Changes in the phosphorylation of guanylyl cyclase-B (GC-B) regulates bone growth in a mouse model

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Abstract

Guanylyl cyclase-B (GC-B), also known as natriuretic peptide receptor 2 (Npr2), is a membrane bound receptor that converts GTP to the second messenger cGMP in response to the extracellular binding of its ligand, C-type natriuretic peptide (CNP). To transmit the extracellular binding signal to stimulate maximum GTP catalysis, GC-B must be phosphorylated on multiple serine and threonine residues, and dephosphorylation inactivates GC-B. In fact, GC-B dephosphorylation is a regulatory element in the resumption of meiosis in ovarian follicles. In this study, we investigated the physiologic effects of a constitutively phosphorylated form of GC-B using a knockin mouse expressing a mutant form of GC-B where all known and putative phosphorylation sites were mutated to the phospho-mimetic glutamate (GC-B$^{7E/7E}$). In this way, we can study the role of GC-B dephosphorylation in regulating chondrogenesis in a mouse model. We observed that the GC-B$^{7E/7E}$ mice have longer naso-anal, femoral, and tibial length at 4, 8, and 16 weeks compared to GC-B$^{WT/WT}$ mice. At 4 weeks, GC-B$^{7E/7E}$ mice were 7% longer than GC-B$^{WT/WT}$ littermates, at all indices. By 8 weeks, GC-B$^{7E/7E}$ mice were 12% longer than GC-B$^{WT/WT}$ mice, and this effect was sustained at 16 weeks. GC-B$^{7E/7E}$ mice also had longer CA5 vertebrae and tails, which grow by a mix of endochondral and membranous ossification, compared to GC-B$^{WT/WT}$ mice. Importantly, there were no differences in cranial width between GC-B$^{WT/WT}$ and GC-B$^{7E/7E}$ mice, consistent with GC-B only being involved in endochondral and not membranous ossification. Together, these data indicate that changes in GC-B phosphorylation regulate endochondral bone growth in mice. By blocking or reducing GC-B dephosphorylation, it may be possible to develop novel therapeutic strategies for disproportionate chondrodysplasias.

Results

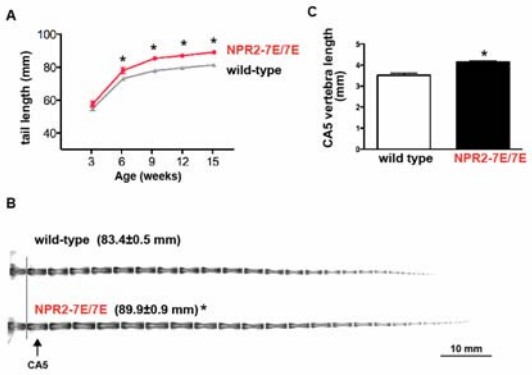

Figure 1. **Increased bone growth in tails of NPR2$^{7E/7E}$ mice.** (A) Tail lengths measured from live NPR2$^{7E/7E}$ and wild-type mice (mean ± s.e.m). There were approximately half females and half males in each group. The results were obtained from 17 NPR2$^{7E/7E}$ and 19 wild-type mice from 7 different litters (6 with heterozygous parents, 1 with wild-type parents). Data were analyzed by two-way ANOVA with Bonferroni correction for multiple comparisons. (B) X-ray images of representative tails from 18-week old NPR2$^{7E/7E}$ and wild-type mice, imaged after euthanizing. The vertical line marks the start of the tail at caudal vertebra number 5 (CA5). Numbers indicate the length of the tails measured from the x-rays (start of CA5 to tip of tail), for 10 NPR2$^{7E/7E}$ and 13 wild-type mice from the set of animals used for (A) (mean ± s.e.m.). Data were analyzed by t-test. (C) CA5 vertebra lengths (mean ± s.e.m.) measured from x-ray images as shown in (B). Asterisks indicate values that are significantly different from the wild-type control (P<0.05).

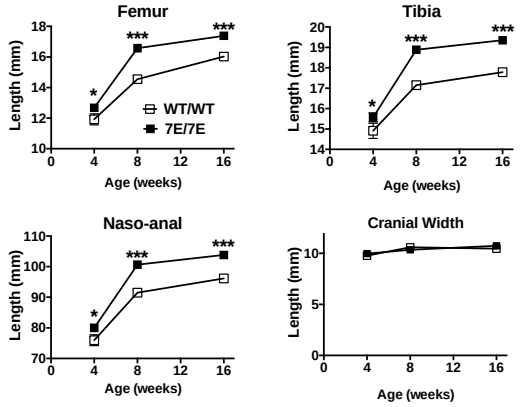

Figure 2. GC-B$^{7E/7E}$ mice have more endochondral ossification than their GC-B$^{WT/WT}$ littermates with no change in membranous ossification. From 4 weeks of age, there is a significant difference between the femoral, tibial, and naso-anal lengths between GC-B$^{WT/WT}$ and GC-B$^{7E/7E}$ mice that is sustained at 16 weeks. However, there is no difference in cranial width between GC-B$^{WT/WT}$ and GC-B$^{7E/7E}$ mice at any age. While femoral, tibial, and to a large extent naso-anal length is attributed to endochondral ossification, cranial width is an indicator of membranous ossification. These data indicate that the GC-B$^{7E/7E}$ mice have greater endochondral ossification than their GC-B$^{WT/WT}$ littermates with no difference in membranous ossification. * and *** indicate p< 0.05 and 0.001, respectively. n= 12-31, depending on genotype and age.

Conclusions

Using this mouse model, where DNA encoding GC-B-7E/7E was inserted into the endogenous gene locus to ensure physiologic GC-B expression levels, we studied the effects of GC-B dephosphorylation on longitudinal bone growth for the first time. Previous studies demonstrated that the GC-B-7E enzyme is activated similarly to the wild type enzyme but is resistant to dephosphorylation-dependent inactivation (1). Furthermore, GC-B$^{7E/7E}$ mice are resistant to luteinizing hormone-dependent inactivation in ovarian follicles (2), a process associated with GC-B dephosphorylation and show a five hour delay in the resumption of meiosis of the oocyte (3). Importantly for this application, preliminary data from our lab has shown that the GC-B$^{7E/7E}$ mice have greater tail, vertebral, naso-anal, femoral, and tibial length than their GC-B$^{WT/WT}$ littermates, all of which involve endochondral ossification. However, there is no difference in cranial width, which occurs solely through membranous ossification.

These data demonstrate the importance of GC-B in regulating endochondral ossification and highlight the role of phosphorylation in regulating GC-B *in vivo*. Studies are ongoing to characterize structural and biomechanical differences between GC-B$^{WT/WT}$ and GC-B$^{7E/7E}$ mice to determine if the bones from the GC-B$^{7E/7E}$ mice, while longer, have less bone mass and are more fragile.

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References

1. Yoder AR, Robinson JW, et al. PloS one. 2012;7(5):e36747. PMID: 22590601
2. Shuhaibar LC, et al. Dev Biol. 2016;409(1):194-201. PMID: 26522847.
3. Egbert JR, et al. Development. 2014;141(18):3594-604. PMID: 25183874