Cortical Bone Micro-Porosity Varies Based on Genetic Mutation in Osteogenesis Imperfecta

Owen Blanchard[1,2], Camden Felgner[1,3], Chris Stephan1, Kenneth Kozloff[1,3], Departments of [1]Orthopaedic Surgery, [2]Biophysics, and [3]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA

Disclosures: Owen Blanchard (N), Kenneth Kozloff (N), Chris Stephan (N), Camden Felgner (N)

Introduction: Osteogenesis imperfecta (OI) is a genetic bone disease characterized by tissue hypermineralization, low bone mineral density, increased bone fragility, skeletal deformities, and imbalanced remodeling. Severity of OI is highly variable, resulting from a variety of genetic mutations. Intracortical porosity plays an important role in governing the biomechanical fragility of OI bone, with porosity arising from lacunar and vascular fractions influencing bone strength and stiffness [1]. The variation of intracortical porosity across multiple genetic forms of the disease has not yet been described. Therefore, the purpose of this study was to evaluate lacunar and vascular characteristics in cortical bone from several OI mice models with varying underlying mutation and severity using ultra-high resolution nanoCT.

Methods: Four OI mouse models, with heterozygous dominance, and their WT counterparts were chosen for this study. Jrt/+ represents severe OI (type III) resulting from a splice site mutation of the COL1A1 gene, and mice are raised on a FVB/NJ background strain. Brtl/+ represents moderate OI (type IV) due to a G349C substitution in COL1A1, and mice are raised on a mixed Sv129/CD-1/C57Bl/6S outbred line. MOV13/+ (Sv129/CD-1/C57Bl/6S) represents mild OI (type I) due to haploinsufficiency in COL1A1. Lastly, G610C/+ (C57Bl/6 background) represents moderate OI (type IV) from a G610C substitution in COL1A2. Left femora were harvested from twenty-four ten-week-old female mice (n=3/genotype). Following harvest, femora were bisected just proximal to the third trochanter, and using this anatomic feature as a common reference point, a 1 mm mid-shaft diaphyseal region of interest was imaged using a Zeiss Xradia Versa 520 3D X-ray Microscope at ~0.97 um isotopic voxel size. 16-bit tiff stacks were generated for each femur and imported into Dragonfly. To eliminate digital artifacts appearing at the distal and proximal ends of the bone, images were cropped to analyze a central 0.75 mm segment. A median smoothing filter was applied to each object image, and the filtered image was segmented with an upper and lower Otsu thresholding algorithm. A 100-2000 um^3 range for lacunar volume was selected based upon a study of mouse cortical bone by Hemmatian et al [2], while porosities greater than 2,000 um^3 were designated as the intracortical vascular canals [2]. Objects smaller than 100 um^3 were considered noise [2]. Femora were evaluated for lacunar density (#/volume intracortical space), average lacunar volume, lacunar volume fraction (# lacunar voxels/total intracortical voxels) and vascular volume fraction (# vascular voxels/vascular+intracortical voxels). Two-way ANOVA was used to evaluate effects of mouse background strain, genotype, and interaction between strain and genotype for each outcome measure. Where positive interactions were observed between strain and genotype, Sidak multiple comparison post-hoc testing evaluated genotype-specific effects.

Results: The total number of lacunae sampled ranged from 29,591 (WT-C57Bl/6) to 50,631 (Jrt/+) across the twenty-four mice (Figure 1). Lacunar Density (LD) demonstrated a significant interaction between strain and genotype ($p = 0.0013$) with Jrt/+ and G610C/+ showing significant genotype-dependent increases in LD compared to their WT controls (Jrt/+: $p = 0.0003$, G610C/+: $p = 0.0074$). In contrast, Brtl/+ and MOV13/+ had unchanged densities compared to their corresponding WT (Figure 2A). Average Lacuna Volume (ALV) showed significant differences across strain ($p < 0.0001$), but in contrast to LD measures, genotype-dependent variations were not observed in any of the groups (not shown). Similarly, there were no significant genotype-dependent variations in LVF or vascular volume fraction (not shown). However, differences across strain were present in both. When pooled across genotype and strain, we observed high linear correlation between lacunar volume fraction and vascular volume fraction ($p < 0.0001$, $R2 = 0.59$) suggesting a strong relationship between intracortical vascularity and osteocyte entrapment during bone formation (Figure 2B).

Discussion: Through sub-micron imaging, four main characteristics, LD, ALV, LVF, and VVF, describing the microscale and vascular porosity of cortical bone in OI mouse models were quantified. We observed strain-dependent variability in the LD, ALV, LVF, and VVF and genotype-dependent variability in the LD of cortical bone in different OI mouse models. A strong correlation between LVF and VVF was observed across strains and genotypes, suggesting a conserved mechanism of osteocyte deposition related to vascular innervation.

Significance: Fragility in osteogenesis imperfecta arises from a variety of factors reflecting macroscale features, bone material properties, and porosity at multiple hierarchical levels. Here, we observed significant differences in lacunar density due to Jrt/+ G610C/+ genotypes, but not in Brtl/+ or MOV13/+. Together, this data suggests that OI mutation may influence osteocyte density and suggests relationships between vascular innervation and osteocyte retention within the bone matrix.

References: [1]Albert C et al 2014 Bone 66:121. [2] Hemmatian H, et al, 2017, PLOS ONE 12(8): e0182996.

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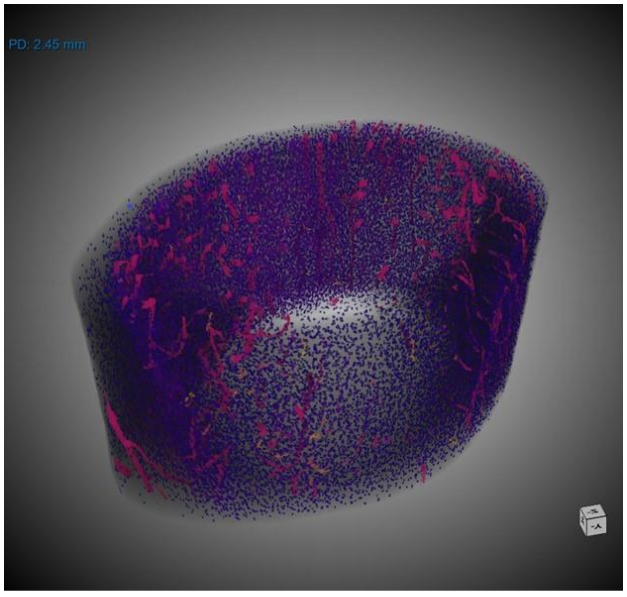

Figure 1: Dragonfly CT scan analysis highlighting lacunae and vascular channels. Translucent black space represents bone, a color spectrum between purple and yellow represents lacunae, and pink objects represent vascular channels.

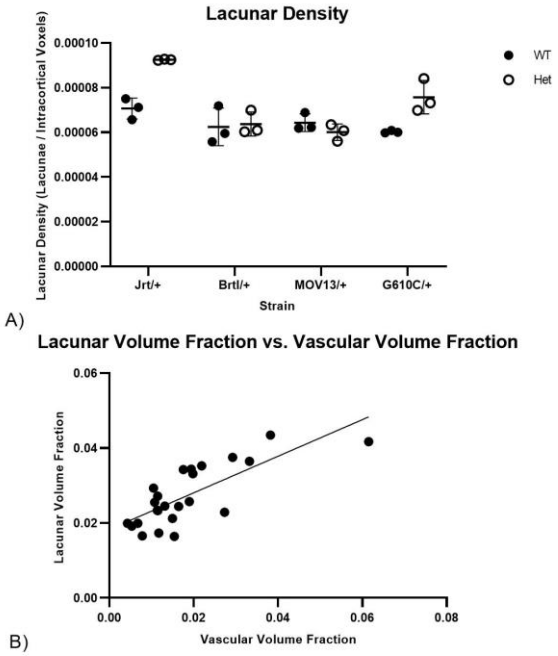

Figure 2: A) Lacunar density was influenced by genotype in Jrt/+ and G610C/+. B) Lacunar Volume Fraction and Vascular Volume Fraction are highly correlated ($p < 0.0001$, $R2 = 0.59$) across strain and genotype.