Progranulin deficiency causes exacerbated osteopenia in Gaucher disease mice

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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, leading 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 Gba1+/D409V/D409V mice, a widely used GD mouse model, through crossing Gm1−/− with Gba1+/D409V/D409V mice, and generated Grn−/−Gba1+/D409V (PG9V) (Fig. 1a) mice and found that the 12-month-old Grn−/−Gba1+/D409V (PG9V) mice developed severe 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, Grm−/− and Grm−/−Gba1+/D409V 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-Analyzer (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.

RESULTS: Grm−/−Gba1+/D409V mice at 12-month-old showed severe behavioral deficits, such as abnormal hindlimb clasping, which was not observed in the age-matched Grm−/− 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 (Gba1+/D409V) mice did not further decrease the bone mass in femur and tibia in comparison with PGRN deficient mice (Grm−/−) (Fig. 2). Intriguingly, in the knee joint, compared with Grm−/− mice, the Grm−/−Gba1+/D409V 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 Gba1 double mutant led to severer cartilage degradation in comparison to Grm−/− mice (Fig. 3c).

DISCUSSION: 12-month-old PGRN and Gba1 double mutant mice developed severe behavioral deficits, in stark contrast to the negligible effects observed in Grm−/− 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 Gba1 mutant Gaucher disease mice was associated with even more pronounced cartilage degradation when compared to Grm−/− 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.

REFERENCES:

Figure 1. PGRN and Gba double mutant mice showed behavioral defects. (a) Schematic of the mouse breeding strategy to generate Grn−/−Gba1+/D409V mice through crossing Grm−/− mice with Gba1+/D409V mice. (b) 12-month-old Grn−/−Gba1+/D409V mice showed hind limb clasping in comparison with age-matched WT, and Grm−/− mice displayed no clapping.

Figure 2. Progranulin deficiency led to bone loss in 12-month-old mouse. (a) Representative reconstructed 3D micro-CT images of femur trabecular bone of WT, Grm−/− and Grm−/−Gba1+/D409V 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, Grm−/− and Grm−/−Gba1+/D409V 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 12-month-old mouse. (a) and (b) Analysis of BV/TV and Tb. N of subchondral bone of WT, Grm−/− and Grm−/−Gba1+/D409V mice. (b) Immunohistochemical staining for Safranin O staining in knee joint section collected from 12-month-old WT, Grm−/− and Grm−/−Gba1+/D409V mice.