Short Communication

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Leucine rich repeat phosphatase 1 (Phlpp1) and the pleckstrin homology (PH) domain inhibit parathyroid hormone receptor 1 (Pth1r) production and signaling during bone development.

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INTRODUCTION

The concentration of PTH(7–34) utilized in vivo was mentioned wrongly in the figure legends of the Weaver et al. (1) publication, according to a reader of the Journal of Bone and Mineral Research (JBMR®). The Supplemental Figures 2, 5, and 6 as well as the legends to Figures 3–5 contain the mistakes. Correct concentration is 100 µg/kg body weight per day, not 100 mg/kg/day, as stated in the Materials and Methods section. The authors regret these mistakes and have updated the figure legends below.

Figure 3 In Phlpp1/ mice, daily PTH(7–34) treatment reverses short limb length. Femur lengths (B,D) and tibia (A,C) in 4-week-old male WT or Phlpp1/U mice were measured daily doses of vehicle (0.1% BSA in PBS) or PTH(7–34) (100 µg/kg body weight/day). At 4 weeks of age, the tibia (E,G) and femur (F,H) lengths of female WT and Phlpp1// mice were measured after receiving the same treatments. 5 mm is the scale bar. Tukey's post-hoc test was used in conjunction with a two-way ANOVA to identify statistically significant differences.

Figure 4: Phlpp1 and Pth1r work together to control the development of growth plates. (A,B) 3D models of the proximal tibial growth plates were created using µCT scans of male 4-week-old WT or Phlpp1/} mice that received daily injections of either vehicle or PTH(7–34) at a dose of 100 µg/kg body weight. The 2D capture's scale bar is 1 mm. The color scale bar measures millimeters. For e, one sample 3D rendering is displayed ach group. (C) The proximal tibia growth plate's proliferative and hypertrophic zones were measured. (D) The proximal tibial growth plate was stained with safranin O/fast green. 50 µm is the scale bar. Either the proliferative zone (P) or the hypertrophic zone (H) is indicated by vertical black lines. (E) For Col10a1, in situ hybridization was carried out. 50 µm is the scale bar. (F,G) From postnatal day 1 (P1) to P5, WT or Phlpp1 / mice were injected daily with either vehicle or PTH(7–34), as previously mentioned. BrdU was given to P5 two hours before the procedure. (F) As shown in G, the percentage of BrdU-positive cells in the proximal tibial growth plate was measured. G scale bar: 50 µm. Tukey's post hoc test was used in conjunction with a two-way ANOVA to identify statistically significant differences.

Figure 5 PTH (7–34) administered daily has little effect on bone mass. (A) Two-dimensional reconstructions using µCT of the distal femur in 4-week-old male WT or Phlpp1/} mice that received daily injections of either vehicle or PTH(7–34) at a dose of 100 µg/kg body weight. One millimeter is the scale bar. (B) Bone volume/tissue volume, (C) trabecular number, and (D) bone mineral density were the trabecular parameters examined by µCT. Tissue mineral density (E), cortical thickness (F), total tissue cross-sectional area (G), and cortical area fraction (H) were among the cortical characteristics. Using a two-way ANOVA and either the Student's test or Tukey's post hoc test, as specified, statistically significant differences were identified.Additional Figure S2 Growth plate images at low magnification. five times magnification photos of (A) the growth plates of male 4-week-old WT or Phlpp1/U mice following Pth1r detection using in situ hybridization (ISH) or immunohistochemistry (IHC). 100 µm is the scale bar. (B,C) WT or Phlpp1// mice, four weeks old, received daily injections of either vehicle or PTH(7–34) at a dose of 100 µg/kg body weight. Safranin O/Fast Green staining was applied to the proximal tibial growth plate in (B), and in (C), ISH was used

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to probe for Col10a1. (D) From postnatal day 1 (P1) through P5, WT or Phlpp1 /} mice were injected with either vehicle or PTH(7–34) daily, as previously mentioned. Two hours before death on P5, BrdU labeling reagent was administered, and BrdU-positive cells were detected.Additional Figure S5 When compared to WT or Phlpp1// mice, the effect of daily PTH(7–34) treatment on restoring limb length in Phlpp1 heterozygotes is moderate. Four-week-old male and female WT (Phlpp1+/+), HET (Phlpp1+/~), or KO (Phlpp1~ /~ mice received daily injections of PTH(7–34) (100 μg/kg body weight/day) or vehicle (0.1% BSA in PBS) to measure (A, E) femur length, (B, F) tibia length, (C, G) body length, and (D, H) body weight. Utilizing Tukey's post hoc test in conjunction with a two-way ANOVA, statistically significant differences were identified.

Additional Figure S6 In Phlpp1 / mice, daily PTH(7–34) treatment reverses low body weight and short body length. (A,C) For 4-week-old male and female WT or Phlpp1/U mice, tail-to-snout body length and (B,D) body weight were measured after daily injections of PTH(7–34) (100 μg/kg body weight/day) or vehicle (0.1% BSA in PBS). Statistically significant differences were identified using Tukey's post hoc analysis in conjunction with a two-way ANOVA.

REFERENCE

1. Weaver S, Taylor E, Zars E, Arnold K, Bradley E, Westendorf J. Pleckstrin homology (PH) domain and leucine rich repeat phosphatase 1 (Phlpp1) suppresses parathyroid hormone receptor 1 (Pth1r) expression and signaling during bone growth. J Bone Miner Res. 2021;36(5):986-989. https://doi.org/10.1002/jbmr.4248.