Loss of BMPR2 expression in skeletal progenitor cells reduces age-related bone loss

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Osteoporosis is a disease of low bone mineral density (BMD) that affects 10 million Americans and accounts for 1.5 million fractures annually. With an additional 34 million Americans at risk for developing the disease, osteoporosis is both a significant health problem and a considerable socioeconomic burden. Current first-line therapies for osteoporosis involve anti-resorptive agents but many patients, such as those with drastically low BMD or high fracture risk, would benefit from augmenting bone formation as well as inhibiting bone loss. We recently reported that targeted deletion of the type 2 BMP receptor BMPR2 in skeletal progenitor cells of the limb bud using Prx1-Cre (*Bmpr2* mutant mice) leads to dramatically increased bone mass and bone formation rate by ten weeks of age in the absence of changes in osteoclast number or function (Lowery *et al.*, *Journal of Cell Science* 2015). In the present study, we examined the impact of *Bmpr2* deletion on age-related bone loss in *Bmpr2* mutant mice. Consistent with our previous results, 55-week-old female *Bmpr2* mutant mice exhibit approximately four-fold higher bone mass in the tibia than control mice. Moreover, the age-related decline in bone mass from 15 weeks to 55 weeks of age in female *Bmpr2* mutant mice is reduced 1.8-fold (CI, 1.5-2.2) compared to control mice. Bone mass of the L5 vertebrae, which is outside the *Prx1*-Cre expression domain, is unchanged in *Bmpr2* mutant mice compared to control mice at all ages examined. Quantification of the serum bone turnover markers Procollagen Type I N-terminal Propeptide (PINP) and Collagen Type I C-telopeptide (CTx) suggest that high bone mass in aging female *Bmpr2* mutant mice is preserved due to a sustained increase in bone formation rate to at least 35 weeks of age with no alteration in bone resorption. Collectively, our findings provide insight into the mechanisms regulating age-related bone loss and suggest that strategies aimed at controlling signaling through BMPR2 have the potential to impact bone mass in the aging adult skeleton.

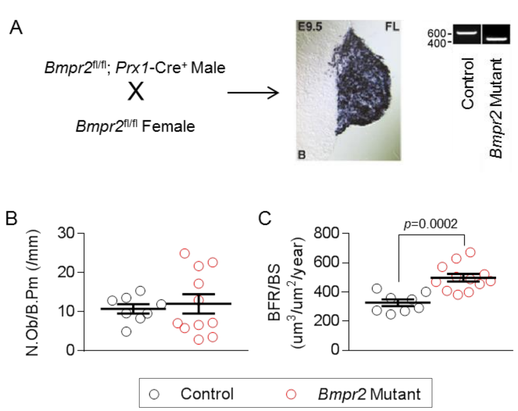

Fig. 1 Conditional deletion of *Bmpr2* in skeletal progenitors leads to high bone mass by ten weeks-of-age due to elevated individual osteoblast activity level. A: At left, *Bmpr2* mutant mice were generated by crossing *Bmpr2*$^{fl/fl}$; *Prx1*-Cre+ males with *Bmpr2*$^{fl/fl}$ females (Lowery *et al.*, *Journal of Cell Science* 2015). At middle, *Prx1*-Cre causes efficient deletion in the mesoderm of the appendicular skeleton by embryonic day 9.5 as evidenced by reporter staining in the forelimb (blue staining; adapted from Logan *et al.*, *Genesis* 2002). At right, the resulting truncated *Bmpr2* transcript is confirmed by RT-PCR in RNA from 15-week-old humerii (Lowery *et al.*, *Journal of Cell Science* 2015). **B, C:** As previously reported (Lowery *et al.*, *Journal of Cell Science* 2015), osteoblast density (B) using standard analysis of number of osteoblasts per mm of bone perimeter (N.Ob/B.Pm (/mm)) and bone formation rate (C, BFR) using standard analysis of BFR relative to bone surface (BFR/BS) in ten-week-old female mice.

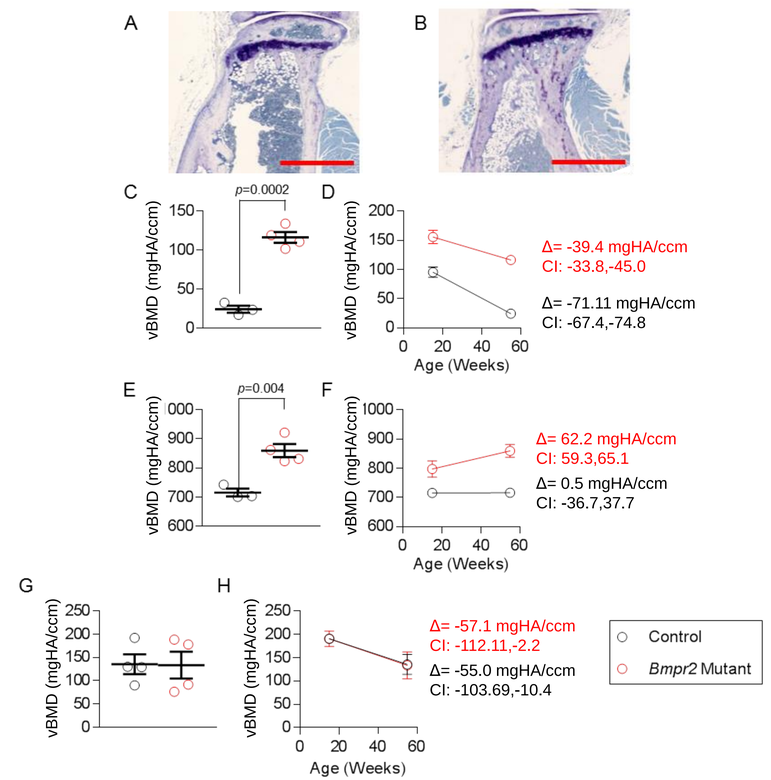

Fig. 2 Reduced age-related decline in bone mass of female *Bmpr2* mutant mice. A, B: Representative histology of tibiae from 55-week-old female control (A) and *Bmpr2* mutant (B) mice. **C-F:** Trabecular (C-D) and mid-shaft (E-F) volumetric bone mineral density (vBMD) quantified by micro-CT in tibiae of females control and *Bmpr2* mutant mice at 55 weeks of age (C, E) and change between 15 and 55 weeks of age (D, F). **G-H:** Volumetric bone mineral density (vBMD) quantified by micro-CT in L5 vertebrae, which is outside of the *Prx1*-Cre expression domain, of females control and *Bmpr2* mutant mice at 55 weeks of age (G) and change between 15 and 55 weeks of age (H).

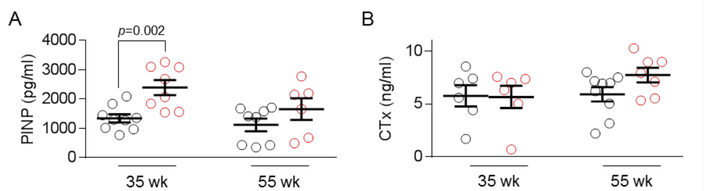

Fig. 3 Sustained increase in bone formation rate in *Bmpr2* mutant mice with no defect in bone resorption rate. A, B: Quantification of the bone formation marker PINP (A) and the bone resoprtion marker CTx (B) in sera of female control and *Bmpr2* mutant mice at 35 and 55 weeks of age using ELISA.

Conclusions:

- Loss of *Bmpr2* in embryonic skeletal progenitor cells leads to high bone mass due to increased osteoblast activity
- *Bmpr2* mutant mice exhibit high bone mass to at least 55 weeks of age and experience reduced age-related bone loss
- Markers of bone formation rate are elevated to at least 35 weeks of age in *Bmpr2* mutant mice with no observed change in bone resorption parameters at any age examined

Current and Future Directions:

- Examination of signal transduction changes associated with loss of BMPR2 in the aging skeleton using western blot and immunohistochemistry
- Identification and characterization of the gene signature associated with sustained increase in bone formation in the absence of BMPR2 expression in the aging skeleton using RNA-Seq and qRT-PCR
- Development of non-genetic means to reduce BMPR2 function and/or expression in the postnatal skeleton

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