THE BONE MORPHOGENETIC PROTEIN RECEPTOR TYPE 1B IS REQUIRED FOR THE DEVELOPMENT OF DIGITS AND THE MAINTENANCE OF ARTICULAR CARTILAGE

Yi, S. E., Daluiski, A., Zhao, M., **Rosen, V., and *+Lyons, K. M.,, *+ Department of Orthopaedic Surgery, UCLA School of Medicine, Los Angeles, CA 90095: (310) 825-5480, FAX (310) 206-8867, klyons@ortho.medsch.ucla.edu, **Genetics Institute, Cambridge MA 01240

Introduction: Members of the bone morphogenetic protein (BMP) family of Transforming Growth Factor ß-related molecules play an essential role in the development of skeletal tissues. At least 15 different BMP family members have been described to date, and many of these have been shown to possess osteoinductive potential. However, the precise role of individual BMP family members in skeletal development is not yet known. BMPs transduce their signals through a receptor complex consisting of type I and type II receptors. Two type I receptors with high affinity for BMP family members have been identified. One of these, BMPRIA, has previously been shown to be essential for early embryonic development. The early embryonic lethality precluded an analysis of the role of this receptor in skeletal development. Previous studies have shown that the other type I receptor, BMPRIB, is expressed in precartilaginous mesenchyme, raising the possibility that this receptor is essential for aspects of skeletal devleopment. In order to test this hypothesis, we have generated mice lacking BMPRIB using gene targeting.

Methods: *Gene targeting*: A targeting vector was constructed in which the first exon of the BMPRIB gene was replaced with a neomycin cassette. ES cells were electroporated, selected in neomycin, and screened by Southern blot analysis. Targeted cells were injected into blastocysts, and the resulting chimeras were bred to test for germline transmission. *Histological analysis*: Embryos and adult tissues were fixed, embedded in Paraplast, sectioned, and stained with hematoxylin and eosin. Thin plastic sections stained with Goldner's trichrome stain were prepared from adult femurs *Morphological analysis*: Cleared skeletal preparations were prepared from newborns. Adult skeletons were examined by autoradiography using a Faxitron. *In situ hybridization*: nonradioactive *in situ* hybridization was performed on frozen sections, whole embryos, or whole limbs using digoxigenin-labeled riboprobes. Probes included sox-9 (a marker for prechondrogenic mesenchyme), gdf5 (a marker for the presumptive joint region), BMPRIB, and PGCP (proteoglycan core protein, a marker for mature cartilage).

Results and Discussion: Mice lacking BMPRIB are viable as adults. However, mutant mice display brachydactyly due to fusion and severe reduction in length of the first and second phalanges (fig 1). This phenotype shows striking similarities to that of the *bp/bp* (brachypodism) mouse, which lacks the BMP family member GDF5 (1), raising the possibility that GDF5 signaling through the BMPRIB receptor is required for the development of digits (fig 1). To explore this possibility in greater detail, we examined whether other aspects of the *bp/bp* phenotype (2) would be found in BMPRIB-/- mice. *bp/bp* mice exhibit defects in joint formation, leading to severe reduction of the first and second phalangeal elements, and joint laxity (2). We have observed that both *bp/bp* and BMPRIB-/- mice exhibit extreme joint laxity leading to frequent dislocations. X-ray analysis of *bp/bp* and BMPRIB-/- mice reveals defects in the shapes of the epiphyses of the long bones (fig 2), suggesting that both GDF5 and BMPRIB are essential for the formation of joints. However, *bp/bp* and BMPRIB-/- mice do exhibit several important phenotypic differences. For example, BMPRIB-/- mice never display polydactyly, whereas this is common in *bp/bp* mice. Moreover, uterine prolapse occurs occasionally in BMPRIB-/- females, but has not been reported in *bp/bp* mice. As uterine prolapse is frequently caused by the degeneration of small ligaments, this phenotype raises the possibility that BMPRIB is required for the maintenance of tendon and ligament. In addition, young (less than one year old) BMPRIB-/- mice generally display severe osteoarthritis. The development of osteoarthritis may be a secondary consequence of joint laxity, or alternatively may be the result of a primary defect in the maintenance of articular cartilage.

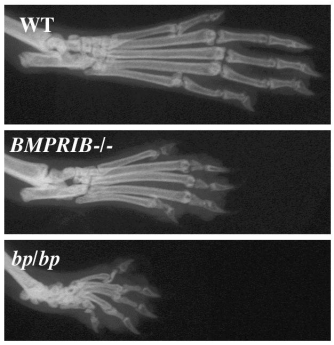
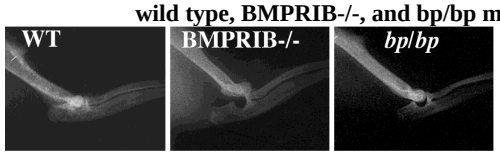


Figure 1: X-rays of forelimb digits from adult wild type, BMPRIB-/-, and bp/bp mice

In order to examine the consequences of loss of BMPRIB to skeletal development on a cellular level, we conducted an analysis of limb development by *in situ* hybridization using molecular markers. The limbs of wild type and BMPRIB-/- mice are indistinguishable until E13-E14. Precartilaginous mesenchyme is present in the limbs of wild type and mutant embryos, demonstrating that loss of BMPRIB does not affect the ability of precartilaginous mesenchyme of the digits to be specified. However, at E13-14, mutants lack a signal for GDF5 in the developing joint separating the first and second phalange. Moreover, the pattern of expression of GFD5 is broadened in this region of the developing digits, raising the possibility that the precartilaginous mesenchyme of the digits in mutants is unable to undergo further development that would normally lead to the development of the joint space.

In summary, the comparative phenotypic analysis of *bp/bp* and BMPRIB-/- mice provides strong evidence that GDF5 signaling through BMPRIB is essential for the formation of digits and joints. However, the observation of important phenotypic differences demonstrates that GDF5 does not signal exclusively through BMPRIB, and that the activation of BMPRIB by ligands other than GDF5 is essential for skeletal development.

Figure 2: Articulation of the humerus with the radius and ulna in wild type, BMPRIB-/-, and bp/bp mice.



References:
1. Storm et al., *Nature* 368:639-642, 1994
2. Grünberg, H. and Lee, A.J., *J. Embryol exp Morph* 30: 119-141

Acknowledgments: supported by NIH grant AR44528

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☒ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.