Heterozygous Inactivation of Gnas Induces Heterotopic Ossification and Impairs Normal Skeletal Development

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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 is the most severe form and is characterized by HO progression into deeper connective tissues including muscle and fascia. The *GNAS* gene shows genomic imprinting and POH is associated with paternal inheritance of the mutation. Mice with paternal inheritance of heterozygous deletion of exon 1 (Gnas $Ex1^{+/-}$) have lower body weight and length, and develop subcutaneous ossifications with age. But whether reduced Gnas expression leads to alterations in the formation or quality of skeletal bone remains undetermined. To investigate the effects of Gnas mutation on skeletal development, we performed μCT and histological analyses to examine the effects of paternal allele inactivation of Gnas on developing bone and cartilage. At postnatal days 1 (P1) and 14 (P14), $Ex1^{+/-}$ mice weighed significantly less than wild-type (wt) littermates. Tibiae from $Ex1^{+/-}$ mice at these ages were significantly shorter in length (15%±4). Trabecular bone parameters, analyzed through μCT scans of the distal epiphyseal region in P14 mice, revealed dramatic reductions in bone volume (36%±11) and bone volume fraction (20%±12). Microarchitecture of trabeculae was altered with a significant decrease in trabecular thickness and a concomitant increase in the structure model index, suggesting that trabecular bone is more rod-like in these mutants than wt littermates. μCT analyses of the femoral mid-diaphysis region showed reduced cortical thickness (20%±10) and cortical bone volume (35%±8) in P14 mutants. Histology of hindlimb sections from P14 mice showed a marked decrease in the length of the hypertrophic zone of the growth plates of $Ex1^{+/-}$ mice. The calvaria of P1 and P14 heterozygous mutants were reduced in size in both antero-posterior and medial-lateral dimensions. Taken together with our previous findings, heterozygous paternal allele inactivation of Gnas not only alters postnatal progenitor cells to form heterotopic ossification, but also adversely affects normal skeletal development that impacts both endochondral and intramembranous bone formation.

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