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Bone Mineral Properties in Growing *Col1a2^{+/-G610C}* Mice, an animal model of Osteogenesis Imperfecta

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Abstract

The *Col1a2^{+/-G610C}* knock-in mouse, models osteogenesis imperfecta in a large old order Amish family (OOA) with type IV OI, caused by a G-to-T transversion at nucleotide 2098, which alters the gly-610 codon in the triple-helical domain of the $\alpha 2(I)$ chain of type I collagen. Mineral and matrix properties of the long bones and vertebrae of male *Col1a2^{+/-G610C}* and their wild-type controls (*Col1a2^{+/+}*), were characterized to gain insight into the role of $\alpha 2$ -chain collagen mutations in mineralization. Additionally, we examined the rescuability of the composition by sclerostin inhibition initiated by crossing *Col1a2^{+/-G610C}* with an *LRP^{+/A214V}* high bone mass allele. At age 10-days, vertebrae and tibia showed few alterations by micro-CT or Fourier transform infrared imaging (FTIRI). At 2-months-of-age, *Col1a2^{+/-G610C}* tibias had 13% fewer

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CONFLICT OF INTEREST

None of the authors have any conflicts of interest with this study.

secondary trabeculae than *Col1a2^{+/+}*, these were thinner (11%) and more widely spaced (20%) than those of *Col1a2^{+/+}* mice. Vertebrae of *Col1a2^{+/G610C}* mice at 2-months also had lower bone volume fraction (38%), trabecular number (13%), thickness (13%) and connectivity density (32%) compared to *Col1a2^{+/+}*. The cortical bone of *Col1a2^{+/G610C}* tibias at 2-months had 3% higher tissue mineral density compared to *Col1a2^{+/+}*; *Col1a2^{+/G610C}* vertebrae had lower cortical thickness (29%), bone area (37%) and polar moment of inertia (38%) relative to *Col1a2^{+/+}*. FTIRI analysis, which provides information on bone chemical composition at ~ 7 μ m-spatial resolution, showed tibias at 10-days, did not differ between genotypes. Comparing identical bone types in *Col1a2^{+/G610C}* to *Col1a2^{+/+}* at 2-months-of-age, tibias showed higher mineral-to-matrix ratio in trabeculae (17%) and cortices (31%), and in vertebral cortices (28%). Collagen maturity was 42% higher at 10-days-of-age in *Col1a2^{+/G610C}* vertebral trabeculae and in 2-month tibial cortices (12%), vertebral trabeculae (42%) and vertebral cortices (12%). Higher acid-phosphate substitution was noted in 10-day-old trabecular bone in vertebrae (31%) and in 2-month old trabecular bone in both tibia (31%) and vertebrae (4%). There was also a 16% lower carbonate-to-phosphate ratio in vertebral trabeculae and a correspondingly higher (22%) carbonate-to-phosphate ratio in 2 month-old vertebral cortices. At age 3-months-of-age, male femurs with both a *Col1a2^{+/G610C}* allele and a *Lrp5* high bone mass allele (*Lrp5^{+/A214V}*) showed an improvement in bone composition, presenting higher trabecular carbonate-to-phosphate ratio (18%) and lower trabecular and cortical acid-phosphate substitutions (8% and 18%, respectively). Together, these results indicate that mutant collagen α 2(I) chain affects both bone quantity and composition, and the usefulness of this model for studies of potential OI therapies such as anti-sclerostin treatments.

Keywords

Osteogenesis imperfecta; FTIRI; Micro-computed tomography; Old order Amish mice; G610C mutation

INTRODUCTION

Osteogenesis imperfecta (OI) is a genetic disorder of connective tissue characterized by fragile and brittle bones¹. Affected individuals may also exhibit an array of associated physical features including short stature, blue sclera, dentinogenesis imperfecta, hearing loss and bone deformities. The disorder is most commonly caused by autosomal dominant mutations in *COL1A1* and *COL1A2* genes, which encode the α 1(I) and α 2(I) chains of type I collagen, respectively. Type I collagen is the most abundant protein in the skeleton, and OI causing mutations can cause phenotypes ranging from mild disease to a perinatal lethality.^{1, 2}

The Lancaster County, PA, Old Order Amish (OOA) community has individuals with an autosomal dominant form of OI, caused by a *COL1A2* missense mutation, that exhibits wide phenotypic variability.^{3,4} To create an animal model of the OOA OI phenotype, mice were generated with the equivalent missense mutation (p.G700C) in *Col1a2*.⁵ This mutation changes an obligatory glycine to a cysteine at the 610th amino acid residue of the triple-helical domain and leads to secretion and bone matrix incorporation of the defective α 2(I) chain in ~ 50% of type I collagen heterotrimers.⁵

Mice with the OOA OI mutation (*Col1a2*^{+/G610C}) display phenotypic variability when crossed into different genetic backgrounds⁵. These mice have been used to test the effects of enhancing Wnt signaling⁶ and those of exercise⁷ on bone properties. Enhancing WNT signaling in these mice has been accomplished both by crossing with the *Lrp5* “high bone mass” allele (*Col1a2*^{+/G610C}; *Lrp5*^{+/A214V}) or by treating with an antibody to sclerostin.⁶ Anti-sclerostin antibodies have also been used to treat other OI mouse models including *Brlt*^{+/8,9,10,11}, *CrTAP*^{-/-}¹², and the severe *Jrt*^{+/} mice¹³. *Jrt*^{+/} mice did not benefit from anti-sclerostin therapy.¹³ This suggested that the anti-sclerostin antibody therapy may benefit some, but not all, forms of OI. While bone mass increased in the treatment arm of several OI models, the effects of blocking sclerostin were reported to be dependent upon animal age, tissue, length of treatment and genotype. Furthermore, it was not clear whether this treatment affected both bone quantity⁶ and composition¹⁰. Mice that are double heterozygous (i.e., *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C}) for the OOA OI mutation and a missense mutation (p.A214V) in *Lrp5* (that causes an increase in bone mass and bone strength compared to wild type mice), had previously been described as having significantly greater bone mass and bone strength compared to mice with the OI mutation alone (i.e., *Col1a2*^{+/G610C}).⁶ Sclerostin inhibition of LRP5 signaling in these *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C} mice was reduced throughout their lives, hence they provide a model for pre-clinical testing of the effect of anti-sclerostin therapies in the absence of other factors. Although these mice were in a different background than those studied in our investigation of the bone composition in the OOA mice at 10 days and 2 months, the availability of embedded bones from the *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C} study, provided the opportunity to ask if the reported improved strength⁶ was only due to the increased quantity of bone, or was it also associated with a change in bone quality (composition) as reported for sclerostin-treated *Brlt*^{+/} mice¹¹.

The objective of the present study is to characterize the morphologic and compositional bone properties of wild-type (*Col1a2*^{+/+}) and OI (*Col1a2*^{+/G610C}) mice, and to address three questions: (1) Is an altered bone phenotype (defined by morphology and composition) present shortly after birth or does it develop later on? (2) Is this altered phenotype common to all types of bone? (3) Is the composition of the aberrant phenotype corrected by sclerostin inhibition, based on the examination of the OI/HBM (*Lrp5*^{+/A214V}; *Col1a2*^{+/G610C}) mice and their corresponding wild-type and OI (*Col1a2*^{+/G610C}) littermates? The answers to these questions will test the hypotheses that: (i) the collagen defect in *Col1a2*^{+/G610C} mice affects matrix mineralization in a site and age-dependent manner, and (ii) the *Lrp5* HBM allele (and hence sclerostin inhibition) improves the composition of matrix mineralization in *Col1a2*^{+/G610C} mice.

METHODS

Animals

Ten-day-old and 2-month-old male *Col1a2*^{+/G610C} and *Col1a2*^{+/+} male littermates and their wild type controls (*Col1a2*^{+/+}), on a mixed CD1/C3H/B6 background, were obtained post-sacrifice from the Bone and Extracellular Matrix Branch, NICHD, NIH (Bethesda, MD). *Col1a2*^{+/G610C} and *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C} male littermates and male *Col1a2*^{+/+}

controls (all on fixed 50% B6/50% SvJ backgrounds)⁶ at 3-months-of-age were obtained post-sacrifice from the Orthopaedic Research Laboratories at Boston Children's Hospital. All protocols were approved by the IACUCs of the institute providing the mice and by the IACUC of the Hospital for Special Surgery. A power study suggested that 5 mice/genotype/age would be sufficient to compare FTIRI variables at an alpha of 0.05 and a power of 80%.

Fracture Counts

Fractures in the long bones of 10-day-old and 2-month-old mice were counted by a single, blinded to genotype, pediatric orthopedic surgeon using whole body, high-resolution, anterior-posterior and lateral radiographs taken via faxitron (Faxitron X-Ray, Wheeling, PA, USA). Fracture counts had not been performed in the published study⁶ of the 3-month-old mice.

Micro-CT

Whole bone morphological traits for the 10-day-old and 2-month-old animals were measured using micro-computed tomography (micro-CT) using global thresholds specific for each age and bone type. The right tibia and lumbar spine were isolated from *Col1a2^{+/G610C}* and *Col1a2^{+/+}* mice, cleaned and scanned at a resolution of 6 μm on a Scanco μCT 35 (Scanco Medical, Brüttisellen, Switzerland). For the 10-day-old tibia, trabecular bone scans were taken 0.9 mm from proximal tibia and cortical bone scans were made 1 mm in the mid diaphysis. For the 2-month-old tibias, trabecular bone scans were made 1.4 mm of length from proximal tibia starting 100 μm from the growth plate, and cortical scans were taken from a length of 1.4 mm in the mid diaphysis. For the vertebral bodies at 10 days, scans were made at a 6 μm resolution; trabecular bone of L3 was segmented using a threshold of 0.4 g/cc. At 2 months, scans were made at a resolution of 10 μm ; trabecular bone of L-3 was segmented using a threshold of 0.5 g/cc. 3D reconstruction and viewing of images was conducted on Scanco micro-CT software (HP, DEC windows Motif 1.6) with volumes of interest defined by a free-hand tool on sequential sections in the metaphyseal and mid-diaphysis of each tibia, and the cortical and cancellous bone of the L3 vertebrae, to determine the cortical and trabecular bone parameters. The trabecular bone parameters calculated using instrument provided software were: bone volume fraction (BV/TV), number, thickness, and spacing of trabeculae (Tb.N, Tb.Th and Tb.Sp, respectively), tissue mineral density (TMD) and connectivity density (Cn. Den), as per the guidelines previously described by Buxsein *et al.*¹⁵ TMD is calculated as mineral content divided by bone volume at a specified threshold. The cortical bone parameters calculated were: cortical thickness (Ct.Th), cortical bone area (Ct.Ar), total cross sectional area inside the periosteal envelope (Tt.Ar), cortical fraction area (Ct.Ar/Tt.Ar), tissue mineral density (TMD), cortical porosity (Ct. Por), polar moment of inertia (pMOI) and bone area per total area (BA/TA).

Fourier transform infra-red spectroscopic imaging (FTIRI)

The chemical composition of the mineral and matrix phases of tibial and vertebral bone from 10-day-old and 2-month-old mice and femoral bone from 3-month-old mice was analyzed using FTIRI. In the 10-day-old and 2-month-old mouse specimens (right tibia and L3 vertebra) samples that had been scanned for micro-CT analysis were dehydrated and

embedded in polymethyl methacrylate (PMMA). Three coronal (tibiae) or 3 sagittal (vertebrae) sections (2 μm thick) were cut using a Leica SM2500 microtome (Leica Microsystems, Wetzlar, Germany) and mounted directly onto BaF₂ windows. For the 3-month-old animal femur specimens, three new 2 μm thick longitudinal sections from PMMA embedded samples previously processed for quantitative histomorphometry⁶ were obtained.

A Spectrum 300 (Perkin Elmer, CT, USA) infrared spectrometer and microscope was used to collect FTIR image-arrays at $\sim 7 \mu\text{m}$ spatial resolution. For long bones, trabecular bone images were acquired at the metaphysis; cortical bone images were taken at mid-diaphysis. Trabecular and cortical bone were also imaged and analyzed separately for L3. Cortical bone scans were performed from the endosteal to periosteal surface. Three separate regions from three different sections of each type of bone were analyzed resulting in 9 images/bone type. Isys4 software (Spectral Dimensions, MD, USA) was used to subtract the embedding media (PMMA), and the infrared images were analyzed to determine 5 FTIR outcome parameter: (1) mineral-to-matrix ratio (area ratio of $[917-1180 \text{ cm}^{-1}]/[1588-1712 \text{ cm}^{-1}]$ peaks), which characterizes tissue mineral content based on the presence of phosphate and is directly related to ash weight¹⁶; (2) carbonate-to-phosphate peak area ratio ($[852-890 \text{ cm}^{-1}]/[917-1180 \text{ cm}^{-1}]$), which, as detailed elsewhere¹⁴, characterizes the extent of carbonate substitution into the hydroxyapatite lattice; (3) collagen cross-link intensity ratio ($1660 \text{ cm}^{-1}/1690 \text{ cm}^{-1}$), which is related to the ratio of non-reducible to reducible enzymatic collagen cross-links as determined by HPLC¹⁷; (4) mineral crystallinity intensity ratio ($1030 \text{ cm}^{-1}/1020 \text{ cm}^{-1}$), which is linked to crystal size and perfection of the hydroxyapatite crystals¹⁶; and (5) acid phosphate substitution intensity ratio ($1128 \text{ cm}^{-1}/1096 \text{ cm}^{-1}$), which characterizes the extent of acid phosphate substitution into the mineral lattice¹⁸.

Statistical analysis

Comparisons were conducted using SAS 9.3 (Cary, N.C.). Fracture counts were compared using unpaired t-tests. For micro-CT measurements, a non-parametric two-sided Wilcoxon Mann-Whitney test was used to compare between genotypes or between animals of different ages. For FTIRI measurements, a GEE (general equation estimate) model was used to account for repeated measures, comparing across genotypes or across ages. Comparison between different bones (e.g., femur vs. tibia, tibia vs. vertebra) was limited to descriptive changes due to the small number of samples available and the differences in background.

RESULTS

Comparison of genotype at 10 Days vs. 2 Months

Fracture counts—No long bone fractures were observed in either 10-day-old *Colla2^{+/G610C}* or *Colla2^{+/+}* mice. The 2-month-old *Colla2^{+/G610C}* mice did sustain fractures, but the difference in fracture count between 2-month-old *Colla2^{+/G610C}* (0.29 ± 0.49) and *Colla2^{+/+}* (0 ± 0) mice did not reach statistical significance ($p = 0.14$).

Micro-CT—Trabecular Bone Trabecular bone measures for tibial and vertebral specimens are depicted in Figure 1A and 1B, respectively. With regard to tibial trabecular measurements, there was no significant difference between 10-day-old *Colla2^{+/G610C}* and

Col1a2^{+/+} mice in terms of BV/TV and the number, thickness and separation of tibial trabeculae (Figure 1A). As to vertebral trabeculae, the 10-day-old *Col1a2*^{+/G610C} mice had higher numbers ($p=0.016$) and smaller separation ($p=0.018$) compared to *Col1a2*^{+/+} mice (Figure 1B).

Two-month-old *Col1a2*^{+/G610C} and *Col1a2*^{+/+} mice each exhibited greater tibial and vertebral trabecular number and separation, and lower connectivity compared to 10-day-old mice with the same genotypes (Table 1). When comparing 2-month-old mice, the *Col1a2*^{+/G610C} mice had a lower tibial bone volume fraction ($p=0.017$), a lower trabecular number ($p=0.061$) and a small, yet significant, higher tissue mineral density ($p=0.013$) compared to *Col1a2*^{+/+} mice (Figure 1A). Vertebral trabeculae in 2-month-old mice exhibited similar patterns with *Col1a2*^{+/G610C} mice having lower bone volume fraction ($p=0.023$), trabecular thickness ($p=0.023$), and connectivity density ($p=0.031$) compared to *Col1a2*^{+/+} mice (Figure 1B).

Cortical Bone: Tibial cortical bone parameters did not differ significantly between 2-month-old *Col1a2*^{+/G610C} and *Col1a2*^{+/+} mice, with the exception of tissue mineral density, which was slightly, but significantly higher ($p=0.013$) in the *Col1a2*^{+/G610C} mice (Figure 2A). Vertebral cortical bone parameters in 2-month-old *Col1a2*^{+/G610C} mice (Figure 2B) exhibited lower cortical thickness ($p=0.009$), polar moment of inertia ($p=0.017$), bone area ($p=0.017$), and total area ($p=0.002$), and a greater tissue mineral density ($p=0.0172$) relative to *Col1a2*^{+/+} mice.

FTIRI—Representative FTIR images were chosen based on the median mineral-to-matrix ratio in a bone section at 2 months from the cortical and trabecular regions of *Col1a2*^{+/G610C} and *Col1a2*^{+/+} mice and are depicted in Figure 3. Visually, the mineral-to-matrix ratio was larger in *Col1a2*^{+/G610C} compared to *Col1a2*^{+/+} bones, with a greater apparent difference between the genotypes noted for cortical bone. Distribution of the carbonate-to-phosphate ratio was more uniform in the cortical bone of *Col1a2*^{+/+} compared to *Col1a2*^{+/G610C} mice. Carbonate-to-phosphate ratio was lower in the trabecular bone from *Col1a2*^{+/G610C} mice, whereas the collagen cross-link ratio was greater in *Col1a2*^{+/G610C} mice. Crystallinity did not appear to be different between *Col1a2*^{+/+} and *Col1a2*^{+/G610C} mice. Acid phosphate substitution was highest along the edges of the cortices and trabeculae for both genotypes, with *Col1a2*^{+/+} mice having an overall greater intensity. Quantification of the data in these images showed mean FTIRI values, stratified for bone region and age, were different when *Col1a2*^{+/+} and *Col1a2*^{+/G610C} mice were compared (Figure 4A and 4B).

Trabecular Bone: In tibial trabeculae of 10-day-old mice, there were no significant differences in mineral-to-matrix, carbonate-to-phosphate, crystallinity, acid-phosphate substitution or in collagen cross-link ratio (Figure 4A), whereas in 2 month-old *Col1a2*^{+/G610C} mice tibial trabeculae had a 17% higher mineral-to-matrix ratio ($p=0.027$) relative to *Col1a2*^{+/+} mice. In the vertebral bodies (Figure 4B), at 10 days, trabecular acid phosphate substitution was higher (31%, $p=0.0007$) and collagen cross-link ratio similarly was higher (42%, $p<0.0001$) compared to *Col1a2*^{+/+} mice. At 2-months of age, vertebral trabeculae in *Col1a2*^{+/G610C} mice had a 31% greater acid phosphate substitution ($p=0.006$),

6% lower crystallinity ($p=0.014$) and 16% lower ($p<0.0001$) carbonate-to-phosphate ratio compared to *Colla2^{+/+}* mice (Figure 4B).

Cortical Bone: The only significant difference between 10-day-old mice tibial cortical bone in the two genotypes was a 12% higher mineral-to-matrix ratio of the *Colla2^{+/G610C}* mice ($p=0.006$). In 2 month-old mice, only the tibial cortical bone mineral-to-matrix ratio of *Colla2^{+/G610C}* mice (Figure 5A), which was 31% higher in *Colla2^{+/G610C}* mice ($p=0.001$), and the collagen cross-link ratio, which was 12% higher in *Colla2^{+/G610C}* mice ($p=0.046$), differed between the two genotypes. Vertebral cortical bone values were only measureable in 2-month-old mice and revealed a 28% greater mineral-to-matrix ratio ($p<0.001$), a 4% higher acid phosphate content ($p<0.001$) and a 22% higher carbonate-to-phosphate ratio ($p=0.016$) in *Colla2^{+/G610C}* compared to *Colla2^{+/+}* mice.

Comparison of Genotype Dependent Changes in Different Bone Types at 2 months

Micro-CT—The relative significant changes in micro-CT properties always occurred in the same direction when different *Colla2^{+/G610C}* and *Colla2^{+/+}* bones were compared (Table 1). Micro-CT comparisons for 2 month old femurs (reported previously⁵) in the same mixed C3H background as mice used to query the effect of the HMB mutation on the *Colla2^{+/G610C}* are included in the table. In all three bones (femur, tibia, vertebrae) the same parameters reported for the femur⁵ were lower for the tibia and vertebrae of *Colla2^{+/G610C}* mice relative to the *Colla2^{+/+}* mice, including cortical and trabecular bone volume fractions, trabecular number, cortical area and polar moment of inertia.

FTIRI—Consistent significant changes between tibia and vertebrae composition in the *Colla2^{+/G610C}* mice relative to *Colla2^{+/+}* mice, were higher mineral-to-matrix ratios and acid phosphate substitution for both trabecular and cortical bone. For cortical bone alone, the consistent changes were higher values of collagen cross-link ratio (Figures 4 and 5).

Effect of enhancing LRP5 signaling on Bone Composition

The effect on femoral FTIRI values of the *Lrp5* high-bone-mass allele values in *Colla2^{+/G610C}* mice was determined by comparing 3-month-old *Colla2^{+/G610C}*, *Lrp5^{+/A214V}*; *Colla2^{+/G610C}*, and *Colla2^{+/+}* femurs⁶ (Figure 6). Relative to *Colla2^{+/+}* mice, the 3-month-old femurs from *Colla2^{+/G610C}* mice had lower trabecular bone carbonate-to-phosphate ratio (17%, $p<0.0001$), and lower crystallinity (6%, $p<0.0001$), while the acid phosphate substitution was higher 32% ($p<0.0001$). In cortical bone there was a 12% higher ($p=0.016$) mineral-to-matrix ratio; a 14% higher value in I collagen cross-link ratio ($p<0.0001$), a 35% higher acid phosphate substitution ($p<0.0001$) and a 6% lower crystallinity ($p=0.0041$), relative to *Colla2^{+/+}* mice.

OI mice with the *Lrp5* high-bone-mass allele had no significant differences in mineral-to-matrix ratio (trabecular or cortical), collagen cross-link ratio (trabecular or cortical) or cortical acid phosphate substitution relative to the *Colla2^{+/+}* mice, although trabecular acid phosphate substitution was still higher, albeit to a lesser extent than in the *Colla2^{+/G610C}* mice. As for the *Colla2^{+/G610C}* mice, the *Lrp5^{+/A214V}*; *Colla2^{+/G610C}* littermates had an 18% higher trabecular bone carbonate-to-phosphate ratio ($p<0.001$), an 18% lower value of

trabecular acid phosphate substitution ($p < 0.05$) and a 8% lower cortical acid phosphate substitution ($p = 0.044$).

DISCUSSION

Skeletal fragility is a hallmark feature of OI. The *Colla2*^{+/G610C} mouse model of OOA OI has previously been shown to have reduced bone mass and bone strength compared to wild-type mice^{5, 6}. Although we did not observe a significant difference in the occurrence of spontaneous fractures between 10-day-old or 2-month-old *Colla2*^{+/G610C} and *Colla2*^{+/+} mice, the *Colla2*^{+/G610C} mice did sustain fractures by 2 months, and our micro-CT data are consistent with *Colla2*^{+/G610C} mice having reduced bone mass and hence reduced bone strength. Here, we report quantitative data on younger (prior to weaning) mice, compare three different bone types (tibia and vertebrae from this study and femurs based on the literature), and relate quantitative data to mechanical properties reported for these mice⁶. We also investigated potential anti-sclerostin effects on bone quality (composition), by comparing FTIR variables in OI mice whose bone mass and strength are increased by a *Lrp5* HBM allele to wild-type and OI control mice.

Comparison of Bone Phenotype in 10 Day and 2 Month old mice

The mineral properties of the *Colla2*^{+/G610C} mice appeared to be normal at 10 days and approached a more brittle bone phenotype with increasing age, as evidenced by the results of the micro-CT and FTIR study. There are few reports of mouse bone morphology or composition prior to weaning. Canalis *et al*¹⁹, based on femoral histomorphometry, reported male wild type mice in a C57BL/6 genetic background had a 216% greater bone volume fraction, a 50% lower trabecular separation, along with a 71% greater trabecular thickness from 10 days to 1 month of age, paralleling our findings in the tibia of *Colla2*^{+/+} mice from 10 days to 2 months. Roemer *et al*²⁰, describing the osteopotential mutant model of OI, provided micro-CT images of 20 day-old mice but no quantitation. The osteopotential OI model is only viable for 20 days. Hence, we previously analyzed their bones by FTIR at 10 days-of-age²¹ and found a severely different phenotype, which prompted the current study of 10-day-old *Colla2*^{+/G610C} animals. The postnatal period from birth to 10 days is one of active endochondral bone formation, which is dependent on a type II collagen matrix, whereas from 10-days until 2-months one expects both endochondral ossification and appositional bone growth, the latter, depending on proper type I collagen. Where a great deal of the bone is derived from endochondral ossification as in the neonate at 10 days, one would not expect a major micro-CT phenotype; however at 2 months-of-age, it was expected.

Only one other FTIR imaging study evaluated mouse bones prior to weaning and that study provided only images without quantification²². In the current study, *Colla2*^{+/G610C} vertebrae showed differences from *Colla2*^{+/+} earlier than the tibiae, perhaps because the vertebrae, mainly trabecular bone, matures faster²³. In both trabeculae and cortices, the mutant collagen heterotrimer in the bone matrix of *Colla2*^{+/G610C} mice may disrupt the normal process of mineralization. Specifically, we suggest that the abnormal matrix makes it energetically more difficult for the first mineral crystals to form²⁴, resulting in reduced bone

formation (modeling). Once the first mineral crystals do form, however, they appear relatively normal in size and perfection (crystallinity), although *Col1a2*^{+/G610C} crystals are smaller than normal in the 3-month-old femur and in the 2-month-old vertebral trabecular bone. Thus, overall and with increasing bone age, there may be fewer larger crystals, reflected by the lower crystallinity and higher acid phosphate substitution in trabeculae. Reduced crystallinity is a well-known feature of osteogenesis imperfecta in both human and animal models of this disease^{25,26,27,28,29,30,31,32}.

The Phenotype of Different Bones

The phenotype of the *Col1a2*^{+/G610C} mouse at 2 months successfully models a mild type IV OI with low fracture incidence, similar to the human pedigree with the same mutation on which the mouse was modeled⁴. Our micro-CT data confirm the osteopenic phenotype previously reported in long bones of older animals. That same data also showed parallel changes in the trabeculae-rich vertebral micro-CT results, with a tendency toward thinner cortices and significantly attenuated secondary trabeculae in 2-month-old *Col1a2*^{+/G610C} compared to *Col1a2*^{+/+} mice, which is consistent with previously findings reported by Daley *et al*⁵ in the 2-month-old femur as shown in Table 1. These comparisons indicated that tibias and femurs change morphologically in the same direction, as do the vertebral bodies, however, not always to the same extent. While these differences could be attributable to the different micro-CT scanners used and the thresholds applied, they indicated that femurs should only be compared to femurs, vertebrae to vertebrae and tibia to tibia.

The FTIRI analyses showed relatively normal mineral and matrix properties in the 10-day-old *Col1a2*^{+/G610C} mice tibias and vertebrae, with differences from wild-type mice becoming more apparent at 2 months of age. We observed a general higher mineral-to-matrix ratio and collagen cross-link maturity in *Col1a2*^{+/G610C} compared to *Col1a2*^{+/+} mice. The former measurement likely reflects a decreased matrix associated with OI, and possibly altered interactions between collagenous and noncollagenous proteins in the mutant mice. The amide I band, used as the denominator to assess the matrix contribution, is influenced by the environment in which the vibrating carbonyl-bonds sit, and hence any change in the association of noncollagenous proteins³³ can alter the position and intensity of this band. The collagen cross-link maturity, which is also elevated in other OI models^{14, 32, 37}, may suggest disorganization and/or over-modification of abnormal mutant collagen fibrils. This parameter, although controversial³⁴ is widely used to study bone material properties and has been shown to correlate to enzymatic cross-links determined by HPLC¹⁷. The ratio is dependent on the environment around the carbonyl bonds in the peptide, and thus could be influenced by alterations in chain properties due to impaired triple-helix formation³⁵, variations in d-spacing³⁶ or changes in collagen-noncollagenous protein interactions³³. Our findings in *Col1a2*^{+/G610C} mice are consistent with those we observed in other OI mouse models, including *Col1a2*^{oim/oim}, *Col1a1*^{Brtl/+}, fro/fro and the *Pedf*^{-/-} mice^{14,21,24,25,26,27,28,29,30,31,37}. Mineral-to-matrix ratio relates to bone mineral density as measured by dual photon absorptiometry and micro-CT³⁸. It is, however, not identical to these modalities, as the entire tissue, rather than the phosphate-mineral-containing tissue is examined, and reflects a measure of bone quantity. While we detected no significant differences in cortical porosity by micro-CT, it could be argued that the 6μm resolution was

unable to detect differences in porosity. Increases in porosity in the OI animals would create an artifactual increase in TMD. This error is unlikely, as the mineral-to-matrix ratio, which is only measured where matrix is present, is also elevated in these mice.

Other differences observed between the *Col1a2*^{+/G610C} and *Col1a2*^{+/+} mice are similarly consistent with their brittle phenotype. Elevated collagen cross-link ratio as seen in cortical and trabecular regions of 2- and, as discussed below, in 3-month-old *Col1a2*^{+/G610C} long bones and 2-month-old vertebrae is also associated with other osteoporosis and fragile bone phenotypes^{39,40,41,42,43,44}.

Limiting Sclerostin Inhibition of Bone Formation by the HBM Mutation

Different bones in the same mice with the same genotype and background showed parallel, yet distinct changes in morphological properties. Thus we limited our comparison of sclerostin-deficient bones to femora. To investigate the effect of sclerostin inhibition we examined the previously characterized 3-month-old femurs from mice expressing *Col1a2*^{+/G610C} and an *Lrp5* high bone mass allele.⁶ The 3-month-old *Col1a2*^{+/G610C} mice femurs, although from mice with a different genetic background, showed comparable defects to the younger animals used in this study, having higher mineral-to-matrix ratio, lower crystallinity and greater acid phosphate substitution. *Lrp5* HBM allele improved bone mass and bone strength in *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C} mice⁶, however, few significant differences were observed in FTIRI variables. Carbonate-to-phosphate ratio, which increases with decreased remodeling³⁹ was higher in the *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C} compared to *Col1a2*^{+/G610C} mice, while acid phosphate substitution, which decreases in older bone¹⁸, was lower, suggesting that the *Lrp5* HBM allele improved remodeling. These data also suggest that, in addition to the previously reported greater bone quantity in *Lrp5*^{+/A214V}; *Col1a2*^{+/G610C} mice⁶, their reported improved strength may also reflect changes in bone composition. The paucity of FTIRI changes may be attributed to the fact that the study was under-powered for this OI mouse model, or because the improvement in mechanical strength is principally due to increased bone quantity rather than quality (i.e., composition). Although limited Raman spectroscopy data were available for the *Col1a2*^{+/G610C} mice, no FTIRI data, namely, the primary outcome, were available for study design. We therefore did a power study based on the data previously obtained from 2-month old mice in the CD1/C3H/B6 background with a different OI-causing mutation¹⁴ to detect a 30% difference in mineral-to-matrix ratio with 80% power (beta = 20%) and alpha = 0.05, and predicted we needed 5 samples/group. We also used data from the *Col1a2*^{+/G610C} mice in the same background as the mice in this study,⁵ to confirm that the planned secondary analysis of micro-CT variables could detect a 50% difference in bone volume fraction with 80% power (beta=20%), thus we used 5–7 samples/group. Based on data presented here, however, a larger sample size (n=16) would have sufficed to show significant differences relative to wild type (or the HBM cross) in all parameters except 2 month tibial carbonate-to-phosphate ratio (cortical and cancellous), 2 month vertebral cancellous collagen cross-links, and 3 month femur cancellous crystallinity.

The overall study, which addressed three questions, has other limitations that should be noted. We used male mice, even though sexual dimorphism has been observed in wild-type

and OI mice^{7,14,45,46}. Additionally, we used femurs from the 3-month-old mice and tibiae and vertebrae from the 10-day-old and 2-month-old mice. However, relative changes in femoral and tibial material properties were said to be similar in other studies^{47,48}. We, here, demonstrate parallel, albeit not identical micro-CT changes in femur, vertebrae and tibia of mice in the same background at 2 months. We did not perform direct comparisons because the 3-month-old mice were in a different background than the younger mice studied. Despite these limitations, the results allow us to characterize the effect of the mutation in the $\alpha 2$ chain of type I collagen on bone composition and suggest that bone composition and bone quantity, can be modified by inhibiting sclerostin activity. We suggest, that improperly formed type I collagen fibrils in the $Col1a2^{+/G610C}$ model do not support initial mineral deposition. Once, the first crystals do form they mature properly, albeit it, in a delayed fashion. Furthermore, the *Lrp5* HBM allele, which improved matrix quantity in $Col1a2^{+/G610C}$ mice⁶, also appears to improve matrix and mineral composition. Similar beneficial effects may occur from therapies that lower the biologic activity of sclerostin, such as sclerostin neutralizing antibodies.

In summary, the mineral and matrix phenotype as characterized by micro-CT and FTIRI demonstrates low fracture counts, mild trabecular osteopenia and altered biomineralization in $Col1a2^{+/G610C}$ compared to $Col1a2^{+/+}$ mice. The effect on bone mass and mineralization in the $Lrp5^{+/A214V}; Col1a2^{+/G610C}$ mice suggests the utility of $Col1a2^{+/G610C}$ mice in testing other potential therapies for mild to moderate forms of OI and supports the concept that inhibiting sclerostin activity may be effective in such models^{6,8,9,10,11,12} and perhaps, in some types of OI in children.

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REFERENCES

1. Forlino A, Marini JC. Osteogenesis imperfecta. Lancet. 2015 Nov 2. pii: S0140-6736(15)00728-X. doi:10.1016/S0140-6736(15)00728-X. [Epub ahead of print].
2. Byers PH, Pyott SM. Recessively inherited forms of osteogenesis imperfecta. Annu Rev Genet. 2012; 46:475–497. [PubMed: 23145505]
3. McBride DJ, Schapiro JR, Streeten EA, Shuldiner AR. Identification of COL1A2 mutation in the Old Order Amish. VII International Conference on Osteogenesis Imperfecta. 1999; 46
4. Streeten EA, McBride DJ, Pollin TI, Ryan K, Shapiro J, Ott S, Mitchell BD, Shuldiner AR, O'Connell JR. Quantitative trait loci for BMD identified by autosome-wide linkage scan to chromosomes 7q and 21q in men from the Amish Family Osteoporosis Study. J Bone Miner Res. 2006; 21:1433–1442. [PubMed: 16939402]
5. Daley E, Streeten EA, Sorkin JD, Kuznetsova N, Shapses SA, Carleton SM, Shuldiner AR, Marini JC, Phillips CL, Goldstein SA, Leikin S, McBride DJ Jr. Variable Bone Fragility Associated With an Amish COL1A2 Variant and a Knock-in Mouse Model. J Bone Miner Res. 2010; 25:247–261. [PubMed: 19594296]
6. Jacobsen CM, Barber LA, Ayturk UM, Roberts HJ, Deal LE, Schwartz MA, Weis M, Eyre D, Zurakowski D, Robling AG, Warman ML. Targeting the LRP5 pathway improves bone properties in

- a mouse model of osteogenesis imperfecta. *J Bone Miner Res.* 2014; 29:2297–2306. [PubMed: 24677211]
7. Jeong Y, Carleton SM, Gentry BA, Yao X, Ferreira JA, Salamango DJ, Weis M, Oestreich AK, Williams AM, McCray MG, Eyre DR, Brown M, Wang Y, Phillips CL. Hindlimb Skeletal Muscle Function and Skeletal Quality and Strength in +/-G610C Mice With and Without Weight-Bearing Exercise. *J Bone Miner Res.* 2015; 30:1874–1886. [PubMed: 25829218]
 8. Sinder BP, Eddy MM, Ominsky MS, Caird MS, Marini JC, Kozloff KM. Sclerostin antibody improves skeletal parameters in a *Brtl/+* mouse model of osteogenesis imperfecta. *J Bone Miner Res.* 2013; 28:73–80. [PubMed: 22836659]
 9. Sinder BP, White LE, Salemi JD, Ominsky MS, Caird MS, Marini JC, Kozloff KM. Adult *Brtl/+* mouse model of osteogenesis imperfecta demonstrates anabolic response to sclerostin antibody treatment with increased bone mass and strength. *Osteoporos Int.* 2014 Aug; 25(8):2097–2107. [PubMed: 24803333]
 10. Sinder BP, Salemi JD, Ominsky MS, Caird MS, Marini JC, Kozloff KM. Rapidly growing *Brtl/+* mouse model of osteogenesis imperfecta improves bone mass and strength with sclerostin antibody treatment. *Bone.* 2015; 71:115–123. [PubMed: 25445450]
 11. Sinder BP, Lloyd WR, Salemi JD, Marini JC, Caird MS, Morris MD, Kozloff KM. Effect of anti-sclerostin therapy and osteogenesis imperfecta on tissue-level properties in growing and adult mice while controlling for tissue age. *Bone.* 2016; 84:222–229. [PubMed: 26769006]
 12. Grafe I, Alexander S, Yang T, Lietman C, Homan EP, Munivez E, Chen Y, Jiang MM, Bertin T, Dawson B, Asuncion F, Ke HZ, Ominsky MS, Lee B. Sclerostin Antibody Treatment Improves the Bone Phenotype of *Crtap*($-/-$) Mice, a Model of Recessive Osteogenesis Imperfecta. *J Bone Miner Res.* 2015 Dec 30.
 13. Roschger A, Roschger P, Keplingter P, Klaushofer K, Abdullah S, Kneissel M, Rauch F. Effect of sclerostin antibody treatment in a mouse model of severe osteogenesis imperfecta. *Bone.* 2014; 66:182–188. [PubMed: 24953712]
 14. Boskey AL, Marino J, Spevak L, Pleshko N, Doty S, Carter EM, Raggio CL. Are Changes in Composition in Response to Treatment of a Mouse Model of Osteogenesis Imperfecta Sex-dependent? *Clin Orthop Relat Res.* 2015; 473:2587–2598. [PubMed: 25903941]
 15. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for Assessment of Bone Microstructure in Rodents Using Micro-Computed Tomography. *JBMR.* 2010; 25:1468–1486.
 16. Faibish D, Gomes A, Boivin G, Binderman I, Boskey A. Infrared imaging of calcified tissue in bone biopsies from adults with osteomalacia. *Bone.* 2005; 36:6–12. [PubMed: 15663997]
 17. Paschalis EP, Tatakis DN, Robins S, Fratzl P, Manjubala I, Zoehrer R, Gamsjaeger S, Buchinger B, Roschger A, Phipps R, Boskey AL, Dall'Ara E, Varga P, Zysset P, Klaushofer K, Roschger P. Lathyrism-induced alterations in collagen cross-links influence the mechanical properties of bone material without affecting the mineral. *Bone.* 2011; 49:1232–1241. [PubMed: 21920485]
 18. Spevak L, Flach CR, Hunter T, Mendelsohn R, Boskey A. Fourier transform infrared spectroscopic imaging parameters describing acid phosphate substitution in biologic hydroxyapatite. *Calcif Tissue Int.* 2013; 92:418–428. [PubMed: 23380987]
 19. Canalis E, Parker K, Zanotti S. Gremlin1 is required for skeletal development and postnatal skeletal homeostasis. *J Cell Physiol.* 2012; 227(1):269–277. [PubMed: 21412775]
 20. Roemer FW, Mohr A, Guermazi A, Jiang Y, Schlechtweg P, Genant HK, Sohaskey ML. Phenotypic characterization of skeletal abnormalities of *Osteopotentia* mutant mice by micro-CT: a descriptive approach with emphasis on reconstruction techniques. *Skeletal Radiol.* 2011; 40:1073–1078. [PubMed: 21207022]
 21. Boskey, AL.; Doty, SB. Chapter 4 - Mineralized Tissue: Histology, Biology and Biochemistry. In: Shapiro, Jay R.; Byers, Peter H.; Glorieux, Francis H.; Sponseller, Paul D., editors. *Osteogenesis Imperfecta: A Translational Approach to Brittle Bone Disease*. London, UK: Elsevier Inc; 2014. p. 31-43. ISBN: 978-0-12-397165-4
 22. Anderson HC, Sipe JB, Hessle L, Dhanyamraju R, Atti E, Camacho NP, Millán JL. Impaired calcification around matrix vesicles of growth plate and bone in alkaline phosphatase-deficient mice. *Am J Pathol.* 2004; 164:841–847. [PubMed: 14982838]

23. Buie HR, Moore CP, Boyd SK. Postpubertal architectural developmental patterns differ between the L3 Vertebra and Proximal Tibia in three Inbred Strains of Mice. *J Bone Min Res*. 2008; 23:2048–2059.
24. Tao J, Battle KC, Pan H, Salter EA, Chien YC, Wierzbicki A, De Yoreo JJ. Energetic basis for the molecular-scale organization of bone. *Proc Natl Acad Sci U S A*. 2015; 112:326–331. [PubMed: 25540415]
25. Carriero A, Zimmermann EA, Paluszny A, Tang SY, Bale H, Busse B, Alliston T, Kazakia G, Ritchie RO, Shefelbine SJ. How tough is brittle bone? Investigating osteogenesis imperfecta in mouse bone. *J Bone Miner Res*. 2014; 29(6):1392–1401. [PubMed: 24420672]
26. Kozloff KM, Carden A, Bergwitz C, Forlino A, Uveges TE, Morris MD, Marini JC, Goldstein SA. Brittle IV mouse model for osteogenesis imperfecta IV demonstrates postpubertal adaptations to improve whole bone strength. *J Bone Miner Res*. 2004; 19:614–622. [PubMed: 15005849]
27. Bogan R, Riddle RC, Li Z, Kumar S, Nandal A, Faugere MC, Boskey A, Crawford SE, Clemens TL. A mouse model for human osteogenesis imperfecta type VI. *J Bone Miner Res*. 2013; 28:1531–1536. [PubMed: 23413146]
28. Camacho NP, Hou L, Toledano TR, Ilg WA, Brayton CF, Raggio CL, Root L, Boskey AL. The material basis for reduced mechanical properties in oim mice bones. *J Bone Miner Res*. 1999; 14:264–272. [PubMed: 9933481]
29. Grabner B, Landis WJ, Roschger P, Rinnerthaler S, Peterlik H, Klaushofer K, Fratzl P. Age- and genotype-dependence of bone material properties in the osteogenesis imperfecta murine model (oim). *Bone*. 2001; 29:453–457. [PubMed: 11704498]
30. Vetter U, Eanes ED, Kopp JB, Termine JD, Robey PG. Changes in apatite crystal size in bones of patients with osteogenesis imperfecta. *Calcif Tissue Int*. 1991; 49:248–250. [PubMed: 1760768]
31. Imbert L, Aurégan JC, Pernelle K, Hoc T. Mechanical and mineral properties of osteogenesis imperfecta human bones at the tissue level. *Bone*. 2014; 65:18–24. [PubMed: 24803077]
32. Coleman RM, Aguilera L, Quinones L, Lukashova L, Poirier C, Boskey AL. Comparison of bone tissue properties in mouse models with collagenous and non-collagenous genetic mutations using FTIRI. *Bone*. 2012; 51:920–928. [PubMed: 22910579]
33. Sweeney SM, Orgel JP, Fertala A, McAuliffe JD, Turner KR, Di Lullo GA, Chen S, Antipova O, Perumal S, Ala-Kokko L, Forlino A, Cabral WA, Barnes AM, Marini JC, San Antonio JD. Candidate cell and matrix interaction domains on the collagen fibril, the predominant protein of vertebrates. *J Biol Chem*. 2008; 283:21187–21197. [PubMed: 18487200]
34. Farlay D, Duclos ME, Gineyts E, Bertholon C, Viguet-Carrin S, Nallala J, Sockalingum GD, Bertrand D, Roger T, Hartmann DJ, Chapurlat R, Boivin G. The ratio 1660/1690 cm⁻¹ measured by infrared microspectroscopy is not specific of enzymatic collagen cross-links in bone tissue. *PLoS One*. 2011; 6(12):e28736. [PubMed: 22194900]
35. Pace JM, Atkinson M, Willing MC, Wallis G, Byers PH. Deletions and duplications of Gly-Xaa-Yaa triplet repeats in the triple helical domains of type I collagen chains disrupt helix formation and result in several types of osteogenesis imperfecta. *Hum Mutat*. 2001; 18:319–326. [PubMed: 11668615]
36. Wallace JM, Orr BG, Marini JC, Holl MM. Nanoscale morphology of Type I collagen is altered in the Brl mouse model of Osteogenesis Imperfecta. *J Struct Biol*. 2011; 173:146–152. [PubMed: 20696252]
37. Boskey AL, Verdelis K, Spevak L, Lukashova L, Beniash E, Yang X, Cabral WA, Marini JC. Mineral and matrix changes in Brl/+ teeth provide insights into mineralization mechanisms. *Biomed Res Int*. 2013; 2013:295812. [PubMed: 23802117]
38. Gourion-Arsiquaud S, Burket JC, Havill LM, DiCarlo E, Doty SB, Mendelsohn R, van der Meulen MC, Boskey AL. Spatial variation in osteonal bone properties relative to tissue and animal age. *J Bone Miner Res*. 2009; 24:1271–1281. [PubMed: 19210217]
39. Isaksson H, Turunen MJ, Rieppo L, Saarakkala S, Tamminen IS, Rieppo J, Kröger H, Jurvelin JS. Infrared spectroscopy indicates altered bone turnover and remodeling activity in renal osteodystrophy. *J Bone Miner Res*. 2010; 25:1360–1366. [PubMed: 20200925]

40. Shi X, Liu XS, Wang X, Guo XE, Niebur GL. Type and orientation of yielded trabeculae during overloading of trabecular bone along orthogonal directions. *J Biomech.* 2010; 43:2460–2466. [PubMed: 20554282]
41. Gamsjaeger S, Srivastava AK, Wergedal JE, Zwerina J, Klaushofer K, Paschalis EP, Tatakis DN. Altered bone material properties in HLA-B27 rats include reduced mineral to matrix ratio and altered collagen cross-links. *J Bone Miner Res.* 2014; 29(11):2382–2391. PubMed PMID: 24771481. [PubMed: 24771481]
42. Misof BM, Gamsjaeger S, Cohen A, Hofstetter B, Roschger P, Stein E, Nickolas TL, Rogers HF, Dempster D, Zhou H, Recker R, Lappe J, McMahon D, Paschalis EP, Fratzl P, Shane E, Klaushofer K. Bone material properties in premenopausal women with idiopathic osteoporosis. *J Bone Miner Res.* 2012 Dec; 27(12):2551–2561. [PubMed: 22777919]
43. Gourion-Arsiquaud S, Faibish D, Myers E, Spevak L, Compston J, Hodsman A, Shane E, Recker RR, Boskey ER, Boskey AL. Use of FTIR spectroscopic imaging to identify parameters associated with fragility fracture. *J Bone Miner Res.* 2009 Sep; 24(9):1565–1571. [PubMed: 19419303]
44. Garcia I, Chiodo V, Ma Y, Boskey A. Evidence of Altered Matrix Composition in Iliac Crest Biopsies from Patients with Idiopathic Juvenile Osteoporosis. *Connect Tissue Res.* 2016 Feb; 57(1):28–37. [PubMed: 26539896]
45. Yao X, Carleton SM, Kettle AD, Melander J, Phillips CL, Wang Y. Gender-dependence of bone structure and properties in adult osteogenesis imperfecta murine model. *Ann Biomed Eng.* 2013; 41:1139–1149. [PubMed: 23536112]
46. Glatt V, Canalis E, Stadmeier L, Buxsein ML. Age-related changes in trabecular architecture differ in female and male C57BL/6J mice. *J Bone Miner Res.* 2007; 22:1197–1207. [PubMed: 17488199]
47. Brodt MD, Ellis CB, Silva MJ. Growing C57Bl/6 mice increase whole bone mechanical properties by increasing geometric and material properties. *J Bone Miner Res.* 1999; 14:2159–2166. [PubMed: 10620076]
48. Battaglia TC, Tsou AC, Taylor EA, Mikic B. Ash content modulation of torsionally derived effective material properties in cortical mouse bone. *J Biomech Eng.* 2003; 125:615–619. [PubMed: 14618920]

HIGHLIGHTS

- Composition and morphology of mouse bones with a G610C collagen mutation were compared to WT.
- At 10d few changes in micro-CT or FTIRI measures in similar bone types in G610C mutants vs. WT.
- At 2mo G610C mutant cortical and trabecular bones were thinner with higher mineral content.
- At 2mo G610C mutant cortical and trabecular bones had altered composition.
- An Lrp5 high-bone-mass allele partly rescued the compositional phenotype in 3 mo-old femurs.

MICRO-CT TRABECULAR VARIABLES

Figure 1.

Micro-CT Trabecular Data: Top: Tibia variables; Bottom: Vertebral (L3) variables: BV/TV = bone volume fraction (%); Tb.N = trabecular number (mm^{-1}); Tb.Sp = trabecular separation (mm); Tb.Th = trabecular thickness (mm); TMD = tissue mineral density; Cn.Den = connectivity density (mm^{-1}). Bar colors: black *Coll1a2*^{+/+}; light grey *Coll1a2*^{+/G610C}. All values are mean $\pm$ SD, * $p < 0.05$ relative to same bone type, same age, different genotype, # $p < 0.05$ relative to same bone type, different age, same genotype; comparisons based on Wilcoxon Mann-Whitney test.

MICRO-CT CORTICAL VARIABLES

Tibia

L3-Vertebra

Figure 2.

Micro CT Cortical Data: Top: Tibia variables Bottom: Vertebral (L3) variables. BV/TV = bone volume fraction (dimensionless); Ct.Th = cortical thickness (mm); TMD = tissue mineral density (g/cc); pMOI = polar moment of inertia (mm⁴); BA = bone area (mm²); BA/TA = ratio of bone area to total area (dimensionless). Bar colors: black – *Col1a2*^{+/+}; light grey *Col1a2*^{+/G610C}. All values are mean ± SD, * p<0.05 relative to same bone type, same age, different genotype., # p<0.05 relative to same bone type, different age, same genotype; comparisons based on Wilcoxon Mann-Whitney test.

Figure 3.

Typical FTIR images of A) trabecular bone and B) cortical bone in *Col1a2*^{+/+} and *Col1a2*^{+/G610C} tibias at 2 months of age. The images have been standardized to the color bar to the right of each group of images for a given parameter. Min/mat = mineral-to-matrix peak area ratio; C/P = carbonate-to-phosphate peak area ratio; XLR= collagen cross-link intensity ratio; XST = crystallinity intensity ratio; HPO4= acid phosphate substitution (intensity ratio).

FTIRI TRABECULAR VARIABLES

Tibia

L3-Vertebra

Figure 4.

FTIRI variables in trabecular bone of the Tibia (Top) and L3 Vertebrae (Bottom). Min/mat = mineral-to-matrix peak area ratio; C:P = carbonate-to-phosphate peak area ratio; XST= crystallinity intensity ratio; HPO4 = acid phosphate substitution (intensity ratio), XLR = collagen cross-link (intensity) ratio. Bar colors: black – *Col1a2*^{+/+}, light grey *Col1a2*^{+/G610C}. All values are mean ± SD, * p<0.05 relative to same bone type, same age, different genotype. Based on GEE model; # p<0.05, 10 days vs. 2 months, same bone, same genotype, GEE model.

FTIRI CORTICAL VARIABLES

Tibia

L3-Vertebra

Figure 5.

FTIRI variables in cortical bone of Tibia (Top) and L3 Vertebrae (Bottom). There were no cortices on the 10-day-old vertebrae. Min/mat = mineral-to-matrix peak area ratio; C:P = carbonate-to-phosphate peak area ratio; XST= crystallinity intensity ratio; HPO4 = acid phosphate substitution (intensity ratio), XLR = collagen cross-link (intensity) ratio. Bar colors: black – *Col1a2*^{+/+}; light grey *Col1a2*^{+/G610C}. All values are mean ± SD, * p<0.05 relative to same bone type, same age, different genotype, based on GEE model; # p<0.05 10 days vs. 2 months, same bone, same genotype GEE model.

FTIRI 3-Month FEMUR VARIABLES

Trabecular

Cortical

Figure 6. FTIRI variables in femoral trabecular (Top) and cortical (Bottom) bone of *Lrp5*^{+/A214V};*Colla2*^{+/G610C} mice (white bars) compared to *Colla2*^{+/G610C} mice (grey bars) and *Colla2*^{+/+} mice (black bars). Min/mat = mineral-to-matrix peak area ratio; C:P= carbonate-to-phosphate peak area ratio; XST= crystallinity intensity ratio; HPO4 = acid phosphate substitution (intensity ratio), XLR = collagen cross-link (intensity) ratio. All values are mean ± SD, * p<0.05 relative to same bone type, same age, different genotype, compared to WT, based on GEE model. ** p<0.05 *Lrp5*^{+/A214V};*Colla2*^{+/G610C} mouse relative to *Colla2*^{+/G610C}, based on GEE model.

TABLE 1

Significant Changes at 2 months in Micro-CT Variables of Different *Col1a2*^{+/G610C} Bones compared to Wild Type Mice

	% Change		
	Femur*	Tibia	Vertebrae
BV/TV trab	-47	-29	-38
Tb.N.	-45	-13	-14
Tb.Th	nr	-11	-12
Tb.Sp	nr	20	11
Cn.Dens	nr	-16	-30
vBMD trab	0	nr	nr
BV/TV cort	-19	-18	-1.3
Ct.Th.	-13	ns	-37
Ct.Ar.	-20	-6.6	-24
BA/TA	nr	4.7	22
Ct.Iyy/Imax	-36	-10	-41
vTMC cort	-19	nr	nr
vTMD cort	1	3	3
Porosity	nr	ns	ns

* Differences between OI and WT calculated from values reported by Daley ET al⁵, compared to data measured in the current study. Only significant values are reported. ns= not significant; nr= not reported

Abbreviations: Trabecular (trab) BV/TV = bone volume fraction; Tb.N = trabecular number; Tb.Sp = trabecular separation, Tb.Th = trabecular thickness; Cn.Dens.=connectivity density; vTMD = volumetric tissue mineral density. Cortical (cort) Ct.Th=cortical thickness; Ct.Ar = cortical area; BA/TA=bone area/total area; Ct. Iyy= cortical moment of inertia in principal direction (also called Imax); TMC= total mineral content