KNOCK-IN OF THE RECURRENT R368X MUTATION OF PRKAR1A THAT REPRESSES cAMP-DEPENDENT PROTEIN KINASE A ACTIVATION : A MODEL OF TYPE 1 ACRODYSOSTOSIS

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OBJECTIVE

Background: In humans, activating mutations in the *PRKAR1A* gene cause acrodysostosis 1 (ACRDYS1), a rare developmental disorder characterized by two main features, renal resistance to PTH and chondrodysplasia caused by PTHrP resistance. These features resemble that of PHP1A. The PRKAR1A mutations cause a reduction in cAMP/PKA activation, similarly to Gsalpha mutations in PHP1A, thereby explaining the phenotypic similarities between both diseases. However, in contrast to PHP1A, ACRDYS1 is associated with increased, not decreased, cAMP production, resulting from an impaired ability of the mutant PRKAR1A to dissociate from the catalytic PKA subunits .

Aim: Establish a mouse model to assess the consequences of the germline expression of a PRKAR1A mutation causing the dominant repression of the cAMP/PKA signaling pathway.

Strategy: Knock-in the p.R368X PRKAR1A mutation, the only recurrent PRKAR1A mutation causing ACRDYS1.

CONCLUSIONS

- The [R368X]/[+] KI mice present the two main features of human ACRDYS1, i.e. renal proximal tubule resistance to PTH and chondrodysplasia.
- Unexpected, this model revealed a striking delay in endochondral ossification in new-born mutant mice.
- We propose that this phenotype results from the persistently elevated cAMP levels, which are likely to induce specific changes in cellular PKA and other cAMP dependent pathways (e.g., EPAC1 and EPAC2)
- These results indicate that PRKAR1A, by tempering intracellular cAMP levels, is a molecular switch at the crossroads of chondrocyte proliferation and differentiation.

METHODS

Prkar1a point mutation R368X knock-in mice : C57Bl/6J genetic background developed by GenOway (Lyon, France). Of note, no litters were obtained when mating HTZ females with either WT or HTZ males (thus no homozygous [R368X]/[R368X] mice)
Skeletal analysis: *Staining and visualization of whole skeletons (Post Natal Day (PND) 1 and 7;* alizarin red or alcian blue).
Histology, Immunohistochemistry, Histomorphometry (PND4, tibial growth plate, H&E, PCNA labeling using Analyze and Image J software)
In vivo Micro-CT analysis of mice. X-ray Micro-CT device (Quantum FX Caliper, Life Sciences, Perkin Elmer, Waltham, MA, USA) using OsiriX imaging , Analyze and Rigaku softwares.
Hormonal and biochemical measurements : Plasma and urinary calcium, phosphorus and creatinine : Modular P Roche analyzer; Plasma intact PTH and TSH: ELISA (Immutopics and Cloud-Clone Corp); cAMP : ELISA (Biotrak Enzyme immunoassay (EIA) System (Amersham).
Western Blot analysis: monoclonal mouse antibodies recognizing the PRKAR1A regulatory subunit, the Prkaca catalytic subunit alpha, PRKAR2A regulatory subunit, and the PRKAR2B regulatory subunit all from BD Transduction Laboratories and ß actin from Sigma
Statistical analysis: GraphPad Prism. Unpaired t-test : Bone mineral density and histological data; ANOVA followed by the Turkey's test; PTH and TSH paired t-test.

References

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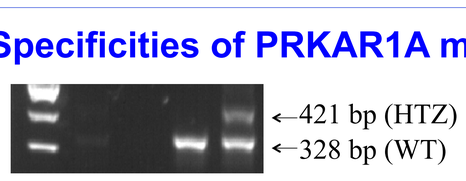
- **Genetic disorders affecting PTH/PTHrP receptor function.** Jüppner H, Silve, C. In: Thakker R, Whyte MP, Eisman J, Igarashi T, editor. Genetics of Bone Biology and Skeletal Disease: Academic Press, Elsevier, Oxford, United Kingdom; 2012.

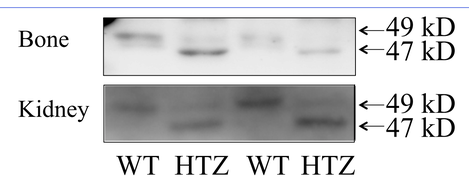
The authors declare no conflicts of interest

RESULTS

1. Specificities of PRKAR1A mutant mice.



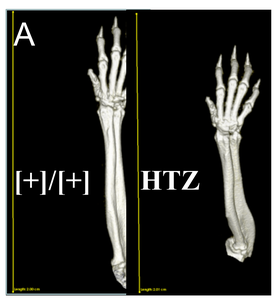
A) PCR Genotyping: Identification of WT and [R368X]/[+] (HTZ) alleles



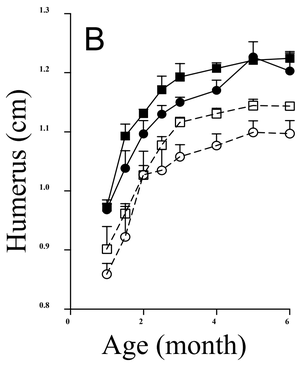
B) PRKAR1A protein expression in kidney and bone extracts from WT and HTZ mice

2. Postnatal growth retardation of HTZ mice and Micro CT quantification of dysostosis.

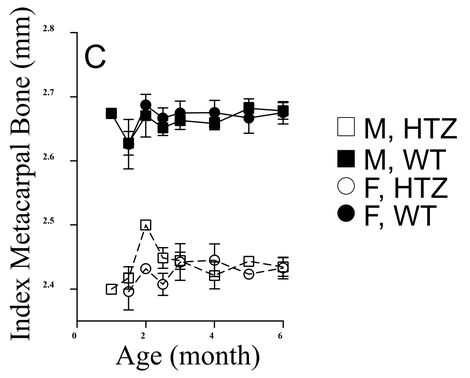
A) 3D volume rendering of fore-limb showing the growth defect in HTZ mice



B) Humerus length



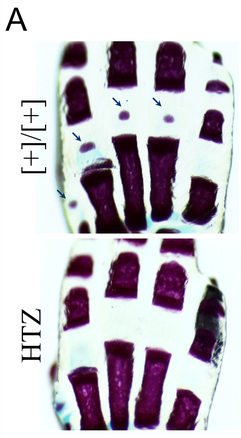
C) Index metacarpal bone



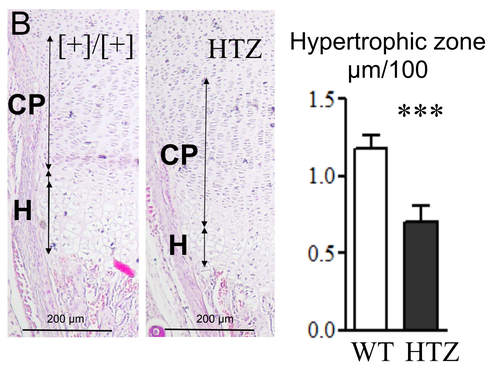
All measures in HTZ mice are significantly reduced compared to values for sex-matched WT mice.

3. Skeletal phenotype of long bone in newborn HTZ mice.

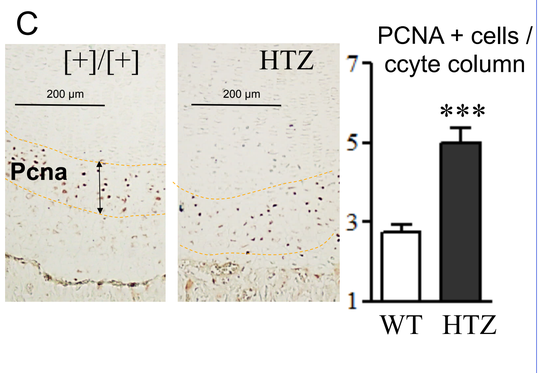
A



A) Alcian blue (cartilage) and Alizarin red (calcified tissue) stained hands at PND 1. Arrows: ossification centers present in WT but not in HTZ mice.

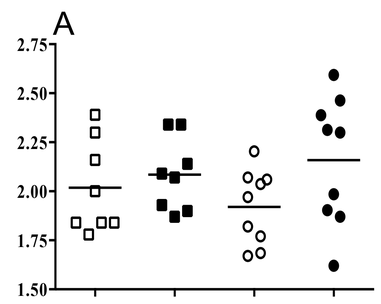


B) H&E staining of tibial sections. H: hypertrophic chondrocytes (ccyte); CP: zone of columnar or proliferative chondrocytes. In mutant growth plate, the height of the hypertrophic zone is decreased (***p<0.001).

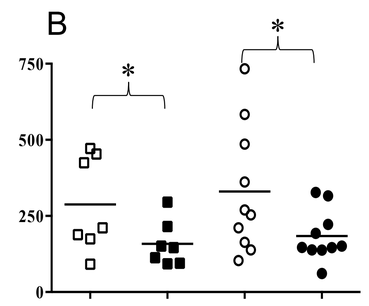


C) PCNA labeling. In WT mice, PCNA positive cells (brown) occupy a circumscribed zone; in the mutant growth plate, PCNA labeling is diffuse and columnar, with positive cells close to the cartilage/osseous junction. The number of PCNA positive cells /hypertrophic chondrocyte columns is Increased in mutant (***p<0.001).

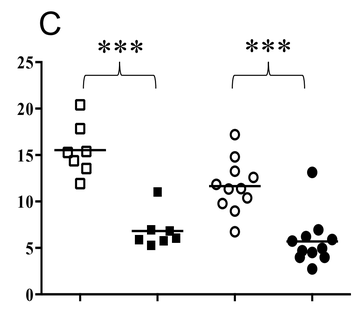
4. Serum and urine biochemistries of WT and HTZ littermates.



A) Plasma calcium (mM)



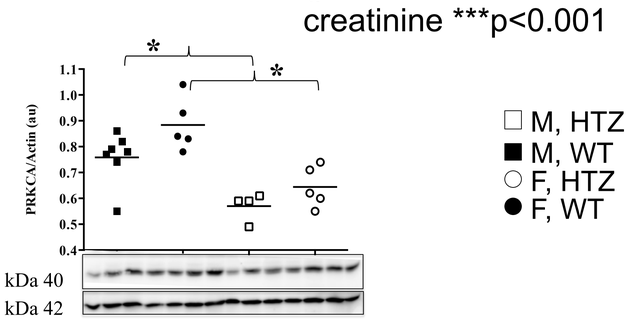
B) Plasma PTH (pg/ml) *p=0.05



C) Basal urinary cAMP (µmoles cAMP/mmoles creatinine ***p<0.001

5. Western blots analysis and quantification of PRKACA in kidney.

PRKACA subunit expression was significantly reduced in kidney extracts from HTZ mice compared with that from wild-type mice. (*p=0.05)



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