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研究題目：PQBP1, an intellectual disability causative gene, affects bone development and growth

目　的：

Polyglutamine tract-binding protein 1（PQBP1）is a nuclear protein involved in transcriptional regulation and has been directly linked to neurodegenerative diseases accompanying dementia and brain atrophy. Clinically, *Pqbp1* mutation is involved in X-linked mental retardation including Golabi-Ito-Hall, Sutherland-Haan, and Renpenning syndromes. It was previously demonstrated that nestin-Cre conditional *Pqbp1*-knockout mice（hereafter referred to as *Pqbp1*-cKOs）exhibited microcephaly, which can mimic the primary microcephaly-like phenotype of patients with intellectual disability. This *Pqbp1*-dependent brain size reduction resulted from an abnormally elongated cell cycle time and decreased total number of cell division times in neural stem cells（NSCs）.

The close relationship between the neuron system and skeletal development has recently been demonstrated. Intriguingly, neurodegenerative disorders are closely related to bone diseases. It has been reported that conditional knockout of Sema3A in osteoblasts results in normal bone mass. In contrast, a lack of Sema3A in neurons resulted in reduced bone mass. These results demonstrate that the expression of neuron-specific Sema3a through the modulation of sensory nerve projections into bone is indispensable for bone formation. However, it remains unclear whether the neurodegenerative disease-related protein PQBP1 plays a role in bone homeostasis. In this study, we aimed to evaluate the physiological contribution of PQBP1 to skeletal homeostasis using *Pqbp1*-cKOs.

対象および方法：

To generate the *Pqbp1*-cKOs, *Pqbp1*-floxed heterozygous female mice were crossed with nestin-Cre transgenic male mice. XY, $X^{Flox}Y$（XFY）, and nes-Cre XY mice（n＝4, respectively）were used as control animals, and *Pqbp1*-cKOs（n＝4）were used as experimental animals.

Longitudinal micro-CT analysis of femurs was performed on four- to ten-week-old mice every two weeks to evaluate the changes in trabecular bone microstructure. To assess the dynamic histomorphometric parameters, double calcein labeling was performed at four days and one day prior to euthanasia. Cephalograms of ten-week-old mice were obtained after sacrifice. For femur length measurements, radiological analysis of the right femur was performed using an inspeXio SMX-100CT（Shimazu, Kyoto, Japan）. Three-dimensional

image analysis software（TRI/3D-BON；Ratoc System Engineering, Tokyo, Japan）was used to reconstruct the CT images and to perform quantitative analysis. Left tibiae were collected for toluidine blue, TRAP and Safranin-O staining. Total mRNA was extracted from right tibial bone marrow. Quantitative analysis of *Alpl, Sp7, Col1a1, Bglap, Sox9, Col2a1, Nfatc1, Tnfsf11, and Tnfrsf11b* mRNA expression was performed. Gapdh was used as a housekeeping gene to normalize the values. The expression values were calculated using the comparative 2-ΔΔCT method.

Differences between the control mice and *Pqbp1*-cKOs were analyzed using one-way ANOVA followed by Tukey's test（$p<0.05$）. All data are presented as means ± SEM. Results with p values <0.05 were considered to be significant.

結　果：

The *Pqbp1*-cKOs exhibited short stature with a reduction in the length of the femur. The skulls of the *Pqbp1*-cKOs showed a significant reduction in total length, width, and height（Fig. 1A and B）. Moreover, the *Pqbp1*-cKOs showed a significant reduction in bone mineral density, bone volume fraction, trabecular numbers, and trabecular thickness, and a significant increase in trabecular separation（Fig. 1C and D）.

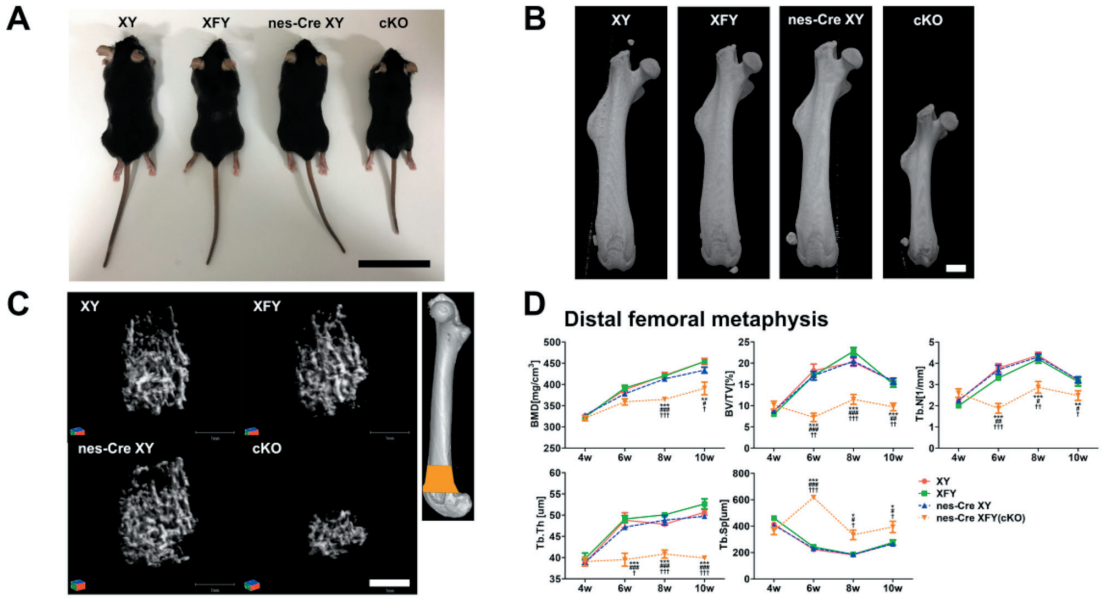

Figure 1.

Dynamic bone histomorphometric analysis showed that mineral apposition rate, bone formation rate per bone surface, and mineralizing surface per bone surface were significantly lower in the *Pqbp1*-cKOs than those in the control mice（Fig. 2A and B）. Quantitative RT-PCR analysis revealed a significant downregulation of *Alpl*（Alp）, *Sp7*（Osx）, *Col1a1* and *Bglap*（Ocn）in the *Pqbp1*-cKOs（Fig. 2C）.

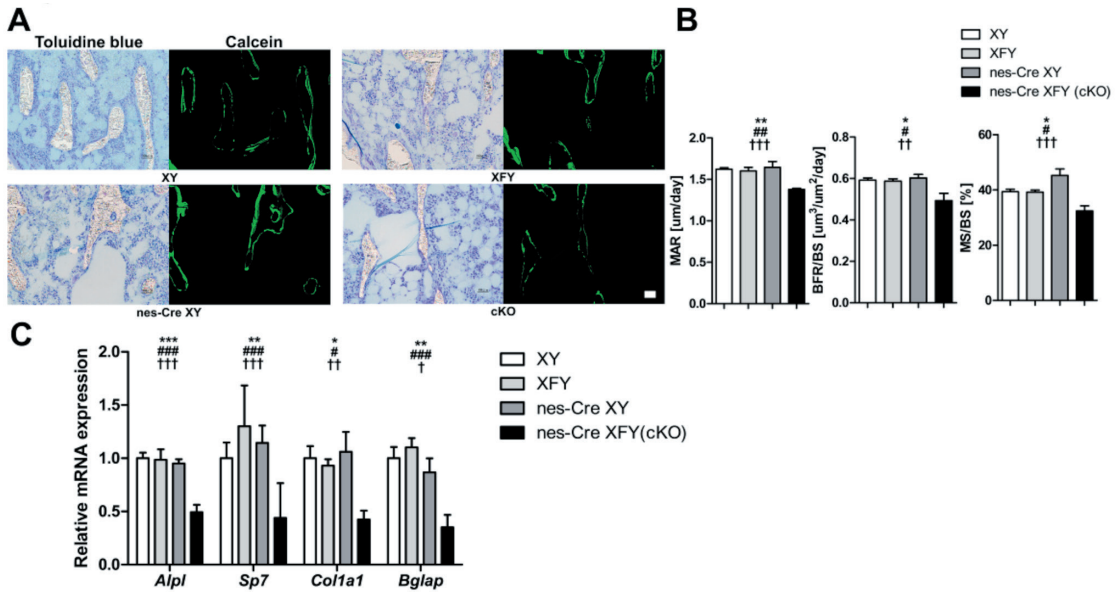

Figure 2.

Histological examination with Safranin-O staining revealed a shortened growth plate with thinner proliferative and hypertrophic zones in the *Pqbp1*-cKOs (Fig. 3A and B). Quantitative RT-PCR analysis showed that both *Sox9* and *Col2a1* were downregulated in the *Pqbp1*-cKOs (Fig. 3C).

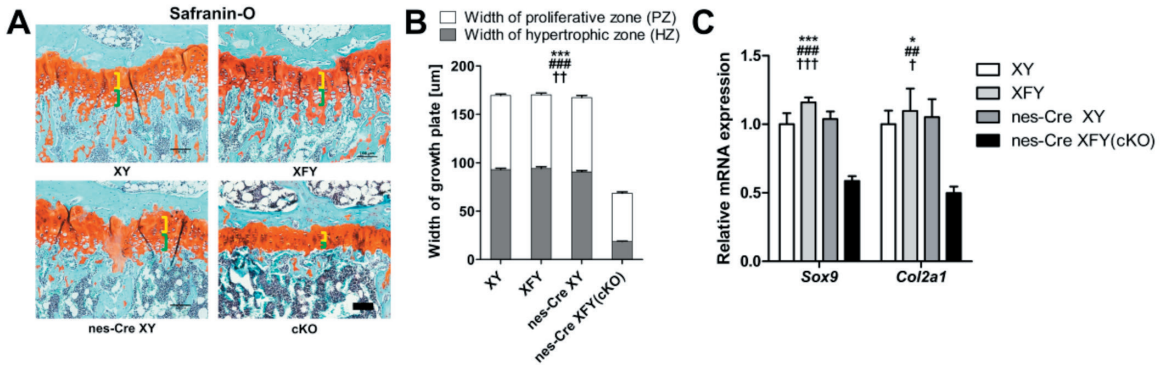

Figure 3.

Histomorphometric analysis showed that the eroded surface per bone surface was normal in the *Pqbp1*-cKOs, with no significant difference in the number of osteoclasts between the control mice and the *Pqbp1*-cKOs (Fig. 4A and B). RT-PCR showed no significant difference in mRNA expression of *Nfatc1*. The expression of *Tnfrsf11b* (Opg) was significantly downregulated in the *Pqbp1*-cKOs, whereas no difference was found in the expression of *Tnfsf11* (Rankl) (Fig. 4C and D).

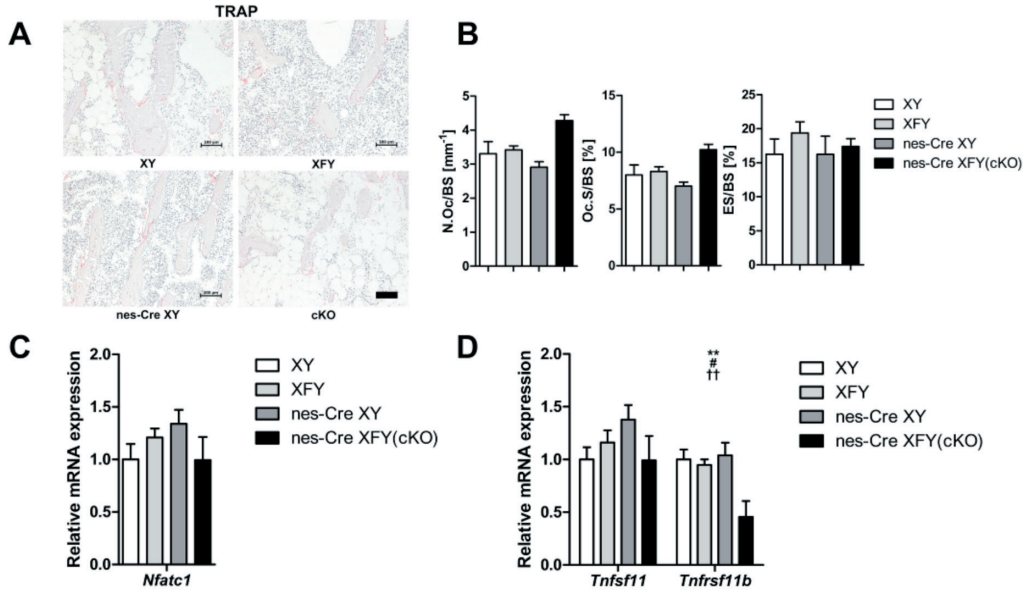

Figure 4.

考　察：

In this study, we found that the *Pqbp1*-cKOs exhibited marked dwarfism with shortened long bones compared with those of the control mice. This finding is consistent with the height measurements from clinical studies, which showed that adult Renpenning syndrome patients were consistently shorter than their unaffected brothers. Cephalometric analysis revealed that the *Pqbp1*-cKOs had brachycephaly and prognathism, features that are reminiscent of Renpenning syndrome symptoms. Furthermore, by analyzing micro-CT data, we found osteoporosis-like bone alterations, including reduced bone mass and changes in trabecular microarchitecture in the *Pqbp1*-cKOs. Quantitative analysis revealed a significant reduction in bone formation as well as chondrocyte deficiency, accompanied by downregulation of osteoblast- and chondrocyte-related gene expression in the *Pqbp1*-cKO mice. In contrast, according to the results of the histomorphometric analysis and RT-PCR, inactivation of *Pqbp1* did not influence osteoclast activation. Taken together, these results indicate that the expression of *Pqbp1* in nestin-expressing cells plays an important role in bone formation and cartilage development.

Mesenchymal stem cells（MSCs）are multipotent stem cells and have the capacity to differentiate into non-hematopoietic cells such as osteoblasts, adipocytes, and chondrocytes. Méndez-Ferrer et al. demonstrated that nestin-expressing bone marrow MSCs（BMMSCs）constitute an essential hematopoietic stem cell（HSC）niche component and promote HSC maintenance. Our results indicate that the bone formation activity of MSC-derived osteoblasts and chondrocytes were impaired in the *Pqbp1*-cKOs. Therefore, it is suggested that PQBP1 deficiency in nestin-expressing BMMSCs may results in impaired osteogenesis and chondrogenesis.

成果発表：

1. 楊新晟、石田宝義、中井雄太、藤田慶大、田川一彦、小野卓史、岡澤均. PQBP1, an intellectual disability causative gene, affects bone development and growth. 第78回日本矯正歯科学会学術大会 2019.11
2. Yang, S. S., Ishida, T., Fujita, K., Nakai, Y., Ono, T. & Okazawa, H.（2020）
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