Compelling Structural and Material Variations in Portuguese Water Dog Femora Hold Promise for Identifying Genetic Linkages Within and Between Sexes

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ABSTRACT INTRODUCTION: Recent studies show significant covariance between the external size, volume, mineralization and stiffness of limb bones that are not wholly explained by one's physique and sexual characteristics [1][2]. For example, in a study of human humeri, radii, second metacarpals, third metacarpals, femora, and tibiae, Jepsen and Schlecht found significant covariance between these characteristics that is not well explained by sexual or physical characteristics, suggesting that genetic factors might be more influential than environmental factors [1] (Fig. 1, taken from [3]). The genetic basis for this relationship has been looked at in mice and showed that genotype-specific difference account for variability in adult bone traits including cortical area, polar moment of inertia, and tissue mineral density[4] However, mice data do not mirror the between-sex differences shown in humans [5]. Consequently, more appropriate animal models are needed. The genetic basis between bone form and function can be difficult to evaluate because it requires large pedigrees with comprehensive DNA analysis, which is not currently possible in humans. Here we present initial results from such a study of femora from a large cohort of Portuguese Water Dogs. The Portuguese Water Dog (PWD) breed is well suited for genetic analysis as it has a well-established pedigree that can be traced back to 31 founders through approximately 24 generations. Through the Georgie Project (http://www. georgieproject.com), the breed has undergone intense genomic study at our institution, and DNA from over 1000 dogs for associations between quantitative trait loci (QTLs) and morphological variations have been analyzed [6]. So far QTLs within the PWD genome have been linked with Addison's disease [7], femoral joint laxity[8], and allometric relationships between the skull and limb bones[6]. The wealth of information available from the PWD also provides opportunities to search for a genetic correlation between bone structure and tissue characteristics in addition to traits of other organ systems. The purpose of this study is to report preliminary data that shows that even a subset (n=30) of our larger sample of femora (n=300) shows the existence of underlying structural and mineral content variations that would be missed if only population data based on sex and body mass distinctions are examined.

METHODS: PWD carcasses were autopsied for various organ pathologies according to prior studies [9]. A sample of 30 femora were randomly picked from our sample of femora (All: 6-17 years old, Mean: 12.6, SD: 2.84; Males (n=13): 6-14 y.o., Mean: 11.77, SD: 2.42; Female (n=17): 6-17 y.o., Mean: 12.47, SD: 3.16). Whole bone measurements included cervico-diaphyseal angle, total bone length, biomechanical length, head diameter, femoral head offset length, diaphyseal sagittal plane bow data, and proximal femoral anteversion angle [10]. 4-6 mm transverse cross sections were taken at the 30%, 50%, and 70% locations of the diaphysis. Each segment was digitally scanned for further cross section analysis, including cortical area, total area, and second moments of area. Pieces were cut from the anterior, posterior, medial, and lateral cortices from the 50% section for determination of ash percentage [11]. Statistical analysis included Spearman correlations, T-tests, and principal component analysis.

RESULTS: In general, mineralization and robustness variations in our sample are greater than expected. When using the entire sample, measures of cortical robustness (cortical area divided by total area) were negatively correlated with age at the 50% location (r= -462, p=0.01) and the 70% location (r= -0.547 and p=0.002). In females, cortical robustness and age were also negatively correlated at the 50% (r= -0.507, p=0.038) and 70% (r= -0.545, p=0.024) locations, however, correlations in males did not exceed significance thresholds but trended the same as females (Fig. 2). Average ash content was found to be statistically higher in males, at 68.4% ash content, than in females, with 67.4% ash content (p=0.012). Average ash was significantly positively related to cortical robustness with the suggestion of differences between males and females (Fig. 3). The preliminary data also suggests that ash content increases with age, with thyroid atrophy and with fibrosis in the liver but decreases with inflammation of the kidney, inflammation of the bowel and/or congestion in the liver.

DISCUSSION: At this time the sample size of 30 PWD femora was too small to assess heritability and genetic linkage, but the large amount of variation in our data is promising for the extension of the present study to our entire cohort. Because of the bottle-neck early in the pedigree of PWDs there is less genetic variation within this breed than seen in other breeds, therefore, there is a simpler genetic architecture that is easier to elucidate. To identify the genetic determinants and associate them with specific locations on the genome, our larger sample size will likely be sufficient. Canines provide a better model for human skeletal adaptation, as opposed to 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. 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. This study is the first step in establishing this population for comparative studies of the genetic vs. epigenetic and extra-genetic (e.g., functional usage) influences on bone adaptation and morphology.

SIGNIFICANCE: The K.J. Jepsen research group at 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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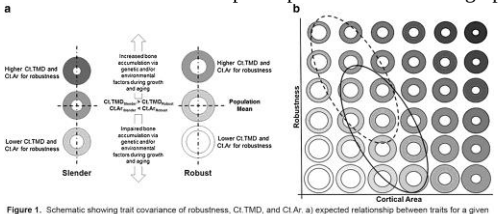

Figure 1. Schematic showing trait covariance of robustness, Ct.TMD, and Ct.Ar. a) expected relationship between traits for a given robustness. b) populational variance of trait sets demonstrating a narrow range that is predictable in relation to robustness. The solid line indicates the range of males within the study population, and the dashed line indicates the range of females. The overlap of the two ellipsoids represents the population average. Gray value differences in a) and b) represent the variation in mineralization, with lighter shades reflecting lower Ct.TMD and darker shades reflecting higher Ct.TMD.

	All		Male		Female	
	R value	p value	R value	p value	R value	p value
Ash vs. CA/TA*	0.380	0.032	0.460	0.114	0.352	0.166
Ash vs. J	-0.052	0.778	-0.229	0.453	-0.375	0.138
Ash vs. Zpol	0.011	0.951	-0.207	0.496	-0.336	0.188
Age vs. CA/TA*	-0.462	0.010	-0.402	0.173	-0.507	0.038
Age vs. J	0.007	0.971	0.111	0.719	0.067	0.798
Age vs. Zpol	-0.076	0.689	0.012	0.969	-0.016	0.953
Age vs. Ash	0.011	0.954	0.005	0.987	0.101	0.700
Body Weight vs. CA/TA*	0.112	0.556	-0.347	0.245	0.148	0.571
Body Weight vs. J	0.696	0.000	0.686	0.010	0.485	0.048
Body Weight vs. Zpol	0.733	0.000	0.719	0.006	0.510	0.037
Body Weight vs. Ash	0.159	0.401	-0.548	0.052	0.031	0.907
Body Weight vs. Age	-0.055	0.775	0.144	0.639	-0.037	0.888

Figure 2. Correlations at 50% location, including the R and p value, in all specimens, males only, and females only. *CA/TA: Cortical area/total area, measure of cortical robustness.

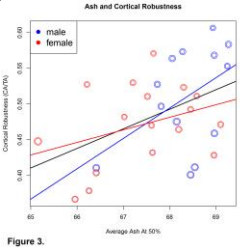

Figure 3.