Role of receptor activity modifying proteins-(RAMPs) in skeletal regulation.

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Introduction:

Receptor activity modifying proteins (RAMPs) are single transmembrane accessory proteins for G-protein coupled receptors (GPCRs). They regulate ligand selectivity, trafficking of receptors and subtle aspects of G-protein activation in a range of partner GPCRs.

Predominant roles of RAMPs include ligand selectivity in receptors for Calcitonin (CT) and its related family of peptides: Calcitonin gene related peptide (CGRP), Amylin (AMY) and Adrenomedullin (AM) which are important in skeletal development and maintenance. Functional receptors for these peptides comprise of a RAMP with either a CT receptor (CTR) or a Calcitonin-like receptor (CLR) (Fig 1).

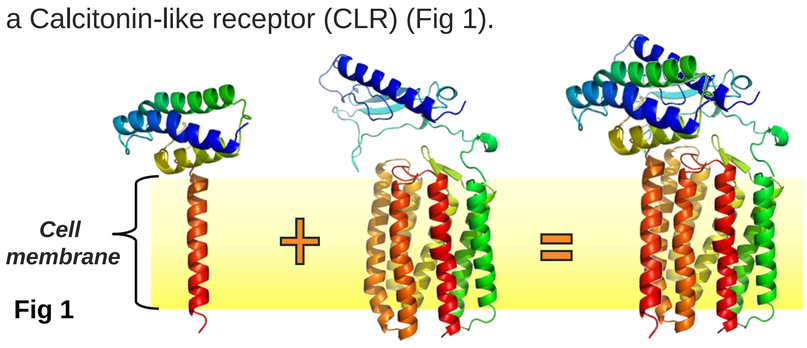

Fig 1

Three mammalian RAMP isoforms have been identified, each of which determine the ligand specificity of their partner GPCR.

RAMP 1/2/3 transgenic mice have distinct phenotypes:

•RAMP1-/- hypertensive & increased serum proinflamatory cytokine levels.

•RAMP2 -/- embryonically lethal - *Hydrops Fetalis.*

•RAMP2 +/- reduced litter size, elevated serum Ca^{2+} levels and prolactin levels.

•RAMP3 -/- normal physiology, older mice do not gain weight.

Aim

To test the hypothesis that knocking out RAMP3 gene in mice results in an altered skeletal phenotype.

Objectives

• Characterise the skeletal phenotype of RAMP3 KO mice at various ages.

• To study skeletal response of RAMP3 KO mice to ovariectomy.

• To investigate whether RAMP3 is a Wnt target by determining protein expression of total β-catenin during osteoblast differentiation.

Methods:

- MicroCT was performed using a Skyscan 1172, to determine bone morphometric parameters. Skeletal bone models were generated using Voxler ™.
- 8 week old RAMP3 -/- and WT mice were injected with Calcein (100mg/kg) twice, one week apart before culling. Distances between dual calcein labels were measured to determine bone apposition rate in the endocorital region of tibiae.
- Ovariectomy (Ovx) were carried out at 12 weeks and microCT analysis was carried out on baseline, sham and Ovx groups.
- Primary osteoblast cultures were obtained from mouse calvaria of both RAMP3 -/- and WT genotype, differentiated in osteogenic media for up to 20 days and β-catenin protein expression was determined by Western blotting. Actin was used as loading control.

Results:

- **Skeletal phenotype of RAMP3-/- mice at 27 days of age (A,B,C).**
- **Dynamic bone histomorphometry of 8 week old RAMP3 -/- and WT mouse tibia (D,E,F).**

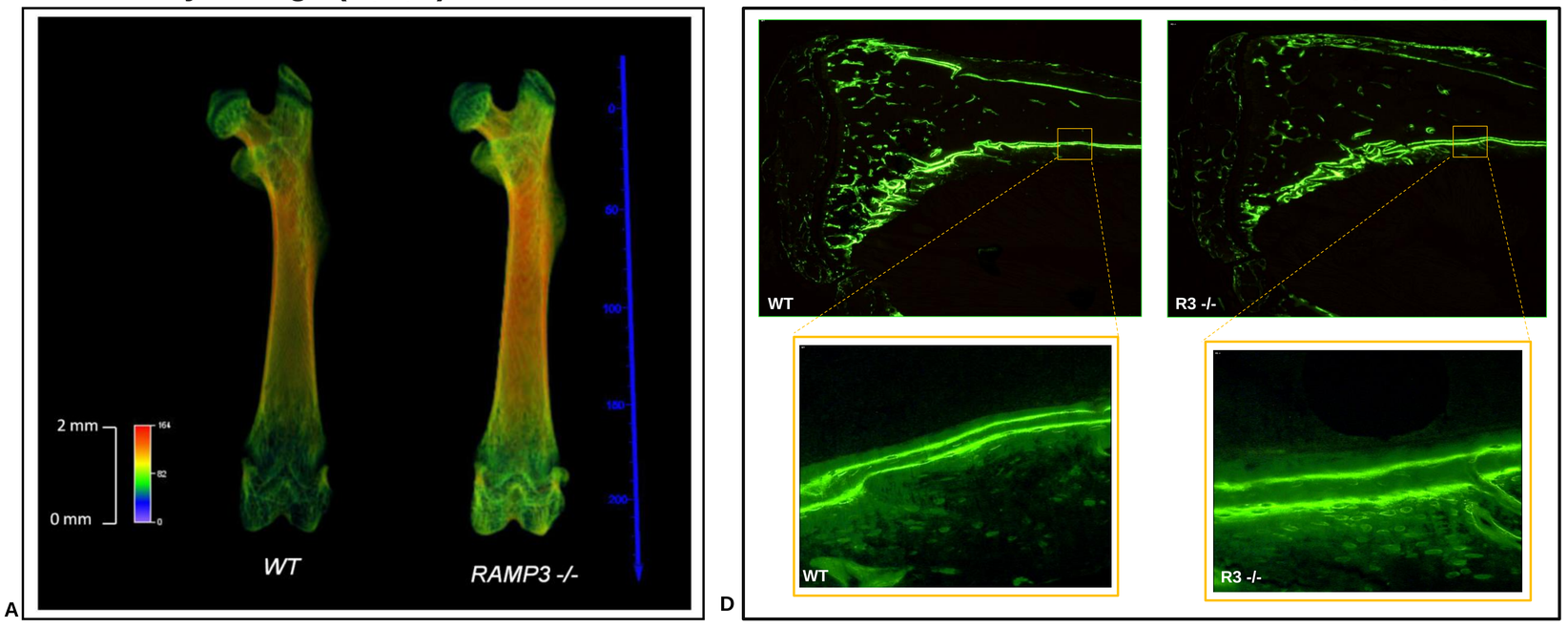

Fig 2

***Fig 2**: RAMP 3 KO mice had higher bone density (increased opacity) and significantly higher femur bone volume (BV) at 27 day old (P value 0.025 considering P value < 0.05 significant) despite having significantly lower whole body weight (P value 0.002) (A,B,C). At 8 weeks, RAMP3 KO mice have revealed significant increase in bone apposition rate (P value 0.0223)(D,F) despite reduces tibia BV (E).*

- ***Skeletal response to ovariectomy.***

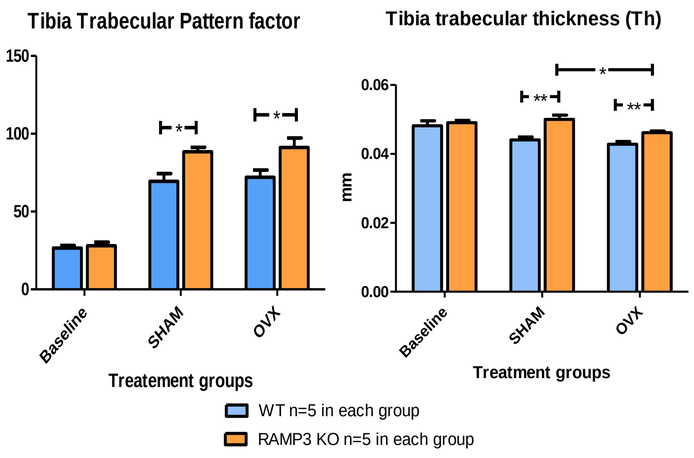

***Fig3.** A significantly higher tibia trabecular pattern factor was observed in R3 KO in both sham and OVX (P value < 0.01) along with significantly higher tibia trabecular thickness (Sham: P value 0.004; Ovx: P value < 0.0045)*

- **Expression of β-catenin in differentiating osteoblasts.**

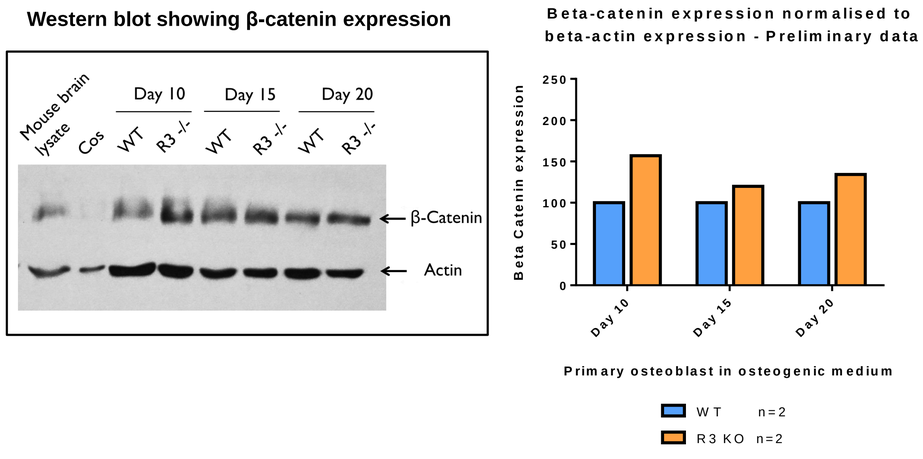

Fig4. Preliminary data suggesting increased β-catenin expression in R3 KO osteoblasts when compared to WTs at all time point throughout differentiation. Actin expression was used as a loading control.

Conclusions and Future work:

- This work provides evidence of accelerated skeletal development in RAMP3 KO mice from an early age, followed by maintenance of a well developed trabecular phenotype and a significant increase in bone apposition rate at older ages (Fig 2,3). We have also shown that this early skeletal development provides protection from bone loss due to ovariectomy (Fig 3).
- Preliminary data suggesting increased expression of total beta-catenin in RAMP3 KO osteoblasts provides novel insight into a possible RAMP3-Wnt pathway interaction (Fig 4).

Hence we have established that RAMP3 is not only a potential therapeutic target for treating skeletal disorders but can also be implicated to be involved in wider physiological consequences.

Future work involves studying skeletal response of RAMP3 KO mice to Wnt and CT hormone stimulation.

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