Micro CT Phenotyping of Degenerative Bone Disease in Mice and Horses

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Background
The ground-breaking studies of Jaenisch and Capecchi in the 1980s revealed the power of introducing specific and targeted modifications to the mouse genome to understand the aetiology and pathogenesis of human disease. More recently, the thickness of equine cartilage has been shown to most closely approximate that of humans, thus identifying the species as the model of choice for cartilage tissue engineering strategies. We have developed micro CT algorithms for the rapid and accurate assessment of cortical and trabecular bone in a murine model of osteoporosis and of degenerative changes in subchondral bone in early stage osteoarthritis in race horses.

Relevance
Up to 70% of the differences in human bone that impact on its remodeling, microarchitecture, biomechanical strength, susceptibility to fracture and capacity for repair have been attributed to differences in genetic background. In previous histological analyses conducted 15 years ago it was shown that C57Bl6 mice carrying one copy of the parathyroid hormone related protein (PTHrP+/−) gene developed osteopenia by four months of age (N. Amizuka et al Dev Biol 1996). Taking advantage of recent developments in micro CT we compared bone mass and architecture in PTHrP+/− mice on C57Bl6 and C3H backgrounds to determine if the ostepenic phenotype was maintained in C3H mice.

Methods
PTHrP+/− and PTHrP−/− mice on C57Bl6 and C3H backgrounds were euthanised at 4 months (young adult) and 12 months (old adult) of age. The femurs were harvested, fixed in 4% paraformaldehyde overnight and scanned at a resolution 5.5 μm on a Skyscan 1172 equipped with a 100kV X-ray source and a 10 megapixel camera. A short program written in custom processing mode of CTAn was developed to automatically separate cortical from trabecular bone. The amount and quality of cortical and trabecular bone was quantified in CTAn using 2D and 3D analysis; 3D reconstruction was done in CTvol and CTVox software (Figure 1).
Results
The custom program to automatically separate cortical from trabecular bone for independent quantification produced consistent and reproducible results. PTHrP+/− mice on the C57Bl6 background had less trabecular bone, which was of poorer quality, than their wild type counterparts at both 4 and 12 months of age. Furthermore, trabecular bone decreased over time in PTHrP+/− mice on the C57Bl6 background. In contrast, there was no difference in trabecular bone between PTHrP+/+ and PTHrP+/− mice on the C3H background at either age, nor did it decrease between 4 and 12 months in PTHrP+/+ mice. Cortical thickness, quantified by average bone area surface in integrated 2D analysis, was the same in PTHrP+/+ and PTHrP+/− mice at 4 and 12 months in mice on the C57Bl6 and C3H backgrounds and did not decline over time as seen for trabecular bone (Fig 2). However, the cortices of the C3H mice were consistently thicker than those of the C57Bl6 mice.

Conclusion
MicroCT represents a rapid, accurate and highly reproducible tool for high throughput, quantitative analysis of bone phenotypes in genetically modified mice. The osteopenic phenotype previously demonstrated in young adult PTHrP+/− mice on a C57Bl6 background was restricted to trabecular bone and exacerbated over time. The absence of osteopenia in PTHrP+/− mice suggests that some osteogenic agent can compensate for PTHrP haploinsufficiency during bone development and also protects the animals from age-related bone loss.

Figure 1: A custom program was developed to select cortical bone in the diaphysis and trabecular bone in metaphysis as the regions of interest (ROIs). Trabecular bone volume/tissue volume (BV/TV) was quantified on 3D reconstructions and the average bone surface area (B.Ar) on 2D images.

![Figure 1: Image showing Regions of Interest (ROI) for cortical bone in the diaphysis and trabecular bone in metaphysis.](image)

Figure 2. Panel A: Age-related trabecular bone loss in PTHrP+/- mice on C57Bl6 background. Trabecular bone volume decreases between 4 (blue) and 12 (red) months in C57Bl6 but not in C3H heterozygotes. Panel B: Cortical bone area does not decrease over time in heterozygotes on either background. C3H mice have significantly more trabecular and cortical bone than C57Bl6 mice at both ages.

![Figure 2: Bar graphs showing trabecular and cortical bone measurements.](image)
Micro CT Phenotyping of Degenerative Cartilage Disease in Horses

Relevance
Degenerative bone diseases, such as osteoporosis or cancer-mediated osteolysis, can be treated effectively with medications that inhibit the activity of catabolic cells in the bone micro-environment. In contrast, degenerative cartilage disease in articulating joints is viewed as a prime area for the development of tissue engineering strategies for regeneration and repair. Interventions with stem cells, scaffolds and biologics must first be validated for efficacy in a pre-clinical animal model of human disease. Like humans, horses develop spontaneous arthritis and the physical and mechanical properties of articular cartilage in equine joints are comparable to those seen in humans. However, little is known about the natural progression of osteoarthritis in either species, particularly if degeneration is initiated in cartilage and/or in sub-chondral bone. We therefore screened the third carpal bone of retired Standardbred race-horses euthanized at a nearby abattoir for micro CT and histological evidence of joint damage.

Methods
Osteochondral cores from the dorsal aspect of the third carpal bone were divided into those with no visible lesion (C control), those with partial thickness defects (EOA early osteoarthritis) and those with full thickness lesions (AOA advanced osteoarthritis) on the basis of macroscopic examination of the articular surface. Cores were fixed in paraformaldehyde and scanned on a Skyscan 1172 at 70 kV and 140 µA with a spatial resolution of 10 µm. Three to five ROIs were selected for quantitative analyses including BV/TV, bone mineral density (BMD), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp). 5µm histological sections were matched to the micro CT images and stained with Safranin O and Fast Green to assess cartilage integrity.

Results
A significant decrease in BMD (less mineral) and lower BV/TV was seen in bone underlying pits at the cartilage-bone interface in EOA and deep in subchondral bone in AOA when compared to remote sites in the same bone. Histological sections demonstrated increased proteoglycan and micro-cracks in the calcified cartilage in the EOA and AOA groups compared with the C group.
Conclusion
Micro CT and histological analysis of the third carpal bone of retired Standardbred racehorses support the premise that degeneration of cartilage and bone occur concurrently during the development of equine OA. Compared with traditional, labor intensive histological analyses (Young et al. Am J Vet Res 1991) quantitative 3D data of calcified cartilage and bone architecture adjacent to lesions on the articular surface provide a rapid and comprehensive assessment of bone architecture and integrity.