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Autosome-wide linkage analysis of hip structural phenotypes in the Old Order Amish ☆

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ABSTRACT

Introduction: Fracture risk is associated with bone mineral density (BMD) and with other indices of bone strength, including hip geometry. While the heritability and associated fracture risk of BMD are well described, less is known about genetic influences of bone geometry. We derived hip structural phenotypes using the Hip Structural Analysis program (HSA) and performed autosome-wide linkage analysis of hip geometric structural phenotypes.

Materials and methods: The Amish Family Osteoporosis Study was designed to identify genes affecting bone health. BMD was measured at the hip using dual X-ray absorptiometry (DXA) in 879 participants (mean age ± SD = 49.8 ± 16.1 years, range 18–91 years) from large multigenerational families. From DXA scans, we computed structural measures of hip geometry at the femoral neck (NN) and shaft (S) by HSA, including cross-sectional area (CSA), endocortical or inner diameter (ID), outer diameter (OD) buckling ratio (BR) and section modulus (*Z*). Genotyping of 731 highly polymorphic microsatellite markers (average spacing of 5.4 cM) and autosome-wide multipoint linkage analysis was performed.

Results: The heritability of HSA-derived hip phenotypes ranged from 40 to 84%. In the group as a whole, autosome-wide linkage analysis suggested evidence of linkage for QTLs related to NN_Z on chromosome 1p36 (LOD = 2.36). In subgroup analysis, ten additional suggestive regions of linkage were found on chromosomes 1, 2, 5, 6, 11, 12, 14, 15 and 17, all with LOD > 2.3 except for our linkage at 17q11.2-13 for men and women age 50 and under for NN_CSA, which had a lower LOD of 2.16, but confirmed a previous linkage report.

Conclusions: We found HSA-derived measures of hip structure to be highly heritable independent of BMD. No strong evidence of linkage was found for any phenotype. Confirmatory evidence of linkage was found on chromosome 17q11.2-12 for NN_CSA. Modest evidence was found for genes affecting hip structural phenotypes at ten other chromosomal locations.

Introduction

Hip fractures cause significant morbidity and mortality in the elderly, and place a major economic burden on the health care system [1,2]. The individual and societal burdens from hip fracture are increasing as the population ages. In fact, the number of hip fractures is expected to increase by 50% in the U.S. from 2005 to 2025, if more aggressive efforts are not taken to treat individuals at risk [3]. The factors contributing to fracture risk are incompletely understood, but since osteoporosis is fundamentally a condition characterized by bones that are reduced in mechanical strength and thus susceptible to fracture, the risk factors must act by degrading mechanical strength. Bone mineral density (BMD) is commonly used in the clinical setting as a strength surrogate due to its strong statistical links with fracture risk, but this measure is structurally ambiguous in that any given BMD value can be produced in a range of different and realistic bone dimensions with different mechanical properties. This makes it difficult to identify from BMD the mechanical differences that might lead to reduced strength. Mechanical properties that are related to bone strength include structural stability, axial bone strength and bending strength, represented by the measurable phenotypes buckling ratio (BR), cross-sectional area (CSA) and section modulus (*Z*) respectively (4). One recent approach, implemented in the Hip

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Structural Analysis (HSA) program, infers geometric properties from two-dimensional images of bone, and derives structural/mechanical properties from these inferred three dimensional projections.

The HSA program was developed specifically to express the mineral mass from a dual-energy X-ray absorptiometry (DXA) scan of the proximal femur in a structural geometry format that can be interpreted using conventional engineering methods [4–6]. HSA used in previous studies has shown reduced hip geometry measures of bone strength (lower bending strength, thinner cortical width), in women with hip fracture compared to those without fracture [7]. In addition, aging is known to be associated not only with a reduction in BMD but also with decreases in HSA-derived cortical thickness, femur width and bending strength (section modulus) [8]. Previous studies have shown that hip geometry-derived measures of bone strength are associated with fracture risk and that this association is at least partly independent of differences in BMD [9–13]).

The significant contributions of genetic factors to osteoporosis and bone mineral density are well accepted [14–16] though the geometric differences underlying the BMD effect are unclear. There is growing evidence to support the importance of heredity on geometric components of bone strength, including suggestive linkages to quantitative trait loci influencing proximal femur geometry in reports of genome-wide linkage analysis from three different research groups [17–21,32]. In the most recent of these reports, the same HSA methods were used as in the current report [21,22]. In aggregate, these linkage studies suggest that genes distinct from those that influence BMD are determinants of hip geometry traits. In this study, we report heritability and autosome-wide linkage analysis using HSA-derived measures of hip geometric strength in a large family-based cohort of Old Order Amish.

Materials and methods

The Amish Family Osteoporosis Study (AFOS)

The AFOS was started in 1997 to identify genetic determinants of osteoporosis [23,24]. The Old Order Amish (OOA) population is an attractive one for genetic studies of complex traits because it is a closed Caucasian founder population with large families who live in close proximity and have a relatively homogeneous lifestyle. OOA study subjects believed to be at risk for osteoporosis by virtue of fracture history or prior BMD measurements were recruited. The diagnosis of osteoporosis was verified by measurement of BMD by DXA. Individuals with a *T* score of ≤−2.5 were identified as "probands". The spouses of probands as well as their first-degree relatives aged≥20 years were asked to participate. This report is restricted to 879 participants of AFOS who received DXA scans and for whom hip structural analysis was performed. Height and weight were taken in standard Amish dress without shoes. Height was measured using a stadiometer. The studies were conducted at the Amish Research Clinic in Strasburg, PA. The protocol was approved by the IRB of the University of Maryland School of Medicine. Informed consent was obtained from all participants.

Dual energy X-ray absorptiometry (DXA)

DXA scans of the hip and whole body were performed by a registered nurse certified in bone densitometry on a Hologic 4500 W scanner (Hologic Inc., Bedford, MA). Hip scans were exported for analysis by the Hip Structure Analysis (HSA) software at the Johns Hopkins University [4]. The coefficient of variation for the total hip BMD scans, determined by 3 sequential measures on one day for each of 15 individuals, was 0.90% and 0.71% for total hip and total body, respectively.

Hip structural analysis (HSA)

The HSA program uses a principle first described by Martin and Burr [6] that mineral mass profiles across the bone axis in a bone mass image are projections of the corresponding cross-section and can partially reveal the geometry of the cross-section. The principle was later modified by Beck et al. [4,5], to derive cross-sectional geometry from femur images acquired from DXA scans of the hip. The proximal femur cross-sections used in this report include the narrow neck (NN), defined as the narrowest point across the femoral neck, and the shaft (S) region located a distance of 1.5 times the width of the femoral neck distal to the intersection of the neck and shaft axes. The structural parameters derived by HSA at the NN and S regions of the hip included bone cross-sectional areas (CSA, cm^3), section modulus (*Z*, cm^3), outer diameter (OD), estimates of the endocortical or inner diameter (ID) and buckling ratio (BR). Outer diameter is measured as the blur corrected width of the bone mass profile. The bone CSA is the area of bone in the cross-section occupied by bone tissue and was measured from the integral of bone mineral in the profile assuming the average tissue mineralization of adult cortex. The section modulus was derived from the cross-sectional moment of inertia measured from the mass profile, divided by d_{max}. d_{max} is the maximum distance from the profile center of mass to the medial or lateral cortical margin. Resistance to stresses due to axial and bending loads is inversely related to the CSA and section modulus, respectively. Although as measured from DXA data, the section modulus only reflects bending resistance in the plane of the DXA image. The buckling ratio, an index of cortical stability was computed as the ratio of d_{max} to average cortical thickness. Average cortical thickness was estimated by assuming that the cross-section is a circular annulus with 60% and 100% of the bone CSA in the cortical shell for the narrow neck and shaft respectively [8]. The endocortical diameter is an estimation of the inside diameter of the cortex derived from the annulus model.

The coefficient of variation of HSA geometry using the Hologic QDR4500 has been reported for the narrow neck (NN) to be 3.0% for BMD, 3.0% for CSA, 4.0% for *Z*, 2.6% for endocortical diameter, and 4.6% for buckling ratio; for shaft (S), 2.2%, 2.3%, 2.5%, 2.8%, 3.5%, respectively [24,25].

To assess the effects of age and sex on hip geometry phenotypes, we initially computed mean values of each geometry-derived phenotype for men and women aged 20–50 years and aged 50 years and over. These two age groups were selected because individuals in the younger group are typically at their peak bone mass, while those in the older group typically experience some degree of bone loss. The effects of age and sex were assessed using analysis of variance. Pearsonian correlations were computed as a measure of the correlations among phenotypes.

Heritability calculation

Genetic effects were estimated simultaneously along with the environmental effects using a pedigree-based likelihood approach [26,27]. Only additive polygenic effects were estimated so that we defined heritability as the proportion of the total trait variance (σ^2_T) attributable to the additive effects of genes (σ^2_A) (i.e., "narrow sense" heritability; $h^2=\sigma^2_A/\sigma^2_T$). Estimation of the additive genetic heritability

Table 1
Mean values (±s.e.) of BMD and hip geometry variables according to age and sex

	Men		Women			
	20–50 (*n*=199)	>50 (*n*=140)	20–50 (*n*=301)	>50 (*n*=239)	Sex-adjusted *p*-value[1]	Age-adjusted *p*-value[2]
Age	38.3±0.5	65.7±0.8	37.6±0.5	65.4±0.6		
Height	67.6±0.2	66.2±0.2	63.5±0.1	61.7±0.2	<0.001	<0.001
Weight	168.4±2.0	170.1±2.6	155.7±1.9	155.9±2.3	0.46	<0.001
Neck						
BMD (g/cm^2)	0.833±0.008	0.729±0.011	0.843±0.007	0.678±0.010	<0.001	0.005
CSA	2.744±0.027	2.466±0.033	2.345±0.020	1.948±0.026	<0.001	<0.001
Z	1.522±0.018	1.374±0.023	1.105±0.011	0.915±0.014	<0.001	<0.001
BR	11.49±0.16	13.94±0.27	9.56±0.12	13.09±0.25	<0.001	<0.001
ID	3.151±0.019	3.294±0.023	2.607±0.014	2.778±0.016	<0.001	<0.001
OD	3.469±0.018	3.569±0.021	2.925±0.012	3.037±0.014	<0.001	<0.001
Shaft						
BMD (g/cm^2)	1.369±0.011	1.336±0.019	1.170±0.008	1.059±0.012	<0.001	<0.001
CSA	4.184±0.034	4.215±0.055	3.191±0.024	2.993±0.034	0.48	<0.001
Z	2.443±0.028	2.563±0.036	1.684±0.015	1.694±0.018	<0.001	<0.001
BR	3.414±0.056	3.663±0.093	3.580±0.035	4.354±0.080	<0.001	<0.001
ID	2.233±0.025	2.391±0.054	2.027±0.013	2.243±0.020	<0.001	<0.001
OD	3.218±0.019	3.345±0.045	2.864±0.010	2.982±0.013	<0.001	<0.001

[1]Age effect; [2]Sex effect; BMD = bone mineral density; CSA = bone cross-sectional area; *Z* = section modulus; BR = buckling ratio; ID = inner diameter; OD = outer diameter.