Osteocyte-Specific Deletion of the $\alpha_2\delta_1$ Auxiliary Voltage Sensitive Calcium Channel Subunit

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ABSTRACT

Context: Skeletal unloading due to disuse, disease, or aging increases bone loss and the risk of skeletal fracture. Conversely, mechanical loading is anabolic to the skeleton, promoting skeletal integrity through increased bone formation. Though osteocytes are the most abundant and mechanosensitive cells within the skeleton, influencing bone formation and resorption, exactly how these forces are transmitted through osteocytes to initiate anabolic responses remains undefined. As calcium influx is the first measurable response of bone cells to mechanical stimuli, voltage sensitive calcium channels (VSCCs) play a critical role in bone formation. Given VSCC activity is influenced by its auxiliary $\alpha_2\delta_1$ subunit, regulating the gating kinetics of the channel's pore-forming (α_1) subunit and forward trafficking of VSCCs to cell membranes, the $\alpha_2\delta_1$ subunit may govern anabolic bone responses. Data showing osteopenia in global $\alpha_2\delta_1$ knockout mouse and decreased mechanosensitivity following $\alpha_2\delta_1$ knockdown in cultured osteocytes support this notion. **Objective & Design**: We hypothesized that osteocyte-specific $\alpha_2\delta_1$ deletion in a mouse model would impair skeletal development, decrease bone formation and mechanosensitivity. **Methods**: Generation of an osteocyte-specific $\alpha_2\delta_1$ knockout was accomplished by crossing mice (C57BL/6) harboring LoxP sequences flanking *Cacna2d1*, the gene encoding $\alpha_2\delta_1$, with mice expressing Cre recombinase under the control of the Dmp1 (10Kb) promoter (*Cacna2d1*$^{fl/fl}$, Dmp1-Cre+). To assess skeletal phenotype and mechanosensitivity, longitudinal whole body and site-specific DXA, *in vivo* µCT (10wk old), and two weeks of tibial loading (16wks) will be conducted before femurs are collected at 20 wks for mechanical testing, *ex vivo* µCT, and quantitative histomorphometry. **Results & Conclusion**: Preliminary analyses show no differences in whole body or site-specific BMD and BMC values between mice over time, suggesting osteocyte-specific $\alpha_2\delta_1$ deletion may not influence skeletal development. However, key differences in mechanosensitivity following tibial loading are expected given the potential role of $\alpha_2\delta_1$ in mechanically-induced bone formation.

BACKGROUND

- Osteocytes are the most abundant and mechanosensitive cells within the skeleton, influencing bone formation and resorption.
- Intracellular calcium influx is the first measurable response of bone cells to mechanical stimuli and voltage sensitive calcium channels (VSCCs) play a critical role in mechanically-induced bone formation (Thompson WR et al. JBMR 2011)

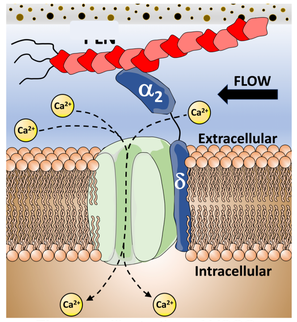

Figure 1: Illustration of VSCC

- The auxiliary VSCC subunit $\alpha_2\delta_1$ (**Fig.1**) regulates channel pore kinetics and cell membrane density.
- Preliminary data from our lab shows osteopenia and decreased mechanosensitivity following global $\alpha_2\delta_1$ deletion.
- **Objective**: The objective of this on-going project is to see the effects of the $\alpha_2\delta_1$ subunit on skeletal development.
- **Hypothesis**: We hypothesized that osteocyte-specific $\alpha_2\delta_1$ deletion in a mouse model would impair skeletal development, decrease bone formation and mechanosensitivity

MATERIALS & METHODS

- Osteocyte-specific $\alpha_2\delta_1$ knockout was accomplished by crossing mice (C57BL/6) harboring LoxP sequences flanking *Cacna2d1*, the gene encoding $\alpha_2\delta_1$, with mice expressing Cre recombinase under the control of the Dmp1 (10Kb) promoter (*Cacna2d1*$^{fl/fl}$, Dmp1-Cre+), and confirmed through PCR and gel electrophoresis (**Fig 2).**

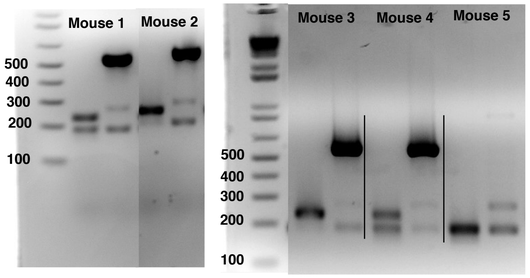

Figure 2: PCR & Gel Electrophoresis. The first column evaluates the *Cacna2d1* gene. 180 BP indicates WT *Cacna2d1*. 220 BP indicates *Cacna2d1* flanked with FL. In the second column the presence of Cre (530BP) is evaluated.

- Whole body and site-specific (L2-L5, right femur) BMD/BMC and soft tissue values were measured by DXA at 6, 9, 12 and 16 weeks age (**Fig 3).**
- In vivo micro-computed tomography (*µ*CT) measurements were collected at 10 weeks of age using a Skyscan *µ*CT machine (**Fig. 4)**
- Mice underwent a 4-week long unilateral tibial loading regiment from 16 to 20 weeks of age (**Fig. 5)**
- Mice were injected (IP) with calcein (10 mg/kg) and alizarin (20 mg/kg) at 17 and 19 weeks of age respectively to assess periosteal and endosteal bone parameters. Following sacrifice, calcein and alizarin labels were used to determine dynamic histomorphometric analyses **(Fig. 6)**
- Statistical analysis performed using Prism to compare DMP1-Cre+ mice (KO) to DMP1-Cre- mice (WT)

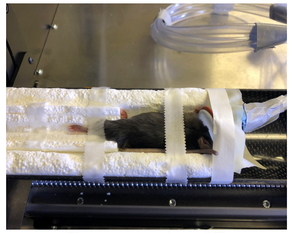

Figure 4: In vivo µCT.

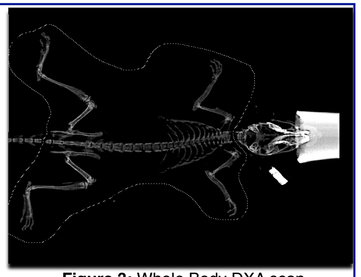

Figure 3: Whole Body DXA scan

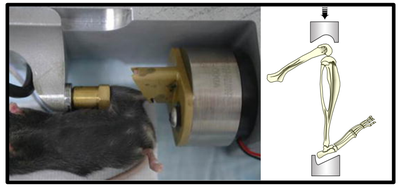

Figure 5: Tibial Loading

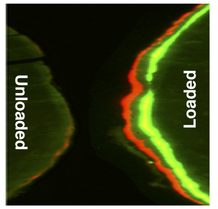

Figure 6:
Dynamic histo example

WHOLE BODY COMPOSITION

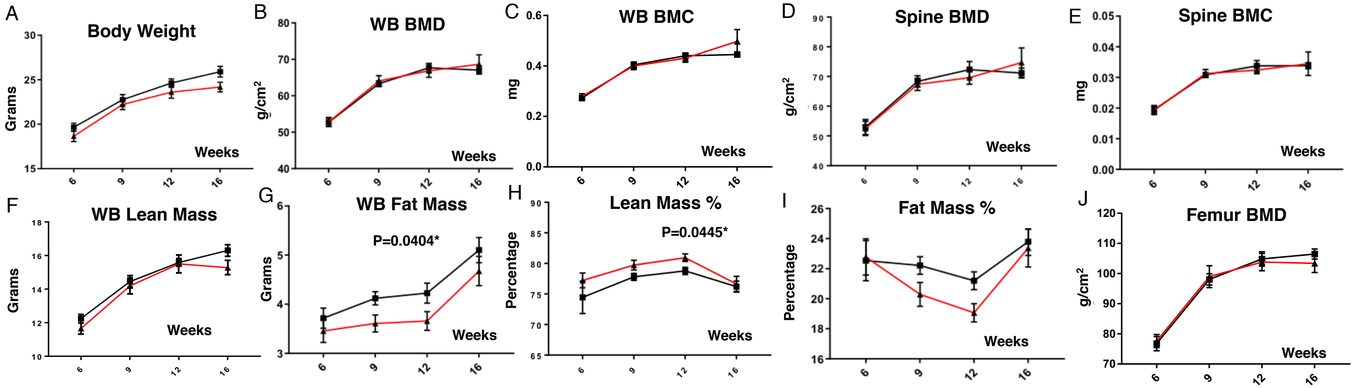

Figure 6: Whole Body Composition. KO (n=8) and WT (n=11) showed no whole body or specific differences in BMD or BMC values (A,B,C,D,F,G,I,J). KO mice did display lower whole body fat mass (E) and increased lean mass percentage (H). Comparable results were shown for femur BMC and in female mice.

CONCLUSIONS

- Previous data has suggested that $\alpha_2\delta_1$ plays a crucial role in proper skeletal development.
- Preliminary analyses show no differences in whole body or site-specific BMD and BMC values between mice over time.
- Data suggests osteocyte-specific $\alpha_2\delta_1$ deletion may not influence skeletal development or bone formation.
- Given the role of osteocytes in bone formation, key differences in mechanosensitivity following tibial loading are expected.