Heterozygous Inactivation of *Gnas* Alters Skeletal Development and Bone Quality

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Introduction

Progressive osseous heteroplasia (POH), Albright hereditary osteodystrophy (AHO), osteoma cutis (OC), and pseudohypoparathyroidism 1a/1c (PHP) form a spectrum of disorders that are caused by heterozygous inactivating mutations in *GNAS*, a gene that encodes multiple transcripts including the α-subunit of the stimulatory G-protein ($G_s\alpha$) of adenylyl cyclase. All these disorders exhibit subcutaneous heterotopic ossification (HO), however, POH has the most severe HO, characterized by HO progression into deeper connective tissues including muscle and fascia. The *GNAS* gene shows genomic imprinting, and POH and AHO are associated with paternal and maternal inheritance of the mutation respectively. Mice with paternally- and maternally-inherited deletion of *Gnas* exon 1 exhibit different phenotypes although both develop subcutaneous ossifications. But whether reduced *Gnas* expression leads to alterations in the formation or maintenance of skeletal bone and how paternal and maternal inheritance of the mutation affects skeletal bone quality remains undetermined.

Methods

We performed μCT and mechanical testing in young (2 week old – P14) and adult (9 months) mice with heterozygous inactivation of paternal ($Ex1^{+p/-}$) or maternal ($Ex1^{m-/+}$) allele of *Gnas* exon 1.

Results

For both mutants, trabecular bone parameters, analyzed through μCT scans of the distal femoral epiphyses in P14 mice, revealed dramatic reductions in total volume (~20%) and bone volume (>25%) with marginal reduction in bone volume fraction (~12%) compared to wildtype littermates. Trabecular microarchitecture was altered with a significant decrease (~9%) in trabecular thickness and a concomitant increase in the structure model index (>10%), suggesting that trabecular bone is more rod-like in *Gnas* deficient mice. In addition, μCT analyses of the femoral mid-diaphysis showed reduced cortical thickness (15%), cortical bone volume (>25%) and bone volume fraction (>13%) in P14 mutants vs. wildtype. Femurs from both P14 mutants exhibited significantly lower strength (stiffness and peak load) by 3-point bending. Although the differences between wildtype and either $Ex1^{+p/-}$ and $Ex1^{m-/+}$ mice were the same at P14, μCT of adult mice revealed differences dependent on the parental origin of the mutation. At 9 months of age, both $Ex1^{+p/-}$ and $Ex1^{m-/+}$ mice showed trabecular bone properties that were now similar to wt. However, cortical bone parameters including bone volume fraction (>10%) and cortical thickness (~14%) and biomechanical strength were significantly lower in mice with paternal inactivation of the *Gnas* at 9 months of age, while mice with maternal inheritance of the *Gnas* mutation showed no differences vs. wildtype in cortical bone.

Discussion

These results indicate that Gsα signaling plays an important role in skeletal bone formation and maintenance and that inheritance of the mutation paternally vs. maternally differentially effects bone remodeling with age. Currently studies are focused on understanding the interaction between Gsα and other signaling pathways and the effects of *Gnas* inheritance in regulating bone quality.