Progranulin deficiency causes exacerbated osteopenia in Gaucher disease mice

Xiangli Zhao[1], Yi Lin[2], Benjamin Liou[2], Ying Sun[2], Chuan-ju Liu[1]*
[1]Department of Orthopaedics, Yale University School of Medicine, New Haven, CT, 06519, USA;
[2]The Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA.

Disclosures: None

INTRODUCTION: Gaucher disease (GD), a common lysosomal storage disease, is caused by mutations of the *GBA1*, encoding glucocerebrosidase (GCase). Mutations in the *GBA1* cause defective GCase function, lead to accumulation of GCase substrate glucosylceramide (GC) and glucosylsphingosine (GS) in GD. Typical visceral manifestations in GD include visceral, hematologic and bone diseases manifested by hepatosplenomegaly, chronic anemia, and osteopenia. Progranulin (PGRN, encoded by *Grn*) is a multi-functional growth factor-like molecular expressed in various cells, playing a critical role in various physiological and disease processes [1]. The levels of PGRN are significantly elevated in cartilage of patient with Osteoarthritis (OA) and rheumatoid arthritis (RA). PGRN deficiency exaggerated surgically induced OA phenotypes [2]. Our previous studies have identified PGRN as a novel modifier of GCase. Aged-PGRN deficient mice displayed GD-like phenotypes in multiple organs [3]. Our recent study showed that PGRN deficiency in *Gba1* mutant mice exacerbates the GD phenotypes [4]. The objective of this study is to examine the potential effects of PGRN on the osteopenia in GD.

METHODS: We deleted PGRN in $Gba^{D409V/D409V}$ mice, a widely used GD mice model, through crossing $Grn^{-/-}$ with $Gba^{D409V/D409V}$ mice, and generated $Grn^{-/-}Gba^{9V/9V}$ (PG9V) **(Fig. 1a)** mice and found that the 12-month-old $Grn^{-/-}Gba^{9V/9V}$ (PG9V) mice developed severer GD phenotypes in comparison with invisible phenotype in the age-matched other control mice. To study the effects of PGRN on the osteopenia in GD, long bones (femur and tibia) and the knee joint were collected from 12-month-old WT, $Grn^{-/-}$ and $Grn^{-/-}Gba^{9V/9V}$ mice. After fixing in 4% paraformaldehyde, the long bones and knee joints were scanned for micro-CT imaging. After reconstruction, the parameters of trabecular bone of femur and tibia, as well as subchondral bone, including bone volume/tissue volume (BV/TV, %), trabecular thickness (Tb.Th), and trabecular number (Tb.N), were quantified using 3D analysis in CT-Analyser (Bruker). Following micro-CT imaging, the knee joints were decalcified with 10% w/v EDTA for 3 weeks prior to paraffin embedding. Serial 6 μm sagittal sections were stained with Safranin O/Fast Green for morphologic analysis.

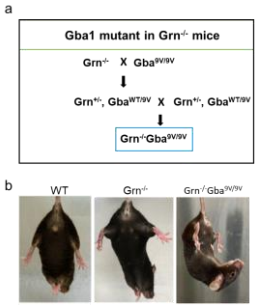

Figure 1. PGRN and Gba double mutant mice showed behavioral defects. (a) Schematic of the mouse breeding strategy to generate $Grn^{-/-}Gba^{9V/9V}$ mice through crossing $Grn^{-/-}$ mice with $Gba^{9V/9V}$ mice. **(b)** 12-month-old $Grn^{-/-}Gba^{9V/9V}$ mice showed hind limb clasping in comparison with age-matched WT, and $Grn^{-/-}$ mice displayed no clasping.

RESULTS: $Grn^{-/-}Gba^{9V/9V}$ mice at 12-mon-old showed severe behavioral deficits, such as abnormal hindlimb clasping, which was not observed in the age-matched $Grn^{-/-}$ or WT mice **(Fig. 1)**. After μCT analysis, we found that PGRN deficiency led to the reduction of bone mass in the long bones, including reduced BV/TV, Tb.Th, and Tb.N in femur (**Fig. 2a and b**) and tibia (**Fig. 2c and d**), compared with age-matched WT mice. However, the PGRN deficiency in *Gba1* mutant ($Gba^{9V/9V}$) mice did not further decrease the bone mass in femur and tibia in comparison with PGRN deficient mice ($Grn^{-/-}$) (**Fig. 2**). Intriguingly, in the knee joint, compared with $Grn^{-/-}$ mice, the $Grn^{-/-}Gba^{9V/9V}$ mice showed more osteopenia, including lower BV/TV and Tb.N (**Fig. 3a and b**). In addition, Safranin O/Fast Green staining demonstrated that PGRN and Gba double mutant led to severer cartilage degradation in comparison to $Grn^{-/-}$ mice (**Fig. 3c**).

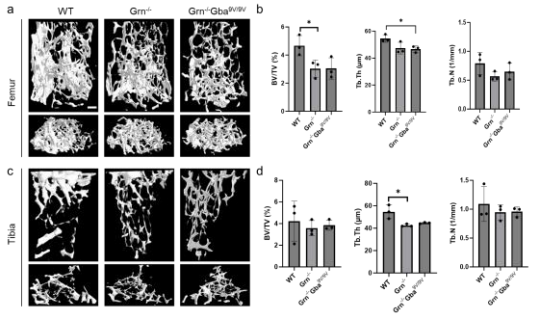

Figure 2. PGRN deficiency led to bone loss in 12-month-old mouse. (a) Representative reconstructed 3D micro-CT images of femur trabecular bone of WT, $Grn^{-/-}$ and $Grn^{-/-}Gba^{9V/9V}$ mice. **(b)** Quantification of BV/TV, Tb. Th and Tb. N of femur trabecular bone. **(c)** Representative reconstructed 3D micro-CT images of tibia trabecular bone of WT, $Grn^{-/-}$ and $Grn^{-/-}Gba^{9V/9V}$ mice. **(b)** Quantification of BV/TV, Tb. Th and Tb. N of tibia trabecular bone. (n = 3 mice for each group). Scale bar = 250 μm. Data are mean ± SD, *P* values are calculated by two-tailed unpaired Student's *t*-test.

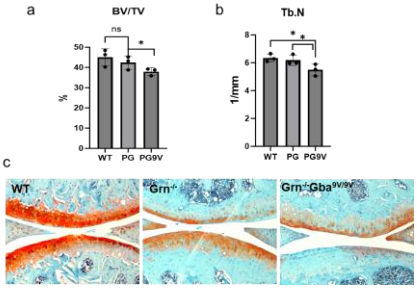

Figure 3. PGRN and Gba double mutant further induced osteoporosis in aged 12m-old mouse. (a) and (b) Analysis of BV/TV and Tb. N of subchondral bone of WT, $Grn^{-/-}$ and $Grn^{-/-}Gba^{9V/9V}$ mice. **(b)** Immunohistochemical staining for Safranin O staining in knee joint section collected from 12-month-old WT, $Grn^{-/-}$ and $Grn^{-/-}Gba^{9V/9V}$ mice.

DISCUSSION: 12-month-old PGRN and Gba double mutant mice developed severe behavioral deficits, in stark contrast to the negligible effects observed in $Grn^{-/-}$ and WT mice. Upon microCT data analysis, it became evident that PGRN deficiency alone resulted in osteopenia when compared to WT mice. Remarkably, the absence of PGRN in Gba mutant Gaucher disease mice was associated with even more pronounced cartilage degradation when compared to $Grn^{-/-}$ mice.

SIGNIFICANCE/CLINICAL RELEVANCE: This study unveils the intricate interaction between PGRN deficiency and Gba mutation, governing musculoskeletal disorders, notably osteopenia, in Gaucher disease. These findings suggest that PGRN might emerge as a novel target for addressing musculoskeletal conditions linked to Gaucher disease.

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