

## Objective

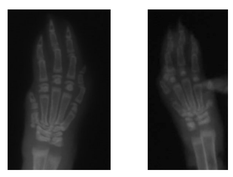
In this study we evaluate improvement of the hard tissues of treated Akp2$^{-/-}$ mice. We have used the lentiviral delivery at 1 day of age approach to examine the correction of bone and teeth.

## Experimental design

### TNAP K.O. model mouse : *Akp2$^{-/-}$*



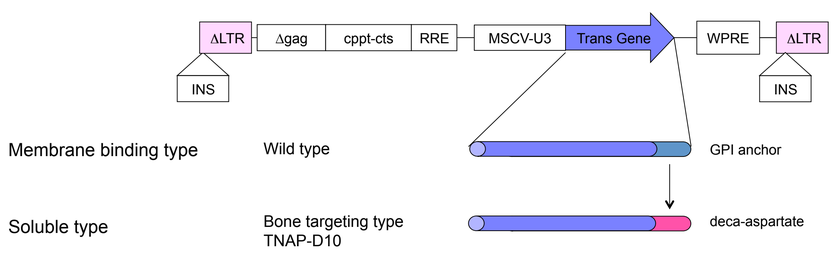

14 days after birth



11 days after birth
Akp2$^{+/-}$ Akp2$^{-/-}$

TNAP knock-out mice (Akp2$^{-/-}$ ) are phenotypically similar to infantile HPP. They are born with a normally mineralized skeletal but develop rickets at 6 days of age and die between days 12 and 16, suffering severe skeletal hypomineralization and epileptic seizure.

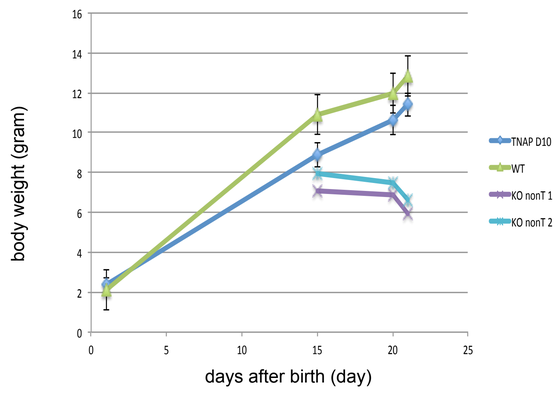
### SIN-HIV1 vector



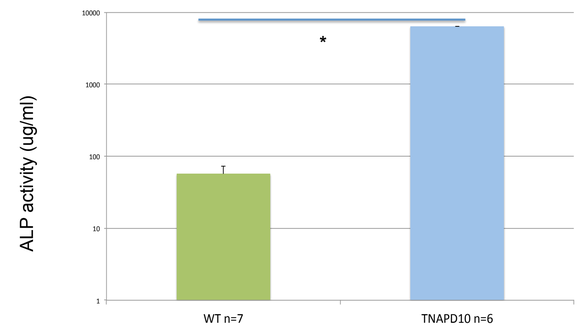

Lentiviral vector (5.0x10$^{7}$TU/100μl ) was injected into the jugular vein of neonatal 1 day old Akp2$^{-/-}$ mice.

## Results

**Body weight**




**ALP activity in plasma on 20 days after injection**



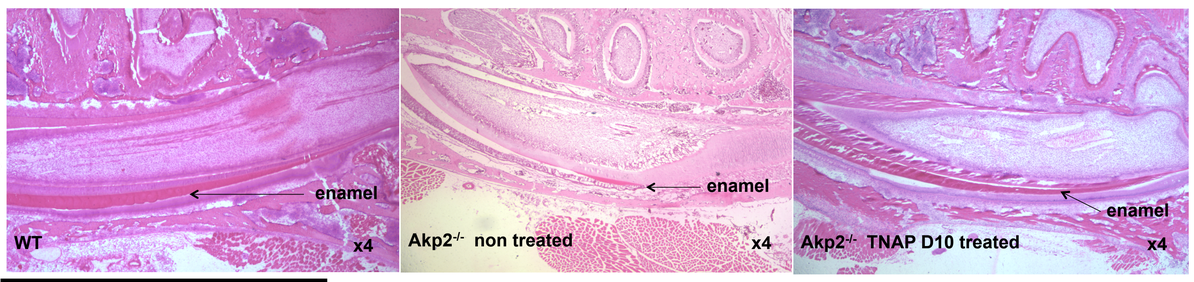

*** P>0.05**

- The body weight of treated Akp2$^{-/-}$ mice (n=6) was comparable to WT (n=5) .
- Treated HPP mice showed healthy growth, while body weight of non-treated Akp2$^{-/-}$ mice (n=2) decreased in after 15 days of age.

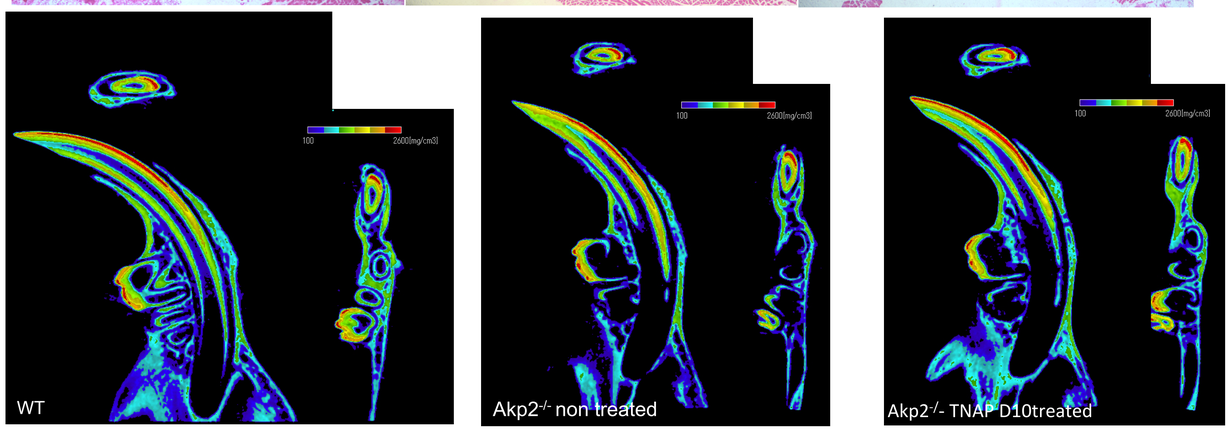
- The ALP activity in plasma of TNAPD10 treated Akp2$^{-/-}$ mice increased 1000 fold. This increased ALP activity is enough to ensure survival of the Akp2$^{-/-}$ mice.

### Comparison of teeth correction

H&E staining



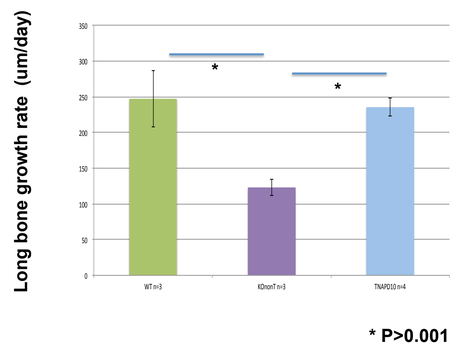

μ CT



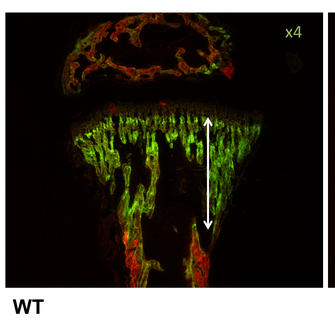

- A thinner enamel layer was shown in Akp2$^{-/-}$ non treated mouse by H & E staining. Treated Akp2$^{-/-}$ mouse had a thicker enamel layer than the Akp2$^{-/-}$ non treated KO mouse measured by the higher mineral density area in the uCT images.
- Enamel defect improves in treated Akp2$^{-/-}$ mouse.

### Comparison of bone correction

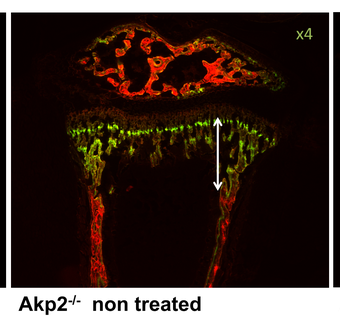
**Long bone growth rate**



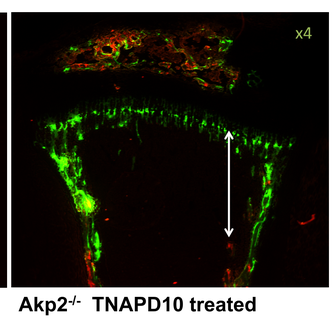

**WT**




**Akp2$^{-/-}$ non treated**




**Akp2$^{-/-}$ TNAPD10 treated**




*** P>0.001**

- Alizarin red was injected subcutaneously into mice 15 days after birth and followed by a calcein injection at 20 days of age. The mice were sacrificed on day 21. 4μm sections were obtained from decalcified tibia samples. We calculated the bone growth rete by measuring the distance between both labels. The results show that WT and treated Akp2$^{-/-}$ long bone growth were significantly higher when compared with Akp2$^{-/-}$ non treated mice. Akp2$^{-/-}$ treated mice growth rate is similar to WT.

## Summary

- Lentiviral vector expressing a bone-targeted TNALP was injected into the jugular vein of newborn Akp2$^{-/-}$ mice 1 day after birth.
- The ALP activity in plasma of the treated Akp2$^{-/-}$ mice increased 1000 fold and this activity level was enough to cure Akp2$^{-/-}$ mice.
- μCT evaluation and histological analysis indicated that the enamel defects were improved by this gene therapy treatment.
- Bone growth rates showed that mineralization in long bone bones is also significantly improved.

## Conclusion

These results indicate that bone-targeted TNAP treatment mediated by lentivirus injection can correct dental symptoms and enhance bone growth in this mouse model of infantile HPP.

## Reference

Millán et al., J. Bone Miner. Res. 23:777-787 (2008)
McKee et al., J. Dental Res. 90:470-476 (2011)
Yamamoto et al., J. Bone Miner. Res. 26; 135-142