Altered bone formation response to mechanical loading in a mouse model of the progeroid disorder gerodermia osteodysplastica

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DISCLOSURES: None.

INTRODUCTION: Gerodermia osteodysplastica (GO) is a segmental progeroid disorder, one of the few monogenic disorders other than osteogenesis imperfecta, which is characterized by congenital bone fragility [1]. GO is caused by homozygous or compound heterozygous mutation in the Gorab gene (SCYL1BP1) [2]. A GO mouse model was created by our group, wherein Gorab is conditionally inactivated in the limb bud mesenchyme by crossing Gorabfl/fl with Ptx1-cre mice [3]. We recently showed in Gorabfl/fl mice that loss of Gorab results in abnormal osteocytes with an unusual round shape and a reduced and disordered canalicular network [4]. Given the crucial role of osteocytes in sensing mechanical signals and orchestrating adaptive bone remodeling [5], the altered morphology of the osteocyte network associated with Gorab deficiency may have a direct effect on bone's mechanoresponsiveness. Here, we hypothesized that loss of Gorab would lead to an impaired anabolic response of bone to mechanical loading. We tested this hypothesis by applying in vivo compressive loading to tibiae of Gorab deficient (Gorabfl/fl) and littermate control (LC) mice and quantifying changes in cortical bone morphology by micro-computed tomography (microCT). Bone formation and resorption was measured by 3D in vivo morphometry using registered in vivo microCT images.

METHODS: Female Gorabfl/fl mice were used for the study, as well as homozygous conditional Gorabfl/fl mice without the cre allele from the same generation, which served as littermate controls. All animals had been backcrossed with C57/B16 mice. All animal procedures were approved by the local animal welfare representative (LaGeSo Berlin, G0021/11). In vivo strain gauge experiments (n=5 mice/genotype) determined that peak loads of -5 N and -11 N engendered strains of +1200 με at the gauge site on the medial surface of the tibial midshaft in Gorabfl/fl and LC mice, respectively. Therefore, the left tibia of 10 wk old female Gorabfl/fl and LC mice (n=10 mice/genotype) underwent daily in vivo cyclic compressive loading (+1200 με, 216 cycles/day at 4 Hz) for 2 weeks (M-F) and the right tibia served as an internal control [6]. In vivo microCT (voxel size: 10.5 μm) was performed at day 0, 5, 10, and 15 to assess the midshaft of the tibia (5% of tibial length). To prevent motion artifacts during microCT scanning, anaesthetized mice were constrained in a custom-made plastic mouse bed. MicroCT scans were segmented and analyzed at each time point to measure morphological parameters including: cortical area (Cl.Ar), cortical thickness (Cl.Th), maximum and minimum principal moments of inertia (Imax and Imin). Consecutive microCT scans of the same bone, acquired at different time points, were geometrically registered to monitor bone formation and resorption, as characterized by MV/BVday0,15, (newly formed bone volume between day 0 and 15 normalized to the bone volume of day 0), EV/BVday0,15 (normalized resorbed bone volume), and RS/BSday0,15 (normalized formed and resorbed bone surfaces). A repeated measure ANOVA was performed to determine the effects of genotype, loading, and their interactions (p < 0.05). Paired t-tests were used to examine the difference between the loaded and control limbs. The percent differences are presented as: (loaded limb–control limb)/control limb)*100%

RESULTS: Gorab deficiency led to a low bone mass phenotype, with Gorabfl/fl mice having a smaller Cl.Ar, Cl.th, Imax and Imin than LC mice (Figs. 1 and 2). Interestingly, the nonloaded control limbs of Gorabfl/fl mice had a greater amount of newly formed bone volume and surface area (MV/BVday0,15, MS/BSday0,15) over the 15 day experimental period than LC mice (Fig. 1). Gorab deficiency affected the bone response to loading. Loading significantly enhanced cortical bone morphology and the bone formation response in LC mice, but not in Gorabfl/fl mice (Fig. 1). In LC mice, the loaded limb had increased volume and surface area of newly formed bone compared to the control limb (MV/BVday0,15, +741%, MS/BSday0,15, +714%), resulting in greater cortical area (Cl.Ar: +11%) and thickness (Cl.th: +11%) (Fig. 1). In Gorabfl/fl mice, all measured morphological parameters (e.g. Cl.Ar, Cl.th) and bone formation parameters (e.g. MV/BVday0,15, MS/BSday0,15) were not significantly different between the loaded and control limbs. There was limited resorption in both LC and Gorabfl/fl mice. Neither loading, nor genotype had any effect on bone resorption.

DISCUSSION: In agreement with previous studies in young C57/B16 mice [6-8], we observed a marked bone formation response to loading in young growing (10 wk old) LC mice. However, the anabolic response to loading is completely absent in the young Gorab deficient mice. Surprisingly, a greater bone formation associated with normal growth, was measured in control limbs of the Gorab deficient mice than LC mice. Similar to what we have observed in young growing C57/B16 mice [8], a reduction in resorption with loading was hardly possible since it was so low in both young LC and Gorabfl/fl mice. These results suggest that low bone mass phenotype of the Gorab deficient mice could be attributable in part to a loss in the capability of the skeleton to respond to mechanical loading.

SIGNIFICANCE: Our results highlight a role of Gorab in regulating the anabolic response to loading and suggest that reduced mechanoresponsiveness may contribute to the low bone mass phenotype observed in GO patients. Future therapeutic strategies to treat GO or other low bone mass diseases could consider strategies to restore or enhance adaptive bone formation.


Fig. 1. Newly formed bone volume (MV/BVday0,15) and bone surface area (MS/BSday0,15) over 15 days in control and loaded tibia of LC and Gorabfl/fl mice. Cortical area (Cl.Ar) and thickness (Cl.Th) at day 15. ANOVA; *genotype, **loading, ***interaction between genotype and loading, *difference between control and loaded limb (paired t-test).

Fig. 2. 3D in vivo morphometry showing bone formation and resorption over 15 days in the midshaft of the control and loaded tibia of 10-wk-old littermate control (LC) and Gorabfl/fl mice.

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