Decreased BMD and Limb Deformities in Mice Carrying Mutations in Both Lrp5 and Lrp6

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ABSTRACT: Humans and mice lacking Lrp5 have low BMD. To evaluate whether Lrp5 and Lrp6 interact genetically to control bone or skeletal development, we created mice carrying mutations in both Lrp5 and the related gene Lrp6. We found that compound mutants had dose-dependent deficits in BMD and limb formation, suggesting functional redundancy between these two genes in bone and limb development.

Introduction: Lrp5 and Lrp6 are closely related members of the low density lipoprotein receptor family and are co-receptors for Wnt ligands. While Lrp5 mutations are associated with low BMD in humans and mice, the role of Lrp6 in bone formation has not been analyzed.

Materials and Methods: To address whether Lrp5 and Lrp6 play complimentary roles in bone and skeletal development, we created mice with mutations in both genes. We inspected limbs of mice from the different genotypic classes of compound mutants to identify abnormalities. DXA and μCT were used to evaluate the effect of mutations in Lrp5 and Lrp6 on BMD and microarchitecture.

Results: Mice heterozygous for mutations in Lrp6 and either heterozygous or homozygous for a mutation in Lrp5 (Lrp6+/−;Lrp5+/− or Lrp6+/−;Lrp5−/−) display limb defects with incomplete penetrance and variable expression. DXA analysis showed that BMD decreased as mice progressively were more deficient in Lrp5 and Lrp6. Lrp6+/−;Lrp5−/− mice were more severely affected than Lrp6+/−;Lrp5−/− mice, whereas Lrp6+/−;Lrp5+/− mice had statistically higher BMD than Lrp6+/−;Lrp5−/− mice and lower BMD compared with wildtype mice and mice heterozygous for either mutation alone.

Conclusions: Lrp6 and Lrp5 genetically interact in limb development in mice. Furthermore, heterozygosity for an inactivating mutation in Lrp6 further reduces BMD in both male and female mice lacking Lrp5.

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Key words: low-density lipoprotein receptor-related proteins 5 and 6, Lrp5, Lrp6, osteoporosis, bone densitometry, knockout mice, Wnt, limb development

INTRODUCTION

Lrp5 and Lrp6 form a distinct subfamily of low-density lipoprotein receptor (LDLR)-like proteins.1–4 They are co-receptors for Wnt ligands, which are a large family of secreted glycoproteins that initiate signaling by binding to members of the Frizzled (Fz) family of seven transmembrane receptors.5 Loss-of-function mutations in mice have shown the importance of Wnt molecules in the development of numerous tissues and organs.6 Lrp6-deficient mice display phenotypes similar to, but not as severe as, those seen in several Wnt gene knockouts, and die between embryonic day 14.5 and birth.7 The Drosophila homolog of Lrp5 and Lrp6, arrow, is required for Wnt signaling in the fly, and loss of arrow phenocopies loss of wingless (wg).8

Mice homozygous for an allele of Lrp5 encoding a truncated version of the protein recapitulate features of the autosomal recessive human disorder osteoporosis-pseudoglioma syndrome (OPPG).9,10 Patients with OPPG have both a markedly decreased BMD and persistence of the embryonic hyaloid vascular system.11–15 Mutations that inactivate the LRP5 gene cause OPPG.16 Further confirming the importance of LRP5 in accruing normal bone mass, families with an autosomal dominant syndrome characterized by extremely high BMD have gain-of-function point mutations in LRP5.17–20 In addition, mice engineered to express a point mutant of Lrp5 associated with high bone mass in humans also develop high bone mass.21

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To better understand the physiological roles of Lrp5 and Lrp6, we crossed mice carrying inactivated alleles of Lrp5 and Lrp6 and characterized the phenotypes of mice carrying compound mutations. Mice deficient for Lrp6 die at birth, so mice of this genotype were not present in the offspring identified at weaning. Mice homozygous for germ-line inactivating mutations in both Lrp6 and Lrp5 die early in embryogenesis with associated defects in gastrulation. In this report, we have focused on an analysis of bone and skeletal phenotypes in mice that survive to adulthood. The most severely affected genotypic class of these mice are those heterozygous for the Lrp6 mutation and homozygous for the mutation in Lrp5 (Lrp6<sup>−/−</sup>:Lrp5<sup>−/−</sup>[6H5M]). They are highly susceptible to the development of limb abnormalities and exhibit reduced BMD compared with mice deficient for Lrp5 alone (Lrp6<sup>−/−</sup>:Lrp5<sup>−/−</sup>[6W5M]). In addition, mice heterozygous for both Lrp6 and Lrp5 mutations (Lrp6<sup>−/+</sup>:Lrp5<sup>−/−</sup>[6H5H]) are also predisposed to these phenotypes. Furthermore, μCT analysis revealed that heterozygosity for mutations in either Lrp6 or Lrp5 leads to changes in trabecular bone. This work documents overlapping roles for Lrp6 and Lrp5 in the formation of the limbs and in the control of bone mass accrual.

**MATERIALS AND METHODS**

*Generation of Lrp5-deficient mice*

A targeting vector (Fig. 1A) designed to replace part of the first coding exon of Lrp5 with a selectable marker was used to create a strain of mice carrying an inactivated allele of Lrp5 (Lrp5<sup>Δ5αmεo</sup>) at Lexicon Genetics. The targeting vector was designed to place the β-galactosidase gene under the control of the Lrp5 promoter, but we were not able to observe β-galactosidase expression, consistent with the observations of others (O Kelly, K Pinson, and W Skarnes, personal communication, 2004). Lrp6-deficient mice (a gift of W Skarnes) have been described. The mice in this report are maintained on a mixed genetic background of 50% C57Bl/6J, 25% 129/Sv, and 25% FVB/N. All experiments performed were done so in compliance with the report are maintained on a mixed genetic background of 50% C57Bl/6J, 25% 129/Sv, and 25% FVB/N. All experiments performed were done so in compliance with the Animal Welfare Act of 1970, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Manual of the National Institutes of Health. All procedures were approved before use by the Institutional Animal Care and Use Committee of the Van Andel Research Institute.

*Genotypic analysis of mutant mice*

DNA was prepared from tail biopsy specimens using an AutoGenprep 960 automated DNA isolation system. PCR of the Lrp5 alleles was carried out in 25 μl total volume containing 2.5 μl 10× PCR buffer (Invitrogen), 2.0 μl DMSO, 1.0 μl 50 mM MgCl₂, 0.625 mM of each nucleotide, 1 U of Taq polymerase (Invitrogen), 6.25 μg/ml of primers Lrp5 F and Neo F1, and 12.5 μl of primer Lrp5 3′-targeted (common primer). The following primers sequences were used: Lrp5 3′-targeted 5′-CCGCTGTCATTCAGGCGCTTG-3′; Lrp5 5′-primer 5′-CTCTTCAAGCCGTTGAGC-3′ (to detect a 430-bp fragment of the wildtype allele) and Neo F1 5′-ACAACAGACTCATCATTGAGC-3′ (to detect a 1000-bp fragment of the mutant allele). Samples were amplified for 34 cycles (94°C for 1 minute, 57.8°C for 1 minute, and 72°C for 1 minute). PCR of the wildtype Lrp6 allele was carried out as above except with no DMSO. The following wildtype primers were used: Lrp6 7757-S primer 5′-AGGTTGTTATGTGTGAGAAGAGGCA-3′ and Lrp6 8085-AS primer 5′-TCCAACTACAAGGCCCTGCACT-3′ (to detect a 325-bp fragment). Samples were amplified for 30 cycles (94°C for 45 s, 56.5°C for 45 s, and 72°C for 1 minute). PCR of the mutant Lrp6 allele was carried out as above except with no DMSO and no MspI. The following mutant primers were used: Lrp6 7757-S primer (see above) and pGT1.8TM-1388 AS 5′-CGGTTATGTCTTTTCTCC -3′ (to detect a 586-bp fragment). Samples were amplified for 30 cycles (94°C for 45 s, 56°C for 45 s, and 72°C for 1 minute). PCR products were visualized by ethidium bromide staining in 1.0% agarose.
rose gels. To ensure that the genotyping was specific for each allele, all initial PCR products were confirmed by sequencing.

**RT-PCR**

Total RNA was isolated from tissues using TRIzol Reagent (Invitrogen). Five-microliter RT reactions were carried out at 42°C for 60 minutes using 0.5 μg total RNA, 2.65 μl DEPC-treated H₂O, 0.5 μl 100 ng/μl anti-sense primer, and 1.6 μl A-mix (160 mM Tris pH 8.3, 128 mM KCl, 25.6 mM MgCl₂, 0.5 μg bovine serum albumin [BSA], 40 mM DTT, 20 U Rnasin, 1.6 mM dNTP, and 1 U AMV). PCR was performed in a final volume of 25 μl: 19.5 μl Taq mix (51.3 mM KCl, 10.25 mM Tris, pH 9.0, 1.54 mM MgCl₂, 0.256 mM dNTP, and 2.5 U Taq polymerase), and 0.5 μl 100 ng/μl sense primer was added to the previous 5-μl RT reaction. The standard cycling conditions used were 94°C for 1 minute, Tm (see below) for 30 s, and 72°C for 45 s. Reaction products were visualized by ethidium bromide staining in 2.0% agarose gels. The sequences of the mouse Lrp5 and β-actin primers used in this study are as follows: MuLrp5 174 S'-TGACTGACCTTGCCCATCC-3'; MuLrp5 697 AS'-CCAGTAAATGCGAGGATCTAC-AATG-3' (product size 524 bp, Tm 58°C, 35 cycles), and β-actin S'-primer 5'-GTCGTACACAGGCAATTGTGGA-TGG-3'; β-actin 3'-primer 5'-GCAATGCCTGGGTAC-ATGTTGG-3' (product size 493 bp, Tm 60°C, 20 cycles). The primer sets were validated by sequencing of the PCR products.

**Immunohistochemistry**

Tissue samples were fixed in formalin overnight and dehydrated through a graded alcohol series in a Ventana Renaissance processor (Ventana Medical Systems). Tissues were paraffin embedded, and 5-μm sections were adhered to glass slides. Lrp5 staining was done as previously described,(23) except that biotinylated anti-rabbit secondary antibodies were added to the sections for 40 minutes at room temperature at a dilution of 1:200 in PBS. The signal was detected with ethidium bromide staining in 2.0% agarose gels. The primer sets were validated by sequencing of the PCR products.

**Skeletal preparations**

Mice were killed, skinned, and eviscerated before fixation in 95% ethanol. Skeletons were stained in Alcian blue (30 mg Alcian blue 8GX in 80 ml 95% ethanol and 20 ml glacial acetic acid) for 48 h. Samples were rinsed twice in 95% ethanol and incubated in 95% ethanol overnight. Alizarin red staining (5 mg Alizarin red in 100 ml 1% KOH) was done overnight. The samples were transferred to 0.5% KOH overnight and moved through graded 0.5% KOH: glycerol (vol/vol) until the skeletons cleared (67:33, 50:50, 33:67, 100% glycerol plus 0.02% sodium azide for storage). Skulls used for histological examination were fixed in formalin, decalcified, and embedded in paraffin as described above.

**BMD**

Mice were anesthetized through inhalation of 2% isoflurane (TW Medical Veterinary Supply) with oxygen (1.0 liter/minute) for 10 minutes before imaging and during the procedure (≈5 minutes). The mice were placed on a specimen tray in a PIXImus II bone densitometer (GE Lunar) for analysis. BMD was calculated by the PIXImus software based on the active bone area in the subcranial region within the total body image and specifically in the femur, humerus, and spine.

**μCT**

Trabecular and cortical bone architecture were assessed at the distal femoral metaphysis and femoral mid-shaft, respectively, using a desktop microtomographic imaging system (μCT40; Scanco Medical AG, Basserdorf, Switzerland). Scans were acquired using a 12-μm isotropic voxel size, with 150 CT slices evaluated at the distal femur and 50 CT slices at the femoral mid-shaft. Morphometric parameters were computed without assumptions regarding the underlying structure. For trabecular bone, we calculated the volume fraction (BV/TV; %), trabecular number (Th.N; mm⁻¹), thickness (Tb. Th; μm), and separation (Tb.Sp; μm). For the femoral mid-shaft, we calculated the total cross-sectional area (TA; mm²), cortical bone area (BA; mm²), medullary area (MA; mm²), and average cortical thickness (Cort Th; μm).

**RESULTS**

The **Lrp5⁵exon¹ allele is a functional null**

Mice carrying an allele of Lrp5 (Lrp5⁵exon¹) lacking both the initiating ATG codon and the signal peptide were created through standard techniques (Fig. 1A). Lrp5⁵exon¹ heterozygotes were intercrossed, and mice homozygous for the mutation were born at the expected frequency. Lrp5⁵exon¹ homozygotes were viable and fertile and appeared similar to, although slightly smaller than, their control littermates (data not shown). To verify that the Lrp5⁵exon¹ allele results in the loss of Lrp5 expression, we analyzed RNA and protein samples from Lrp5⁵exon¹ homozygotes. As expected, RT-PCR analysis indicated that Lrp5⁵exon¹ homozygotes lacked mRNA sequences corresponding to the first coding exon of Lrp5 (Fig. 1B). To confirm that a functional protein could not be produced from this allele, we performed immunohistochemistry on tissue sections from these mice (Fig. 1C). As previously shown,(24) Kupffer cells in the liver exhibited strong positive staining for Lrp5 in wildtype mice. In contrast, livers from Lrp5⁵exon¹ homozy-
gotes (Lrp5\(^{-/-}\)) had no immunoreactivity for Lrp5. The antibody used was prepared against a 253 amino acid portion of the extracellular domain of mouse Lrp5 that corresponds to amino acids 281–533.(23) This lack of antibody reactivity is consistent with the Lrp5\(^{ exon 1}\) allele being a functional null (Lrp5\(^{-/-}\)).

Mice homozygous for other mutant alleles of Lrp5 have decreased BMD.(9,10) Consistent with these reports,(9,10) we found that our Lrp5\(^{-/-}\) mice exhibited decreased BMD compared with both wildtype and heterozygous littermates. Total body DXA analysis using a PIXIImus II densitometer (GE Lunar, Madison, WI, USA)\(^{25,26}\) suggested that Lrp5\(^{-/-}\) mice did not have statistically lower BMD compared with their wildtype littermates (Fig. 4); however, more sensitive \(\mu\)CT analysis revealed decreased trabecular bone volume fraction (BTV/TV) in Lrp5\(^{-/-}\) mice compared with wildtype littermates (Table 1).

To determine when differences in BMD first become apparent, we examined litters of mice born from matings of Lrp5 heterozygotes crossed to Lrp5-deficient homozygotes. Thus, in these litters, one-half the offspring were heterozygous and one-half were homozygous for the mutation. We began total body DXA analysis of these mice at 21 days of age and found that differences in BMD became significant at 29 days of age (\(p < 0.03\)) and persisted thereafter (Fig. 2A). Consistent with other reports,(9,10) we also found that Lrp5-deficient mice showed persistence of the hyaloid vasculature system (Figs. 2B–2E).

**Limb phenotypes of Lrp6/Lrp5 mutant mice**

Lrp6 heterozygous mice\(^{71}\) were crossed to the Lrp5\(^{-/-}\) mice to generate Lrp6/Lrp5 double heterozygous (6H5H) mice, which were intercrossed to obtain all possible genotypes. Mice of the genotypic classes Lrp6\(^{+/+}\)/Lrp5\(^{-/-}\) (6W5W), Lrp6\(^{+/-}\)/Lrp5\(^{-/-}\) (6H5W), Lrp6\(^{+/+}\)/Lrp5\(^{-/-}\) (6W5H), and Lrp6\(^{+/+}\)/Lrp5\(^{+/-}\) (6W5M) appeared normal. However, the Lrp6\(^{+/-}\)/Lrp5\(^{-/-}\) (6H5H) and Lrp6\(^{+/-}\)/Lrp5\(^{+/-}\) (6H5M) mice displayed limb deformities. We scored these defects based on visual inspection of intact limbs (Fig. 3). The limb phenotype was not fully penetrant; 35% of 6H5H mice and 72% of 6H5M mice were affected. Interestingly, the severity of the limb deformities followed a pattern along both the left-right and the anterior-posterior axes. Of the 6H5H animals analyzed (\(n = 121\)), 27% had affected right forelimbs, 1% had affected left forelimbs, and 7% had both forelimbs affected. None of the 6H5H animals showed hindlimb defects. Of the 6H5M animals analyzed (\(n = 23\)), 36% had all limbs affected, 27% had defects in both forelimbs, and 9% had forelimbs and the right hindlimb affected. Whereas right-sided defects could occur in the absence of a left-sided defect, the converse was not observed in either the 6H5H or 6H5M animals (Figs. 3A–3D).

To characterize the skeletal abnormalities in the Lrp6/ Lrp5 mutant mice, we prepared skeletal whole mounts of 6W5H, 6H5W, 6W5M, 6H5H, and 6H5M mice. All limbs

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**TABLE 1. TRABECULAR BONE PARAMETERS DETERMINED BY HIGH-RESOLUTION \(\mu\)CT ANALYSIS OF DISTAL FEMURS FROM 3-MONTH-OLD Lrp6/ Lrp5 WILDTYPE AND MUTANT FEMALE MICE**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>B.T/V (%)</th>
<th>Tb.N (n/mm)</th>
<th>Tb.Th ((\mu))</th>
<th>Tb.Sp ((\mu))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lrp6(^{+/-})/Lrp5(^{-/-})</td>
<td>2</td>
<td>7.2 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td>45.5 ± 4.3</td>
<td>266 ± 19</td>
</tr>
<tr>
<td>Lrp6(^{+/+})/Lrp5(^{-/-})</td>
<td>2</td>
<td>4.5 ± 0.2*</td>
<td>3.0 ± 0.3</td>
<td>41.3 ± 5.5</td>
<td>343 ± 37</td>
</tr>
<tr>
<td>Lrp6(^{+/-})/Lrp5(^{+/-})</td>
<td>3</td>
<td>5.6 ± 0.3*</td>
<td>3.7 ± 0.1</td>
<td>41.2 ± 0.7</td>
<td>274 ± 8</td>
</tr>
<tr>
<td>Lrp6(^{-/-})/Lrp5(^{-/-})</td>
<td>4</td>
<td>3.4 ± 0.1*</td>
<td>2.6 ± 0.3*</td>
<td>40.2 ± 1.4</td>
<td>408 ± 37*</td>
</tr>
<tr>
<td>Lrp6(^{-/-})/Lrp5(^{-/-})</td>
<td>2</td>
<td>3.5 ± 0.4*</td>
<td>2.5 ± 0.2*</td>
<td>36.8 ± 1.0</td>
<td>422 ± 21*</td>
</tr>
<tr>
<td>Lrp6(^{-/-})/Lrp5(^{-/-})</td>
<td>2</td>
<td>3.9 ± 0.2*</td>
<td>2.6 ± 0.03*</td>
<td>41.9 ± 5.0</td>
<td>402 ± 7*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
* \(p < 0.05\) compared with wildtype mice.
† \(p < 0.01\) compared with wildtype mice.

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**FIG. 2.** Lrp5-deficient mice recapitulate aspects of the human osteoporosis pseudoglioma syndrome. (A) Total body BMD of heterozygous (HET) and mutant (MUT) Lrp5 mice was determined within the same litter over time (\(p < 0.03\) at 29 days). \(p\) values were determined using a two-sample \(t\)-test assuming equal variances. (B–E). Persistent eye vascularization in Lrp5-deficient mice (C and E) relative to wildtype controls (B and D). (B and C) Immunofluorescent staining for the endothelial associated markers P1H12 (green) and FactorVIII (red). (D and E) Hematoxylin and eosin staining of adjacent sections. Note the presence of material in the vitreous of the Lrp5-deficient mouse. L, lens; V, vitreous; R, retina.
of genotypes 6W5H, 6H5W, and 6W5M seemed normal (Figs. 3E–3H). The severity of the skeletal limb defects varied significantly in the 6H5H and 6H5M mice, ranging from apparently normal limbs to the absence of an entire limb. The majority of the animals presented between these two extremes with synostosis or reduction deformity of the postaxial digits. Interestingly, the deformities were more severe in the right forelimb than the left forelimb. Typically, in both the affected 6H5H and 6H5M mice digits 4 and/or 5 were missing in the forelimbs (Figs. 3I–3J, 3M, and 3N). The hindlimbs of the 6H5H mice seemed normal (Figs. 3K and 3L), but often only one digit was present in the hindlimbs of the affected 6H5M mice (Figs. 3O and 3P). In more severe cases, truncations extended into more proximal regions of the limb. In forelimbs, the hamate and/or triquetral carpal bones were missing or the bones were fused (Figs. 3M and 3N). In hindlimbs, the fibula was absent, and the talus, calcaneus, and other tarsal bones were either malformed or absent (Figs. 3O and 3P).

Lrp6 haplo-insufficiency independently reduces bone mass and further accentuates the low BMD phenotype observed in Lrp5-deficient mice

To determine the effects of Lrp6 heterozygosity in mice with and without mutations in Lrp5, we performed an initial screen using total body DXA analysis on 6H5W, 6W5H, 6H5H, 6W5M, 6H5M mice and wildtype littermates (6W5W). We analyzed cohorts of age-matched male and female mice between 14 and 18 weeks of age. We chose this age based on the fact that we and others found that mice between these ages do not exhibit statistically significant increases in BMD. Using DXA, it appeared that 6W5W, 6H5W, and 6W5H mice had similar BMDs (Fig. 4). However, more sensitive µCT analyses revealed significant reductions of BV/TV in the 6W5H and 6H5W mice (Table 1). In contrast, DXA and µCT both detected reduced BMD in
the three other genotypic classes: 6H5H, 6W5M, and 6H5M (Tables 1–3; Fig. 4). Overall, the BMD and μCT studies indicate that Lrp6 and Lrp5 independently affect bone mass and that Lrp6 haploinsufficiency further exacerbates the low bone mass phenotype seen in Lrp5 mutant mice. The consequence of Lrp5 and Lrp6 genotypic effects on bone mass can be summarized as follows: 6W5M > 6H5W = 6W5H > 6H5H > 6W5M > 6H5M.

To ensure that total body BMD values were not affected by the absence of skeletal elements in the 6H5M mice, equivalent regions of the femur, humerus, and spine were measured independently for both 6W5M (mice without limb defects) and 6H5M mice (mice with limb defects). Significant differences in BMD were observed between both male and female 6W5M and 6H5M mice for all regions measured, suggesting that BMD values are not disproportionately reduced in mice with limb defects (Table 3). To further assess BMD, we compared the thickness of skull sections from 3-month-old 6W5H, 6H5H, 6W5M, and 6H5M female littermates. Histological examination was consistent with the lowered BMD detected by DXA (Fig. 5).

**DISCUSSION**

Our studies show that Lrp6 and Lrp5 have overlapping roles during limb development and bone mass accrual. The mutant limb phenotype observed in Lrp6/Lrp5 6H5H and 6H5M mice resembles, but does not fully recapitulate, the conditional loss of Wnt3 expression in the limb ectoderm, the conditional removal of β-catenin in the ventral ectoderm, (27) and Wnt-7a deficiency. (28) That 6H5H and 6H5M limbs have an incompletely penetrant phenotype supports there being a dosage effect for the Wnt signaling cascade during patterning. Pinson et al. (7) noted a similar dosage effect of Lrp6 on a hypomorphic Wnt3a mutant background. Failure to fully recapitulate the features of individual Wnt deficiencies is likely explained by the presence of at least one functional Lrp allele in these animals. The endoplasmic reticulum protein MESD has been shown to be an essential chaperone for normal LRP expression. (29) Mice completely lacking MESD die during gastrulation and have a phenotype similar to that of Wnt3-deficient embryos. (29) This observation, plus the early embryonic lethality of 6M5M mice, (22) further supports the role of LRP5 in Wnt signaling. Wnt signaling is inhibited at the level of the LRP5 and 6 receptors by the Dickkopf protein family Dkk. (30) Therefore, it is interesting to note that mice lacking Dkk1 develop postaxial polydactyly. (31) The converse of the postaxial reductions seen in the Lrp6/Lrp5 mutant mice.

We screened our cohort of mutant mice for alterations in BMD using DXA analysis and performed μCT analysis in a smaller sample of mice from all genotypic groups. μCT analysis revealed that heterozygosity for mutations in either

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Cort Th (µm)</th>
<th>Total area (mm²)</th>
<th>CortBone area (mm²)</th>
<th>Medullary area (mm²)</th>
<th>BA/TA (%)</th>
<th>MA/TA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lrp6+/+; Lrp5+/+</td>
<td>2</td>
<td>184 ± 10</td>
<td>1.5 ± 0.20</td>
<td>0.7 ± 0.03</td>
<td>0.8 ± 0.20</td>
<td>47 ± 5.2</td>
<td>53 ± 5.2</td>
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<tr>
<td>Lrp6+/+; Lrp5+/+</td>
<td>2</td>
<td>161 ± 25</td>
<td>1.3 ± 0.05</td>
<td>0.6 ± 0.09</td>
<td>0.8 ± 0.04</td>
<td>43 ± 5.0</td>
<td>57 ± 5.0</td>
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<tr>
<td>Lrp6+/+; Lrp5+/+</td>
<td>3</td>
<td>164 ± 5.0</td>
<td>1.4 ± 0.07</td>
<td>0.6 ± 0.02</td>
<td>0.8 ± 0.06</td>
<td>43 ± 1.7</td>
<td>57 ± 1.7</td>
</tr>
<tr>
<td>Lrp6+/+; Lrp5+/+</td>
<td>4</td>
<td>164 ± 2.0*</td>
<td>1.4 ± 0.05</td>
<td>0.6 ± 0.01</td>
<td>0.8 ± 0.04</td>
<td>44 ± 1.0</td>
<td>56 ± 1.0</td>
</tr>
<tr>
<td>Lrp6+/+; Lrp5+/+</td>
<td>3</td>
<td>139 ± 13*</td>
<td>1.4 ± 0.10</td>
<td>0.5 ± 0.07</td>
<td>0.9 ± 0.04</td>
<td>37 ± 2.2</td>
<td>63 ± 2.2</td>
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<tr>
<td>Lrp6+/+; Lrp5+/+</td>
<td>2</td>
<td>116 ± 12*</td>
<td>1.2 ± 0.04</td>
<td>0.4 ± 0.05*</td>
<td>0.8 ± 0.01</td>
<td>34 ± 2.8</td>
<td>66 ± 2.8</td>
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</table>

Values are mean ± SEM.
* p < 0.05 compared with wildtype mice.

<table>
<thead>
<tr>
<th>Table 3. BMD VALUES FOR THE FEMUR, HUMERUS, AND SPINE IN MALE AND FEMALE 6W5M AND 6H5M MICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
</tr>
<tr>
<td>6W5M</td>
</tr>
<tr>
<td>0.0547</td>
</tr>
<tr>
<td>p &lt; 0.0008</td>
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</table>

BMD values (g/cm²) were determined using the PIXIImus II densitometer. This represents an age-matched cohort of mice between 14 and 18 weeks old. p values were determined using a two-sample t-test assuming equal variances.
Lrp5 or Lrp6 is sufficient to cause noticeable changes in bone parameters that may not be detected by DXA. Other investigators have also found DXA to be less sensitive than other imaging methods for detecting subtle effects on bone mass.\(^3\)\(^2\)\(^3\). That Lrp6 also mediates bone accrual, or homeostasis, is shown by the reduced bone mass in 6H5W mice and further reductions in bone mass among Lrp5 mutant mice when placed on a heterozygous Lrp6 background. Interestingly, mice lacking Lrp5 (6W5M) had similar trabecular bone measurements as those heterozygous for both mutations (6H5H), but dramatically reduced cortical bone thickness (Table 2). This was further accentuated by heterozygosity for the Lrp6 mutation in Lrp5-deficient mice (6H5M). These observations imply that, although Lrp5 and Lrp6 can both affect bone accrual, their actions may occur at nonredundant sites (i.e., cortical versus trabecular growth). Consequently, careful examination of Lrp6 (in addition to Lrp5) is warranted in studies designed to uncover the mechanisms underlying the bone mass accrual and loss in humans. To date, the perinatal lethality of Lrp6\(^{-/-}\) mice has precluded studies that solely study the role of this receptor on bone growth and homeostasis. However, such studies should be possible by conditionally inactivating alleles using Cre-lox technology and bone-specific promoters.\(^3\)\(^3\) Our results clearly show that Lrp5 and Lrp6 affect bone mass accrual; however, the precise mechanisms by which this occurs have not yet been elucidated. The Wnt signaling cascade remains the attractive candidate pathway for this process.\(^3\)\(^4\) Further reinforcing this idea, mice lacking the Wnt inhibitor sFRP1 have increased BMD.\(^3\)\(^5\) It has been shown that Lrp5, like other LRP family members,\(^2\)\(^4\) can bind ligands other than Wnt’s.\(^3\)\(^6\) Consequently, non-Wnt ligands may also be capable of activating Lrp6/Lrp5 signaling in bone. Several reports have also linked the function of the Wnt signaling pathway to the proper regulation of stem cell pluripotency.\(^3\)\(^7\)–\(^3\)\(^9\) We speculate that the defects seen in these mice and in human OPPG patients may be caused by defects in maintaining the proper balance of pluripotent osteogenic stem cells with terminally differentiated osteoblasts.

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