Impaired *c-Kit* Signaling Couples Bone Resorption to Bone Formation through Wnt10b in *Kit*$^{W\text{-}sh/W\text{-}sh}$ Mice

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Background

c-Kit, a receptor tyrosine kinase belonging to the platelet-derived growth factor (PDGF) and the colony-stimulating factor 1 (CSF-1) receptor family, is a product of the gene at the *Dominant White Spotting* (*W*) locus.

A number of naturally occurring loss-of-function mutations in *c-Kit* have been identified in mice and humans. The *W* mutation is a null mutation that causes deletion of the transmembrane domain of the *c-Kit* receptor, while W^{v} is a point mutation in the kinase domain of the receptor resulting in impaired activity of the receptor. Cells that express the W^{v} mutation do not respond to c-Kit ligand in proliferation and apoptosis, presumably due to inability of the receptor to initiate signal transduction. *W-sash* (W^{sh}), an allele of *W,* is an inversion mutation upstream of the *c-Kit* promoter region that affects a key regulatory element, resulting in cell-type-specific altered expression of the gene.

Previous studies indicated that female WBB6F1/J-*Kit*$^{W/W\text{-}v}$ (W/W^{v}) mice carrying a compound mutation in *c-Kit* were osteopenic at 14 weeks of age. However, these mice were infertile because of lacking germ cells in ovary and had reduced estrogen and progesterone levels leading to increased FSH level. It was unclear whether the observed skeletal phenotype resulted from cell-autonomous effects in osteoclasts or was a consequence of changes in sex hormone level.

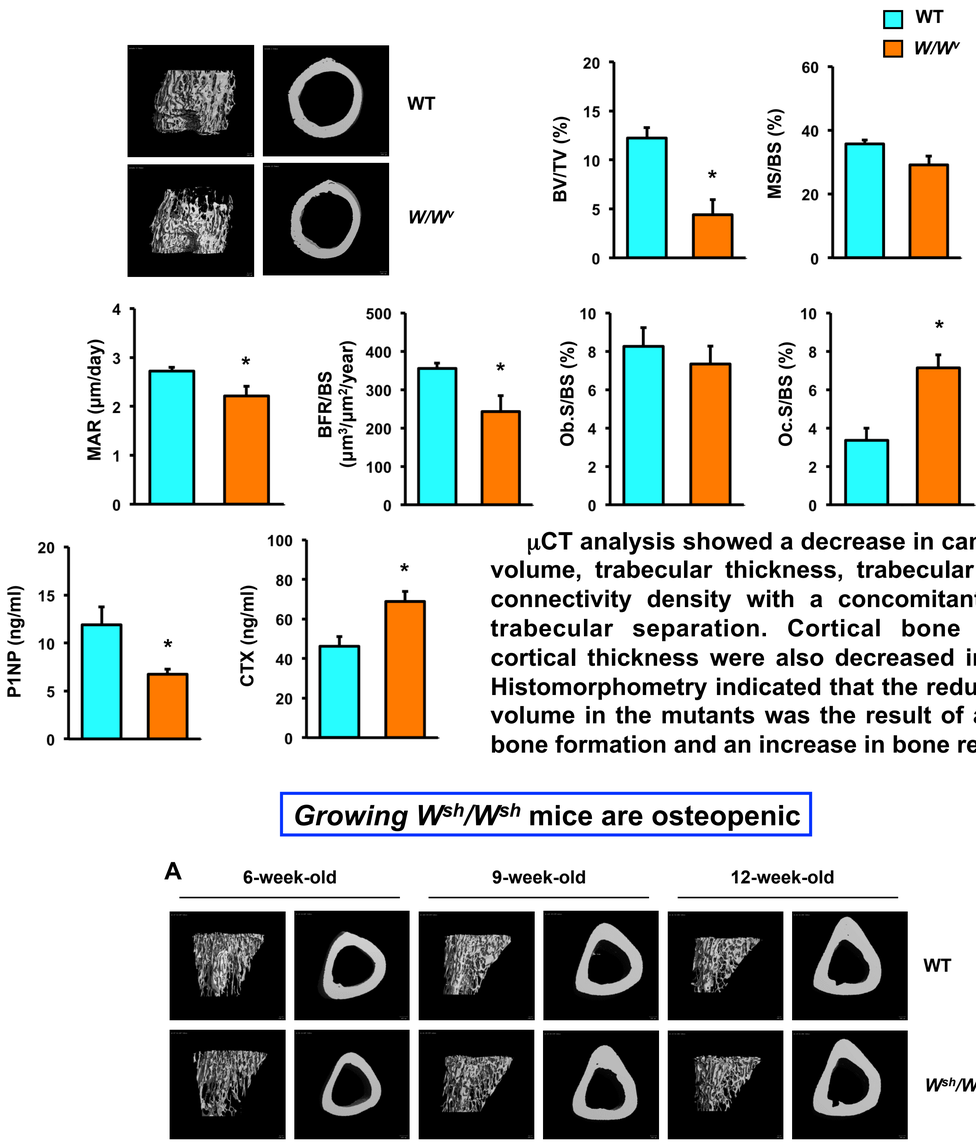
Materials and Methods

W^{sh}/W^{sh}, W/W^{v} and WBB6F1/J-*Kit*$^{+/+}$ wildtype (WT) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). W^{sh}/+ mice were crossed with W^{sh}/+ mice to generate W^{sh}/W^{sh} mice and littermate controls. W/W^{v} and W^{sh}/W^{sh} mice are white, black-eyed and lack coat pigment whereas their controls are black. Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Harvard Medical School.

Mice were subcutaneously injected with 20 mg/kg calcein and 40 mg/kg demeclocycline and inter-labeling periods were 4, 5 and 6 days for 6-, 9- and 13-week-old mice, respectively. At the end of the experiment, mice were weighed and anesthetized with isoflurane. Blood samples were collected and centrifuged and serum was kept at -80°C for determination of P1NP and CTX. Tibiae and femora were removed. Right femora and tibiae of W/W^{v} mice were fixed in 70% alcohol for μCT analysis and bone histomorphometry, respectively. For W^{sh}/W^{sh} mice, left tibiae were used for μCT analysis whereas right tibiae were analyzed for bone histomorphometry. Left femora were frozen in liquid nitrogen and stored at -80°C until processed for RNA isolation and qPCR analysis in W^{sh}/W^{sh} mice.

Results

W/W^{v} mice exhibit osteopenic phenotype



μCT analysis showed a decrease in cancellous bone volume, trabecular thickness, trabecular number and connectivity density with a concomitant increase in trabecular separation. Cortical bone volume and cortical thickness were also decreased in W/W^{v} mice. Histomorphometry indicated that the reduction in bone volume in the mutants was the result of a decrease in bone formation and an increase in bone resorption.

Growing W^{sh}/W^{sh} mice are osteopenic

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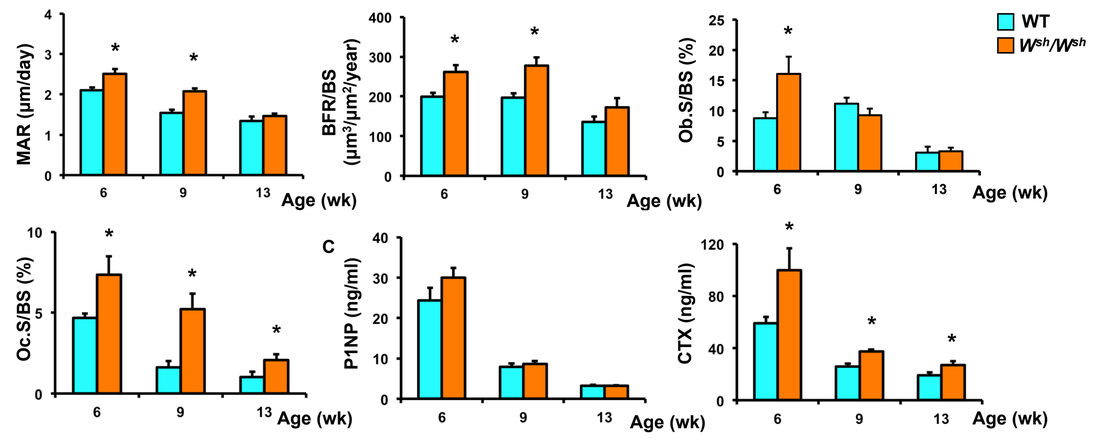
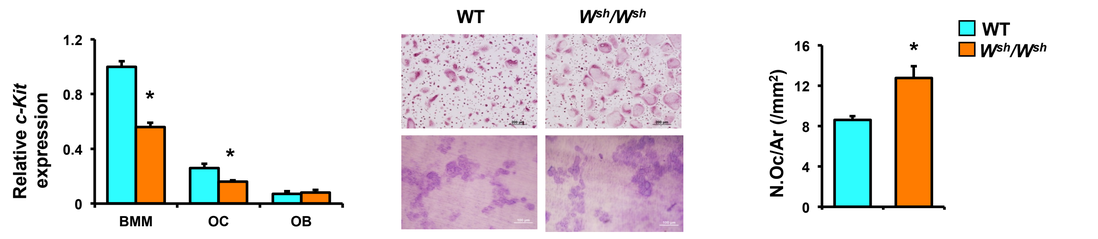
Results

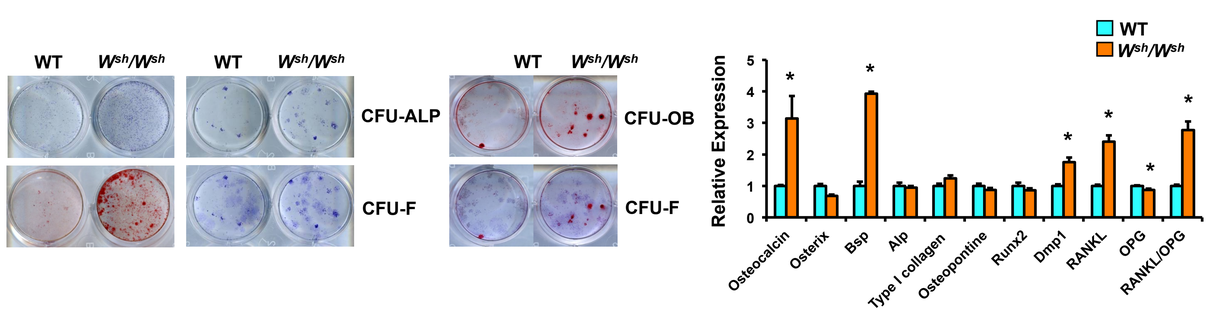
W^{sh} mutation increases bone formation and bone resorption in growing mice



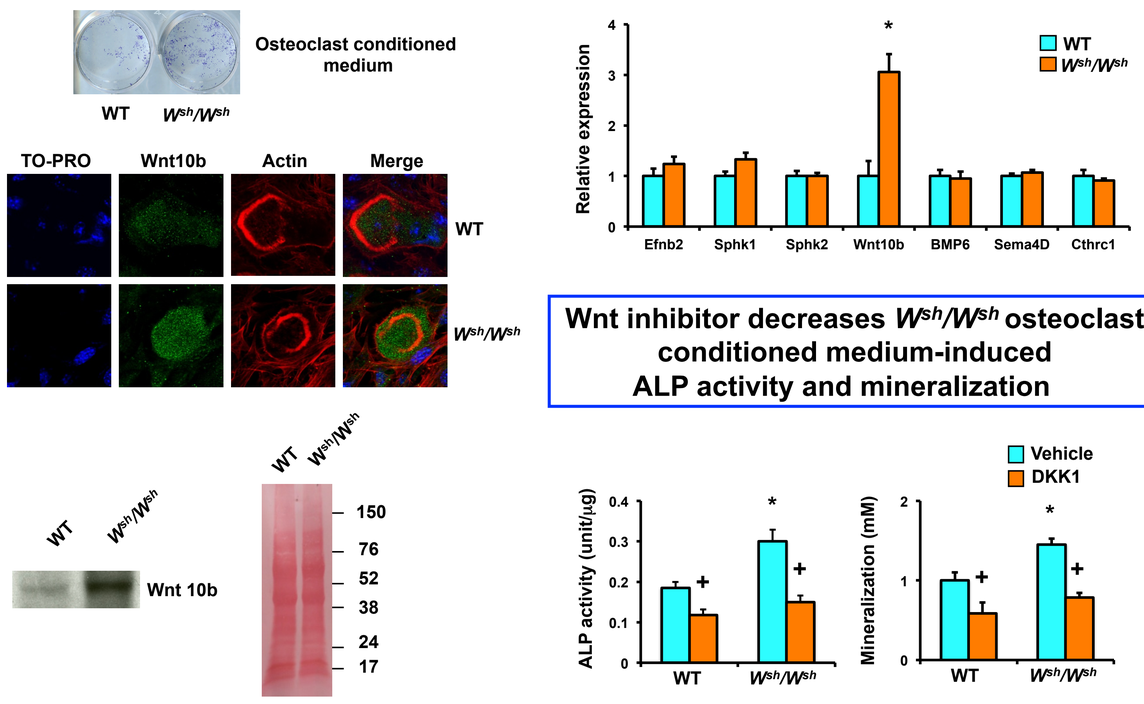
c-Kit mutation increases osteoclast differentiation



Mutation of *c-Kit* increases osteoblast progenitors and differentiation



Osteoclast-coupling factor Wnt 10b is increased in W^{sh}/W^{sh} osteoclasts



Wnt inhibitor decreases W^{sh}/W^{sh} osteoclast conditioned medium-induced ALP activity and mineralization

Summary

Our data suggest that c-Kit plays a crucial role in bone homeostasis. Loss-of-function mutation of *c-Kit* resulted in decreased cortical and cancellous bone volume in W/W^{v} mice. The reduction in cancellous bone volume was the result of a marked decrease in osteoblast surface and increase in osteoclast surface. However, these mice are sterile. To gain more insight into the precise role of *c-Kit* in bone metabolism, we used W^{sh}/W^{sh} mice that possess an inversion mutation upstream of the *c-Kit* region and are fertile. This mutation of *c-Kit*, which reduced *c-Kit* expression in BMM and osteoclasts but had no effect on expression in osteoblasts, resulted in osteopenia associated with increased bone formation and increased bone resorption in growing W^{sh}/W^{sh} mice. The skeletal phenotype was milder when animals were mature. The increase in osteoclast number was a consequence of increased RANKL/OPG ratio in osteoblasts. It appears that the alteration in osteoclast-osteoblast coupling mechanism contributes to increased bone formation in W^{sh}/W^{sh} mice. Mutation of *c-Kit* stimulated Wnt10b production from osteoclasts to promote osteoblast mineralization and subsequently bone formation. Blocking Wnt10b markedly inhibited the increase in ALP activity and mineralization that were induced by W^{sh}/W^{sh} osteoclast conditioned medium.