

# Variation in Cross-sectional Morphology in Adult Portuguese Water Dog Femora Across a Broad Age Range

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**INTRODUCTION:** Being able to predict the risk for fracture will aid in reducing fracture incidence [1]. While studies have been done to better understand cortical robustness throughout bone growth and aging and its use in predicting fractures [1,2], these studies have lacked genetic data and large sample sizes. Here we present the results of a study of femora from a large cohort of Portuguese Water Dogs (n=314). These Portuguese Water Dog (PWD) femora were obtained through the Georgie Project (<http://www.georgieproject.com>) [3,4]. PWDs were chosen because they have a pedigree that can be traced back to 31 founders through 24 generations. The sex, weight, and age at time of death are known for all the dogs in our study. We chose this cohort due to the availability of genetic data on the PWDs. The purpose of this study was to analyze the cross-sectional morphology of the PWD femur as a model to better understand age-related changes in robustness of the shaft (medullary, cortical, and total cross-sectional area), which has implications for the emergence of osteoporosis in humans. We sought to then determine if age-related patterns described in humans are also seen in our PWD sample [5,6]. If the patterns are present in our sample, are they strongly genetically linked and how applicable this is to humans?

**METHODS:** With IACUC approval, PWD carcasses were autopsied for various organ pathologies in prior studies [3,4] (n=314, age range 2-18 years, Male: n=130, Female: n=184, soft tissue manually removed). The right femur from each dog was examined for the presence of femoral head arthritis and cross-sectional morphology at 50% shaft length. Cross-sectional measurements included: Total cross-sectional area (TA), cortical area (CA), medullary area (MA) and total femur length (Ltot). Relative cortical area was calculated as CA/TA. All statistics were estimated using custom scripts in R(1) (<http://www.R-project.org/>). Pearson correlation coefficients were estimated using the 'cor' function, robust line fitting was done with the 'rlm' function. The significance of the sex difference was estimated using a two-sided, unpaired, unequal variance T test (the 't.test' function) (\* p < 0.05; \*\* < 0.01; \*\*\* < 0.001). A genetic estimate (the effect of clustering by genotype) was conducted on the current data set. Additional detailed genetic analyses are in progress.

**RESULTS:** As shown at the far left (quartiles) in Figs. 1-3, CA, MA, and TA are each significantly greater in males than females. In each sex and in the total sample, the age-related changes TA and MA were minor (no statistical significance detected). By contrast, CA significantly decreased in females and in the overall/total sample (p<0.001), and CA/TA significantly decreases in both sexes. When the data were re-analyzed with respect to differences in bone length (Ltot; as CA/Ltot; MA/Ltot; TA/Ltot) the age-related variations in each sex (and the total sample) for TA remained the same (no significant relationships) and MA showed a minor, though significant (p<0.05), decrease with age in females. Overall there was no strong influence of the presence of hip arthritis (see solid dots in the figures). Preliminary results of the genetics analysis are consistent with previous findings that there is a genetic component regulating the trade-off between limb-bone length and width in this population, and MA has the highest genetic component and TA is much lower. While CA has no genetic component, it is related to age but the age relationship seems to be different between males and females.

**DISCUSSION:** These results are compelling because they do not differ when compared to humans, especially in terms of cortical bone loss (in terms of CA). The robust genetic analysis that is currently underway in our laboratory on this PWD sample will help determine potential genetic bases for the variations shown here and whether or not they can be translated to human limb bones. Because of the bottle-neck early in the pedigree of PWD's there is less genetic variation within this breed than seen in other breeds and there is a simpler genetic architecture that is easier to elucidate. Canines provide a better model for human skeletal adaptation, as opposed to smaller mammals (like mice), due to their increased size and similar genetic disease phenotypes, in addition dogs have similar histomorphology (e.g., secondary osteons) and skeletal physiological responses to hormones. Additionally, as opposed to mice, our sample of canine femora mirror the sexually dimorphic pattern seen in human femoral bone loss [7,8]. PWDs are the ideal candidate because of their well-established pedigree and vast amount of genetic work that has already been done at our institution.

**SIGNIFICANCE/CLINICAL RELEVANCE:** The K.J. Jepsen research group at the U. of Michigan has established covariance between the external size, volume, mineralization, and stiffness of limb bones is not explained by sex or physique. The PWD pedigree holds promise for determining the strength of genetic linkages to these covariant characteristics within and between sexes.

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