HHS Public Access

Author manuscript

Bone. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Bone. 2018 April ; 109: 86–90. doi:10.1016/j.bone.2017.11.019.

Ablation of Gsα signaling in osteoclast progenitor cells adversely affects skeletal bone maintenance

Girish Ramaswamy[1,2], **John Fong**[1,2], **Niambi Brewer**[1,2], **Hyunsoo Kim**[3], **Deyu Zhang**[1,2], **Yongwon Choi**[3], **Frederick S. Kaplan**[1,2,4], and **Eileen M. Shore**[1,2,5]

[1]Department of Orthopaedic Surgery, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA USA

[2]Center for Research in FOP and Related Disorders, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA USA

[3]Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA USA

[4]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA USA

[5]Department of Genetics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA USA

Abstract

Gsα, the alpha stimulatory subunit of heterotrimeric G proteins that activates downstream signaling through the adenylyl cyclase and cAMP/PKA pathway, plays an important role in bone development and remodeling. The role of Gsα in mesenchymal stem cell (MSC) differentiation to osteoblasts has been demonstrated in several mouse models of Gsα inactivation. Previously, using mice with heterozygous germline deletion of Gsα (*Gnas*$^{+/p-}$), we identified a novel additional role for Gsα in bone remodeling, and showed the importance of *Gnas* in maintaining bone quality by regulating osteoclast differentiation and function. In this study, we show that postnatal deletion of Gsα (*CreERT2;Gnas*$^{fl/fl}$) leads to reduction in trabecular bone quality parameters and increased trabecular osteoclast numbers. Furthermore, mice with deletion of Gsα specifically in cells of the macrophage/osteoclast lineage (*LysM-Cre;Gnas*$^{fl/fl}$) showed reduced trabecular bone quality and increased trabecular osteoclasts, but to a reduced extent compared to the *CreERT2;Gnas*$^{fl/fl}$ global knockout. This demonstrates that while Gsα has a cell autonomous role in osteclasts in regulating bone quality, Gsα expression in other cell types additionally contribute. In both of these mouse models, cortical bone was more subtly affected than trabecular bone. Our results support that Gsα is required postnatally to maintain trabecular bone quality and that Gsα function to maintain trabecular bone is regulated in part through a specific activity in osteoclasts.

Corresponding Author: Eileen M. Shore, PhD, University of Pennsylvania, Department of Orthopaedics, 115A Stemmler Hall, 3450 Hamilton Walk, Philadelphia, PA 19104-6081, phone: 215-898-2331, fax: 215-573-2133, shore@pennmedicine.upenn.edu.

Keywords

GNAS; Gsα; LysM; CreERT2; osteoclast; bone remodeling; trabecular bone

Introduction

Skeletal bone formation and maintenance occur through the processes of bone modeling and remodeling which are directed by coordinated activities of osteoblasts, osteoclasts, and osteocytes. Rates of bone formation by osteoblasts and bone resorption by osteoclasts are balanced and regulated through a network of signaling pathways to maintain bone quality [1–3]. *GNAS*, which encodes the α-subunit of stimulatory G-protein (Gsα) of adenylyl cyclase and activates cAMP signaling, plays important roles in skeletal development and maintenance. Previously, mouse models with global, as well as osteoblast- and osteocyte-specific, deletion of *Gnas* revealed important functions of the Gsα pathway in various stages of skeletal bone formation including mesenchymal stem cell commitment, osteoblast differentiation, and mineralization [4–8].

In additional to osteoblasts and osteogenesis, cAMP/PKA signaling has also been shown to regulate osteoclastogenesis, and other pathways such as Wnt/β-catenin and calmodulin-dependent kinase have been shown to act as upstream regulators of this process[9,10]. Using a mouse model with heterozygous germline inactivation of Gsα, we demonstrated that Gsα maintains bone quality by regulating osteoclast differentiation and bone resorption activity [11]. However, given that the mouse model used globally reduced Gsα expression, we could not distinguish the contributions of a cell-autonomous role of Gsα in osteoclasts versus additional cell types.

In this study, we investigated the effects of osteoclast-specific reduction of Gsα on bone maintenance through two *in vivo* approaches. Since osteoclasts do not begin to remodel bone and regulate skeletal bone quality until post-natal life, we induced postnatal global deletion of *Gnas* (*CreERT2;Gnas*$^{fl/fl}$) to bypass effects of decreased Gsα during embryonic skeletal development. We additionally induced Gsα deletion specifically in cells of the macrophage/osteoclast lineage. In both models, we examined the impact of *Gnas* deletion on skeletal bone quality and osteoclasts. Our data suggest that postnatal Gsα deletion during bone remodeling stages adversely impacts trabecular bone quality and concomitantly increases *in vivo* osteoclast number. Similar effects were detected with specific macrophage/osteoclast lineage *Gnas* deletion indicating that Gsα influences trabecular bone quality at least in part by Gsα function in osteoclasts.

Methods

Animals

All animal experiments were performed in accordance with the relevant regulations and guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC), University of Pennsylvania.

CreERT2 mice (Jackson Laboratories, stock no. 008463) [12] were crossed to *Gnas*$^{fl/fl}$ mice [4–8] to generate *CreERT2;Gnas*$^{fl/+}$ mice which were subsequently crossed with *Gnas*$^{fl/fl}$ mice to obtain *CreERT2;Gnas*$^{fl/fl}$ mice. Cre-negative mice from the same litters were used as controls. At 4 weeks of age, mice were injected intraperitoneally with tamoxifen dissolved in corn oil (1 mg/100 μl) on three successive days at 50 μg/g body weight. Three mice were harvested at 4 weeks and at 6 weeks post-injection. LysM-Cre mice (Jackson Laboratories, stock no. 004781) [13] were crossed with *Gnas*$^{fl/fl}$ mice to generate *LysM-Cre;Gnas*$^{fl/+}$ mice which were subsequently crossed with *Gnas*$^{fl/fl}$ mice to obtain *LysM-Cre;Gnas*$^{fl/fl}$ mice and Cre-negative (*Gnas*$^{fl/fl}$) control mice. Mice were analyzed at 3–4 weeks of age by histology and at 12 weeks by micro-computed tomography. Both male and female mice were used in these studies. All mouse lines were maintained on a C57BL/6 background (crosses used mice from Jackson Laboratories, stock no. 000664).

Micro-computed tomography (μCT)

Femurs from *CreERT2;Gnas*$^{fl/fl}$ and Cre-negative littermates at 6 weeks after tamoxifen injections or from 12 week old *LysM-Cre;Gnas*$^{fl/fl}$ and Cre-negative littermates were processed for μCT scanning and bone volume quantification using previously described methods [11].

Histology

One femur per mouse was harvested for μCT; each contralateral limb was processed for histology. TRAP staining was performed using the leukocyte acid phosphatase kit (Sigma 387A) as described previously [11]. Briefly, paraffin embedded slides were de-paraffinized, hydrated through a graded series of ethanol to deionized water and then stained following the recommended procedure. After staining for TRAP for 1 hour, slides were rinsed thoroughly with deionized water, counterstained with methyl green, and allowed to air dry. Femurs from *CreERT2;Gnas*$^{fl/fl}$ and Cre negative mice harvested at 4 weeks after tamoxifen injections and femurs from 3–4 week old *LysM-Cre;Gnas*$^{fl/fl}$ and Cre negative littermates were stained for TRAP and multi-nucleated osteoclasts (≥3 nuclei) were counted along trabeculae in the distal femur proximal to the growth plate.

Real-time PCR

Real-time PCR to measure Gsα expression was performed as described previously. Briefly, soft tissue was removed from femur and tibiae, ends were cut, and bone marrow was flushed out. The mid-diaphyseal region was frozen in liquid nitrogen then crushed in a tissue lyser (TissueLyser LT, Qiagen). RNA was extracted with Trizol, and cDNA was prepared using High-Capacity RNA-to-cDNA kit (Thermo Fisher Scientific). Gsα primer sequences were published previously [11]. Data were normalized to Gapdh.

Statistical Analyses

Analyses of *CreERT2;Gnas*$^{fl/fl}$ and Cre-negative controls: In order to accommodate for the small litter size and the higher variation between the multiple litters, Student’s t-test was performed to compare μCT data after setting the Cre-negative littermate controls to 1.

Unpaired two-sided Student's t-test was performed using absolute osteoclast numbers per mm. For real time qRT-PCR data, Cre-negative littermate controls were set to 1.

Analyses of *LysM-Cre;Gnas*$^{fl/fl}$ and Cre-negative controls: Similar to above, unpaired two-sided Student's t-test was performed to compare μCT, real time qRT-PCR data, and osteoclast numbers after setting the littermate controls to 1.

Results

Postnatal homozygous deletion of Gnas/Gsα adversely alters trabecular bone

Since osteoclasts do not begin to remodel bone and regulate skeletal bone quality until post-natal life, we induced global deletion of *Gnas* (*CreERT2;Gnas*$^{fl/fl}$) post-natally in order to examine effects of *Gnas*/Gsα depletion independently of embryonic skeletal development. At 4 weeks of age, *CreERT2;Gnas*$^{fl/fl}$ mice and control (Cre-negative) mice were treated with tamoxifen to induce *Gnas* deletion. RNA from mid-diaphyseal cortical bone harvested 6 weeks after the injection showed a significant decrease in Gsα mRNA expression by RT-PCR in *CreERT2;Gnas*$^{fl/fl}$ mice compared to control (Cre-negative) bone (Figure 1A). To investigate the postnatal role of Gsα on bone remodeling, we examined the femurs at 6 weeks after tamoxifen injections by μCT (Figure 1B). Trabecular bone volume (20% reduction) and bone volume fraction (30% reduction) were significantly reduced in *CreERT2;Gnas*$^{fl/fl}$ mice compared to controls (Figure 1C). Furthermore, trabecular architecture showed dramatic changes between the genotypes, with *CreERT2;Gnas*$^{fl/fl}$ mice demonstrating significantly reduced trabecular number (10% lower) and trabecular thickness (15% lower) and increased trabecular spacing (15% higher) compared to littermate controls (Figure 1D–F). Of note, total volume at the distal femur was 15% higher in the mutants compared to controls suggesting that bone is increased overall (Supplementary Figure 1A) in the homozygous mutant mice.

In comparison to the impact on trabecular bone, effects of *Gnas* inactivation on cortical bone at the mid-diaphysis of femur were more subtle. While total volume measured at the mid-diaphysis was significantly increased (12%) in mutants (Supplementary Figure 1B), bone volume fraction (BV/TV) and cortical thickness were marginally reduced in the *CreERT2;Gnas*$^{fl/fl}$ mice when compared with age-matched littermate controls (Supplementary Figure 1C, D), suggesting an overall loss of cortical bone in mutants.

CreERT2;Gnas$^{fl/fl}$ mice have increased osteoclast numbers

Previously, we determined that mice with germline heterozygous inactivation of *Gnas*/Gsα had increased endocortical osteoclast numbers that were associated with decreased cortical bone quality [11]. In order to determine whether bone quality and osteoclast numbers are altered by *Gnas* inactivation only during post-natal bone remodeling, femurs from *CreERT2;Gnas*$^{fl/fl}$ and littermate controls at 4 weeks after tamoxifen treatment were subjected to TRAP staining for osteoclast activity and the number of trabecular osteoclasts were counted. The numbers of multi-nucleated trabecular osteoclasts were significantly increased (Figures 2A, B) in *CreERT2;Gnas*$^{fl/fl}$ mice, supporting that postnatal homozygous

deletion of Gsα affects bone maintenance and trabecular bone quality by increasing the numbers of bone-resorbing osteoclasts.

Deletion of Gnas/Gsα specifically in macrophage/osteoclast lineage cells negatively affects trabecular bone quality but less severely than in CreERT2;Gnas$^{fl/fl}$ mice

Increased trabecular osteoclasts in *CreERT2;Gnas*$^{fl/fl}$ mice, along with our previous data from mice with germline heterozygous *Gnas*/Gsα deletion showing increased osteoclast numbers in vivo and enhanced osteoclast differentiation in vitro, led us to investigate whether *Gnas*/Gsα has a cell-autonomous role in osteoclasts to regulate bone quality. We generated mice with homozygous deletion of Gsα in cells of the macrophage/osteoclast lineage using the LysM-Cre mouse model. Macrophages (pre-osteoclasts) isolated from *LysM-Cre;Gnas*$^{fl/fl}$ mice showed a 80% decrease in Gsα mRNA expression by RT-PCR compared to Cre-negative cells, confirming the deletion of Gsα in the mutants (Figure 3A). MicroCT scans of femurs (Figure 3B) showed a statistically significant decrease in trabecular BV/TV (18%) in 12-week-old *LysM-Cre;Gnas*$^{fl/fl}$ mice compared to controls (Figure 3C). In addition, trabecular microarchitecture parameters, trabecular number, and trabecular thickness were marginally decreased (8%, $p \leq 0.1$) while trabecular spacing was marginally increased (10%, $p < 0.1$) compared to littermate controls (Figure 3D–F). These results were, however, less pronounced than the effects in *CreERT2;Gnas*$^{fl/fl}$ mice.

TRAP staining for osteoclast activity appeared increased in *CreERT2;Gnas*$^{fl/fl}$ trabecular bone compared to control (Figure 3G). Although poor viability of mutant mice limited our ability to examine a larger sample size, quantification of multi-nucleated osteoclasts (from n=4 *LysM-Cre;Gnas*$^{fl/fl}$ and n=4 controls) identified a trend towards increased numbers in *LysM-Cre;Gnas*$^{fl/fl}$ mice ($p < 0.2$) compared to controls (Figure 3H). Reduced viability also limited our ability to examine *LysM-Cre;Gnas*$^{fl/fl}$ cells in osteoclast differentiation and pit formation assays, however data using cells heterozygous for *Gnas* knockout (n = 3 *LysM-Cre;Gnas*$^{fl/+}$), showed enhanced differentiation, increased numbers of TRAP+ multi-nucleated cells, and higher pit formation/resorption area by mutant cells compared to littermate Cre-negative controls (n = 2) (also see Supplementary Figure 2 and Supplementary Information).

The more subtle effect on osteoclast functions by *LysM-Cre;Gnas*$^{fl/fl}$ or *LysM-Cre;Gnas*$^{fl/+}$ mice compared with the statistically significant effects observed in *CreERT2;Gnas*$^{fl/fl}$ mice supports that while *Gnas* inactivation confers cell-autonomous osteoclast-specific effects on osteoclast function, *Gnas* additionally acts in other cell types that contribute to the regulation of osteoclast activity and function.

Discussion

The roles of *GNAS*/Gsα in skeletal development and maintenance have been demonstrated in MSCs, osteoblasts, and osteocytes through several mouse models [4–8]. Previously, we identified a role for Gsα in bone remodeling and maintenance of bone quality in regulating osteoclast activity and differentiation. In this follow up investigation, we further show that Gsα regulates bone quality at least in part through cell-specific functions in osteoclasts [11].

These investigations additionally reveal that Gsα is important not only during bone development, but also during postnatal bone remodeling.

Since osteoclasts do not begin to remodel bone and regulate skeletal bone quality until postnatal life [1–3,14], this investigation used a conditional *Gnas* knockout mouse model and induced deletion of *Gnas*/Gsα postnatally. When *Gnas*/Gsα was globally deleted through the CreERT2 recombination system, trabecular bone quality was significantly reduced along with significantly elevated osteoclast numbers. This delineates a role for Gsα in maintaining bone quality during postnatal remodeling independently of skeletal development.

Our earlier study [11] was the first to show that Gsα regulates skeletal development and maintenance through modulating osteoclast number and function. *In vitro* differentiation studies suggested that the role of Gsα in osteoclasts is cell-autonomous. However, the effects of deletion of Gsα in other cell types including osteoblasts on the overall skeletal phenotype and the potential impact of global Gsα deletion on osteoblast-osteoclast coupling precluded us from confirming a solely cell-autonomous role for Gsα in osteoclasts. In the current study, to overcome this limitation, we deleted Gsα in cells of the macrophage-lineage using LysM-Cre to inactivate *Gnas.* Mice with Cre driven by the LysM promoter has previously been used to study bone quality in several studies and has been shown to be reliable in gene deletion in macrophage/osteoclast-lineage cells [9,15,16].

Both conditional *Gnas* knockout mouse models used in this study demonstrated enhanced osteoclast function and activity upon *Gnas* inactivation. Postnatal global Gsα deletion during bone remodeling stages was shown to adversely impact trabecular bone quality and concomitantly increase *in vivo* osteoclast numbers. Specific macrophage/osteoclast lineage *Gnas* deletion also enhance osteoclast functions and negatively affected trabecular bone, however to a reduced degree, indicating that while *Gnas*/Gsα in osteoclasts influences trabecular bone quality, *Gnas* function in additional cell types also contribute.

Studies of osteoblast differentiation and mineralization were not performed in this study in either of the two mouse models. In our previous study, we also showed that Gsα regulates osteoclast differentiation and function through enhancement of Nfatc1 levels via the PKA signaling pathway [11]. Future experiments on osteoblast differentiation and function, osteoblast-osteoclast coupling, and downstream signals regulating bone remodeling would uncover the various cellular and signaling mechanisms by which Gsα impacts bone maintenance.

Of particular note, our previous studies in mice with global germline heterozygous deletion of Gsα showed a more severe effect in cortical bone than trabecular bone [11]. In contrast, *CreERT2;Gnas*$^{fl/fl}$ mice showed a more severe adverse effect on trabecular bone quality and a subtle effect on cortical bone. This could be due to a varied number of reasons including differences in mouse ages and the time points examined, the levels of Gsα expression, and/or differing effects of these differences on osteoblasts, osteoclasts and other cell types. A significant impact on skeletal bone that depends on the levels of Gnas signaling through early development may also be a major influence on bone quality in cortical vs. trabecular

bone. After initial skeletal development, trabecular bone undergoes more active remodeling than cortical bone [17]. Hence the effect of postnatal deletion of Gsα could plausibly have a larger impact on trabecular bone remodeling than cortical bone.

Surprisingly, despite decreased trabecular bone volume fraction and microarchitecture, *CreERT2;Gnas*$^{fl/fl}$ mice showed increased total volume at both the distal femur and mid-diaphysis suggesting an increase in overall size of the bone. With aging, there is increased osteoclast activity leading to loss of both trabecular and cortical bone (endosteal resorption at the mid-diaphysis). In order to compensate for the bone loss, the mid-diaphyseal region undergoes periosteal expansion via appositional growth which helps maintain the mechanical strength of long bones [1,2]. In the Gsα mutants, we observe a similar phenotype with increased osteoclasts and loss of bone. Although we did not measure bone strength by mechanical testing in the two mouse models in this current work, we did perform three-point bending tests in mice with heterozygous deletion of Gsα [11] and showed reduction in mechanical strength parameters (stiffness and peak load) in the Gsα mutants compared to littermate controls. Postnatal deletion of Gsα could cause a decrease in mechanical strength that would support the decreased trabecular and cortical bone that we observe by μCT. Hence, the increase in total bone volume at both the distal femur and mid-diaphysis could be an adaptive response to maintain integrity of the bone.

Overall, our data demonstrate that *Gnas* is an important regulator of postnatal skeletal bone remodeling. Our studies additionally support that *Gnas*/Gsα activity in osteoclasts plays important roles in trabecular bone quality and architecture, but also that cells in addition to osteoclasts contribute to postnatal maintenance of bone quality. The identities of the relevant interacting cells and the mechanisms through which *Gnas*/Gsα signaling in these cells are important considerations for future investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by Progressive Osseous Heteroplasia Association (POHA), the Center for Research in FOP and Related Disorders, the Ian Cali Endowment for Research in FOP and Related Disorders, the Cali-Weldon Professorship of FOP Research (EMS), the Penn Center for Musculoskeletal Diseases (NIH/NIAMS P30-AR06919), and US National Institute of Health grant R01-AR046831 (EMS).

References

1. Eriksen EF. Cellular mechanisms of bone remodeling. Rev Endocr Metab Disord. 2010; 11:219–227. DOI: 10.1007/s11154-010-9153-1 [PubMed: 21188536]
2. Seeman E. Bone modeling and remodeling. Crit Rev Eukaryot Gene Expr. 2009; 19:219–233. [PubMed: 19883366]
3. Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. Bonekey Rep. 2014; 3doi: 10.1038/bonekey.2013.215
4. Wu JY, Aarnisalo P, Bastepe M, Sinha P, Fulzele K, Selig MK, Chen M, Poulton IJ, Purton LE, Sims NA, Weinstein LS, Kronenberg HM. Gsα enhances commitment of mesenchymal progenitors to the osteoblast lineage but restrains osteoblast differentiation in mice. J Clin Invest. 2011; 121:3492–3504. DOI: 10.1172/JCI46406 [PubMed: 21804192]

5. Sinha P, Aarnisalo P, Chubb R, Ono N, Fulzele K, Selig M, Saeed H, Chen M, Weinstein LS, Pajevic PD, Kronenberg HM, Wu JY. Loss of Gs(alpha) early in the osteoblast lineage favors adipogenic differentiation of mesenchymal progenitors and committed osteoblast precursors. J Bone Miner Res. 2014; 29:2414–2426. DOI: 10.1002/jbmr.2270 [PubMed: 24806274]
6. Sinha P, Aarnisalo P, Chubb R, Poulton IJ, Guo J, Nachtrab G, Kimura T, Swami S, Saeed H, Chen M, Weinstein LS, Schipani E, Sims NA, Kronenberg HM, Wu JY. Loss of Gsα in the postnatal skeleton leads to low bone mass and a blunted response to anabolic parathyroid hormone therapy. J Biol Chem. 2016; 291:1631–1642. DOI: 10.1074/jbc.M115.679753 [PubMed: 26598522]
7. Sakamoto A, Chen M, Nakamura T, Xie T, Karsenty G, Weinstein LS. Deficiency of the G-protein α-subunit Gsα in osteoblasts leads to differential effects on trabecular and cortical bone. J Biol Chem. 2005; 280:21369–21375. DOI: 10.1074/jbc.M500346200 [PubMed: 15797856]
8. Fulzele K, Krause DS, Panaroni C, Saini V, Barry KJ, Liu XL, Lotinun S, Baron R, Bonewald L, Feng JQ, Chen M, Weinstein LS, Wu JY, Kronenberg HM, Scadden DT, Pajevic PD. Myelopoiesis is regulated by osteocytes through Gs alpha-dependent signaling. Blood. 2013; 121:930–939. DOI: 10.1182/blood-2012-06-437160 [PubMed: 23160461]
9. Weivoda MM, Ruan M, Hachfeld CM, Pederson L, Howe A, Davey RA, Zajac JD, Kobayashi Y, Williams BO, Westendorf JJ, Khosla S, Oursler MJ. Wnt Signaling Inhibits Osteoclast Differentiation by Activating Canonical and Noncanonical cAMP/PKA Pathways. J Bone Miner Res. 2016; 31:65–75. DOI: 10.1002/jbmr.2599 [PubMed: 26189772]
10. Yoon SH, Ryu JY, Lee Y, Lee ZH, Kim HH. Adenylate cyclase and calmodulin-dependent kinase have opposite effects on osteoclastogenesis by regulating the PKA-NFATc1 pathway. J Bone Miner Res. 2011; 26:1217–1229. DOI: 10.1002/jbmr.310 [PubMed: 21611964]
11. Ramaswamy G, Kim H, Zhang D, Lounev V, Wu JY, Choi Y, Kaplan FS, Pignolo RJ, Shore EM. Gs α Controls Cortical Bone Quality by Regulating Osteoclast Differentiation via cAMP / PKA and β-Catenin Pathways. Sci Rep. 2017; 7:45140.doi: 10.1038/srep45140 [PubMed: 28338087]
12. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. Restoration of p53 function leads to tumour regression in vivo. Nature. 2007; 445:661–665. DOI: 10.1038/nature05541 [PubMed: 17251932]
13. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Förster I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. Transgenic Res. 1999; 8:265–277. DOI: 10.1023/A:1008942828960 [PubMed: 10621974]
14. Ke HZ. In vivo characterization of skeletal phenotype of genetically modified mice. J Bone Miner Metab. 2005; 23:84–89. DOI: 10.1007/BF03026330
15. Soung DY, Kalinowski J, Baniwal SK, Jacome-Galarza CE, Frenkel B, Lorenzo J, Drissi H. Runx1-Mediated Regulation of Osteoclast Differentiation and Function. Mol Endocrinol. 2014; 28:546–553. DOI: 10.1210/me.2013-1305 [PubMed: 24606124]
16. Ucer S, Iyer S, Bartell SM, Martin-Millan M, Han L, Kim H-N, Weinstein RS, Jilka RL, O'Brien CA, Almeida M, Manolagas SC. The Effects of Androgens on Murine Cortical BoneDoNot Require AR or ERa Signaling in Osteoblasts and Osteoclasts. J Bone Miner Res. 2015; 30:1138–1149. DOI: 10.1002/jbmr.2485 [PubMed: 25704845]
17. Clarke B. Normal bone anatomy and physiology. Clin J Am Soc Nephrol. 2008; 3(Suppl 3):S131–9. DOI: 10.2215/CJN.04151206 [PubMed: 18988698]

Highlights

- Postnatal Gsα deletion impacts skeletal bone maintenance by reducing trabecular bone and increasing osteoclasts.
- Gsα deletion in osteoclast progenitor cells decreases trabecular bone and increases osteoclasts.
- Phenotype is less severe in mice with specific osteoclast Gsα deletion compared to postnatal global knockout.
- Trabecular bone quality is regulated in part by Gsα activity in osteoclasts.

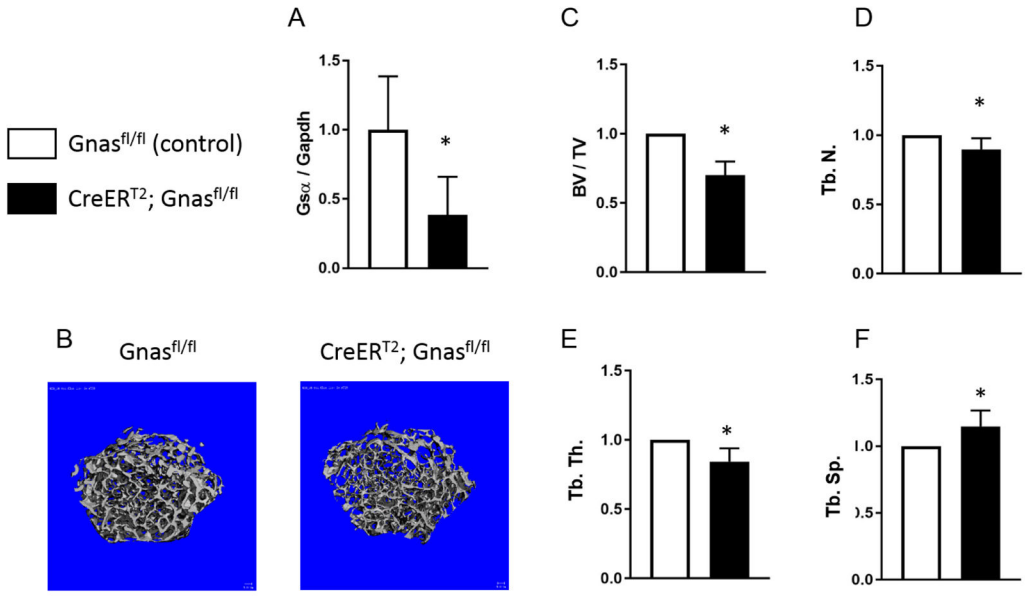

Figure 1. Postnatal homozygous deletion of *Gnas*/Gsα negatively affects trabecular bone quality

(A) Reduced Gsα mRNA expression in bone samples from *CreERT2;Gnas*$^{fl/fl}$ mice compared to control (Cre-negative) *Gnas*$^{fl/fl}$ samples was confirmed by RT-PCR (n = 5 per genotype). (B) Representative μCT scans of control and *CreERT2;Gnas*$^{fl/fl}$ mice at 6 weeks after tamoxifen injections (n = 5 per genotype) showing cross-section of trabecular bone from distal femurs. (C–F) Measured trabecular bone parameters show reduced bone volume fraction (BV/TV), trabecular number (Tb.N/mm), and trabecular thickness (Tb.Th) and increased trabecular spacing (Tb.Sp, mm) in *CreERT2;Gnas*$^{fl/fl}$. *$p < 0.05$.

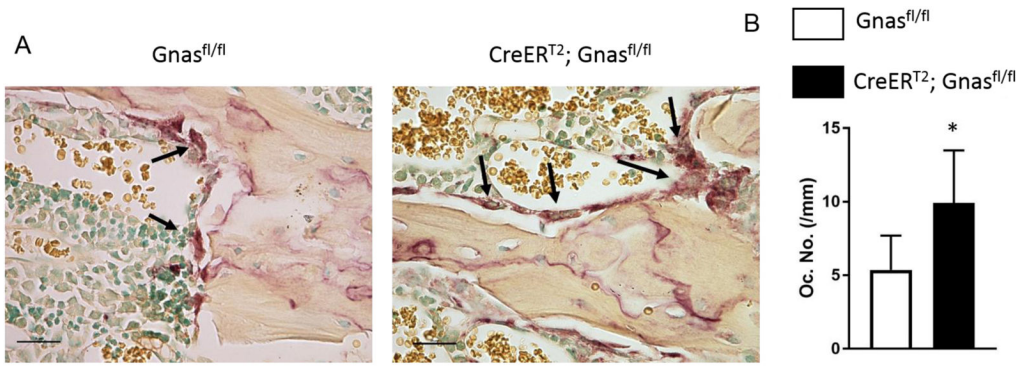

Figure 2. Mice with postnatal homozygous deletion of *Gnas*/Gsα show increased trabecular osteoclast numbers

(A) TRAP staining of femurs at 4 weeks after tamoxifen injection shows multi-nucleated osteoclasts along trabecular bone. (B) Quantification of trabecular osteoclast numbers (Oc.N/mm) at 4 weeks after tamoxifen injection in control and *CreERT2;Gnas*$^{fl/fl}$ mice (n= 4 per genotype). *$p < 0.05$

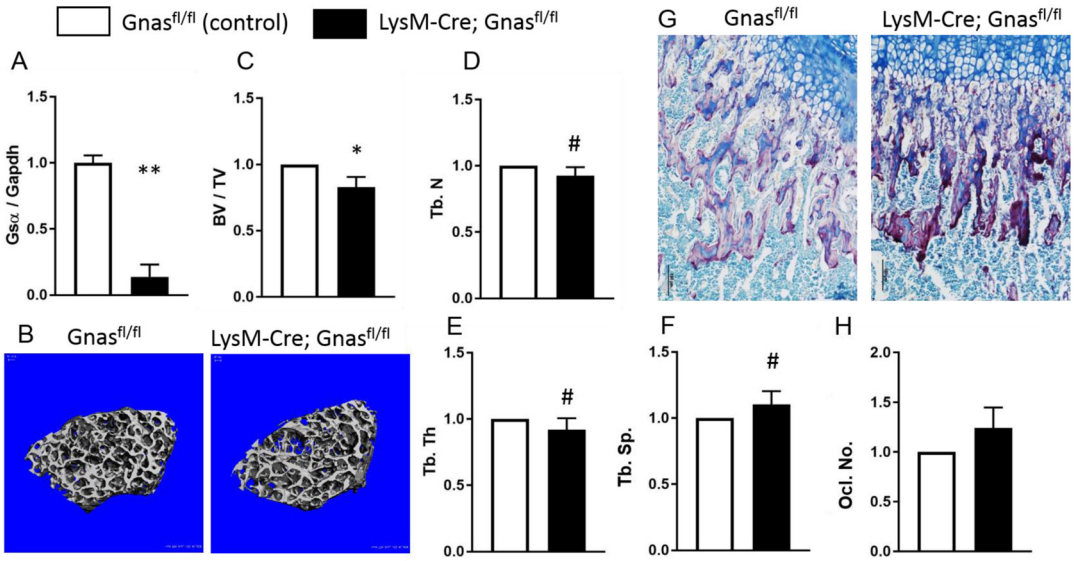

Figure 3. Macrophage-specific *Gnas*/Gsα deletion reduced trabecular bone quality less severely than global deletion of *Gnas*/Gsα

(A) Gsα mRNA expression in bone marrow macrophages (osteoclast lineage cells) isolated from *LysMCre;Gnas*$^{fl/fl}$ mice is reduced relative to controls (Cre-negative; n= 4 per genotype). (B) Representative μCT scans of 12-week-old control and *LysMCre;Gnas*$^{fl/fl}$ mice showing cross-sections of trabecular bone from distal femurs. (C–F) Measured trabecular bone parameters show reduced bone volume fraction (BV/TV), trabecular number (Tb.N, and trabecular thickness (Tb.Th) and increased trabecular spacing (Tb.Sp) in *LysMCre;Gnas*$^{fl/fl}$ (n= 5 per genotype). **p< 0.01; *p < 0.05; #p≤0.1