Homozygous Deletion of Dickkopf-1 Results in a High Bone Mass Phenotype.

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Introduction

Dickkopf-1 (DKK1) antagonizes Wnt/β-catenin signaling activity via interaction with the Lrp5/6 co-receptor and is thus a negative regulator of osteoblast differentiation and bone formation. Complete deletion of Dkk1 activity leads to early embryonic lethality and thereby precludes a proper investigation of its role in post natal bone. Recently, adult mice with complete absence of Dkk1 function have been generated by genetically reducing the activity of Wnt3 during embryonic development[1]. Over 50% of mice of the Dkk1-/-; Wnt3+/- genotype (HOM/HET) are found viable. We examined the bone phenotype associated with complete loss of Dkk1 in comparison to Dkk1+/+;Wnt3+/+ (WT/WT), Dkk1+/-;Wnt3+/+ (HET/WT) and the Dkk1+/+;Wnt3+/- (WT/HET) mice.

Hypothesis

Mice homozygous for Dkk1 and heterozygous for Wnt3 will have a high bone mass phenotype compared to WT/WT. WT/HET mice will have no postnatal skeletal phenotype.

Methods

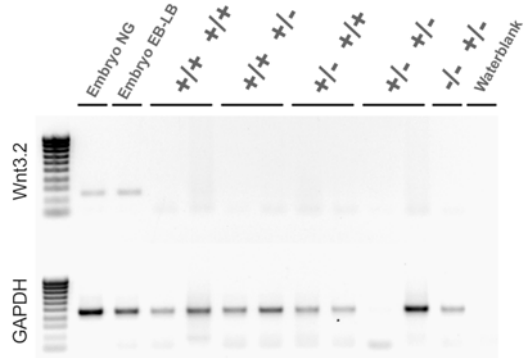
Male and female mice were examined for skeletal changes at 15 weeks of age. Mice were dosed with dual calcein labels to examine bone formation parameters. Samples of long bones were harvested and fixed for radiographic and histological analysis. RNA was extracted from calvaria of all mice to assess the presence of Wnt3 in postnatal bone.

Radiographs were used to measure femoral length. DXA scans examined whole body BMC and BMD. QCT scans examined BMD, BMC and bone volume in individual femora. MicroCT (μCT) scans were used to examine trabecular and cortical bone volume and architecture. Tibiae were processed for decalcified histology and femora for undecalcified histology. Osteoclast numbers and growth plate height were examined in tibia, whilst mineral apposition rate was assessed in the distal femur on both trabecular bone and endosteal cortical bone.

Results

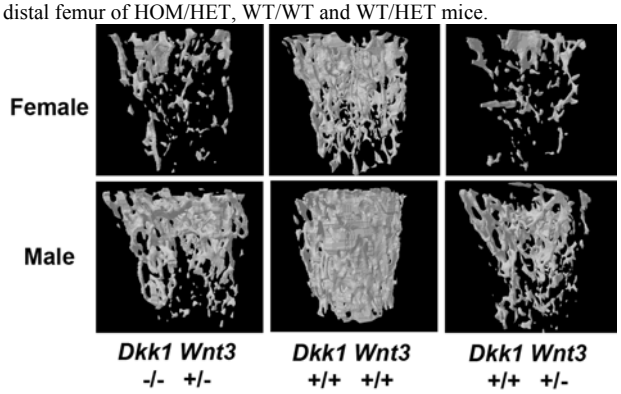
Analysis of calvarial RNA showed no postnatal expression of Wnt3 in any genotype, as such this tissue in HOM/HET mice is functionally null only for Dkk1(Figure 1). Both male and female HOM/HET mice showed no change in body mass compared to WT/WT, however male HOM/HET mice showed a 4% reduction in femur length along with an 18% decrease in growth plate height compared to WT/WT ($p<0.05$).

Figure 1. Representative gel image demonstrating a lack of RNA for Wnt3 in postnatal calvaria from all genotypes



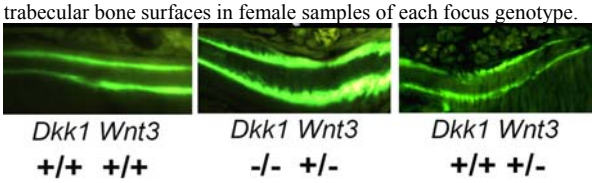
Whole body BMC was increased 12% in male and 16% in female HOM/HET mice ($p<0.05$) and BMD was increased 14% in male HOM/HET mice ($p<0.01$) compared to WT/WT. QCT scans of metaphyseal bone revealed increases in trabecular BMC of 67% in female ($p<0.01$ vs WT/WT) and 64% in male HOM/HET mice ($p=0.053$ vs WT/WT). Diaphyseal cortical bone volume was increased by 31% and 27% in female and male HOM/HET mice respectively ($p<0.01$ vs WT/WT). Concurrently, cortical thickness was increased 22% in female ($p<0.01$ vs WT/WT) and 20% ($p<0.05$ vs WT/WT) in male HOM/HET mice.

Figure 2. Representative 3D μCT images of trabecular bone from the distal femur of HOM/HET, WT/WT and WT/HET mice.



μCT analysis of distal femoral metaphyseal bone revealed a 416% increase in female and a 247% increase in male HOM/HET mice compared to WT/WT for 3D bone volume (BV) ($p<0.01$), which was associated increases of 304% in female and 166% in male in trabecular number in HOM/HET mice ($p<0.05$, Figure 2). Cortical bone volume in the femoral midshaft was also increased 25% and cortical thickness 16% in female HOM/HET mice compared to WT/WT ($p<0.05$). Histological analysis of distal femora showed a 47% increase in trabecular MAR in female and a 50% increase in male HOM/HET mice compared to WT/WT ($p<0.05$, Figure 3). Cortical bone MAR was not significantly altered in HOM/HET mice. Differences in bone resorption have not been revealed between genotypes. Importantly no skeletal phenotype has been detected in WT/HET control mice for any parameters measured.

Figure 3. Representative images of double fluorescent labels on trabecular bone surfaces in female samples of each focus genotype.



Discussion

Complete deletion of Dkk1 in the presence of reduced Wnt3 expression produces viable healthy mice with an extremely high bone mass. Analysis of bone mass through the use of DXA, QCT and μCT revealed increases of up to 4 fold in trabecular bone volume with increased trabecular number in mutant HOM/HET mice compared to WT/WT controls. These increases were a direct result of increased bone formation with no alteration in the histological assessment of bone resorption. Both the lack of postnatal skeletal expression of Wnt3 and absence of a skeletal phenotype in WT/HET mice suggest Wnt3 does not have a functional role in the postnatal skeleton.

Conclusion

This study is the first to present data on the postnatal skeletal phenotype of mice homozygous for deletion of Dickkopf-1. The extreme high bone mass phenotype seen in these mice due to enhanced bone formation confirms a pivotal role for Dkk1 as a negative regulator of bone formation. Further examination of limbs from these mice is underway to assess their mechanical properties. In addition, in vitro examination of primary cells will expand on the phenotype of these mice and provide further mechanism for the role Dkk1 plays in osteoblast differentiation.

References

1. Lewis S et al. Development 2008

Acknowledgements

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