GENETIC ARCHITECTURE OF BONE STRENGTH RELATED PHENOTYPES: TOBAGO FAMILY HEALTH STUDY

by

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Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2007

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

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It was defended on

July 30th, 2007

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Abstract

Background: Populations of African ancestry have greater bone strength and lower osteoporotic fracture risk than other ethnic groups but there is little information about skeletal health among individuals of African heritage.

Methods: Univariate, bivariate and multivariate analytical methods under the variance components framework were employed to dissect the genetic and environment determinants for DXA and pQCT measured bone strength related phenotypes. Our analyses were performed on phenotypic and genotypic data on 471 individuals aged 18+ from 8 large, multigenerational Afro-Caribbean families.

Results: The major conclusions of this study are that (1) compared to Caucasians and African Americans, Afro-Caribbeans have the highest peak areal BMD and slowest bone loss rate, but heritabilities of many bone strength related traits are similar among different populations, and (2) genes and environmental factors differentially affect trabecular versus cortical traits, and also

BMD versus bone size. These conclusions are supported by differences in heritability and genetic correlation estimates among these bone categories, differential effects of environmental risk factors, as well as associations with different candidate genes. We also evaluated the capability of two multivariate analysis methods for uncovering underlying genetic factors using both simulated and real family data. We concluded Factor Analysis behaves better for both simulated and real data compare to Principal Component Analysis. The residual strategy increases the probability that composite phenotypes detect underlying genetic components if no gene-environment interaction is involved. And most importantly, composite phenotypes from multivariate analysis demonstrated their capabilities to capture more and stronger association signals in real data analysis.

Public health significance: Our work has identified the facts that environmental risk factors and genetic determinants may differentially affect various bone compartments and types of bone phenotypes. This information will contribute to the understanding of the underlying genetic architecture of osteoporosis and hence lead to better methods of treatment and prevention of the disease.

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PREFACE

At the beginning of my dissertation, I would like to take this opportunity here to express my sincere appreciations to the following organization, groups, and individuals.

This study was supported by Grants R03-AR050107 and R01-AR049747 from the National Institute of Arthritis and Musculoskeletal Diseases.

My greatest appreciations to my PhD committee chair Dr. Candace Kammerer and other committee members: Dr. Joseph Zmuda, Dr. Eleanor Feingold, Dr. Clareann Bunker and Dr. Stewart Anderson. In past five years of my PhD career, I was educated, impressed and inspired everyday by these outstanding scientists: their talented creativity, dedication and enthusiasm in research, as well as the science integrity and rigidness. Having advisors like them is a real treasure of my entire life.

I am deeply grateful for the cooperation of the families participating in the Tobago Family Health Project. Also many thanks to Dr. Alan Patrick, Dr. Victor Wheeler, all nurses and other staff members in Tobago research center.

I also want to express my special gratitude to my wife, Ms. Jiang Lu. Without your love and support, I wouldn't be the person I am today.

Thanks to all my former and current colleagues: John Shaffer, Margaret Kenney, Ankur Mukherjee from Dr. Kammerer's lab; Laura Yerges, Dr. Iva Miljkovic-Gacic, Dr. Susan Moffett, Yah-Tying Sheu, Jennifer Oakley, Alicia McDonald, Angelina Daily, Marie Wilkerson and Dr. Michael Okobia from Dr. Zmuda's lab.

Finally, thank you, my family members and all my friends.

1.0 INTRODUCTION

1.1 OSTEOPOROSIS

Osteoporosis is a systemic skeletal disease characterized by low bone mass and the microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fractures. (Consensus 1993) [3] The pathogenesis of fragility fracture almost always involves trauma and is not necessarily associated with reduced bone mass. Thus, fragility fracture should neither be used synonymously nor interchangeably as a phenotype for osteoporosis [2].

Osteoporosis affects the elderly, both sexes, and all racial groups. Although most American women under the age of 50 have normal bone marrow density (BMD), 27% are osteopenic (Osteopenia is low bone density. If not treated, it may result in osteoporosis.) 70% are osteoporotic at the hip, lumbar spine, or forearm by the age of 80 years. Epidemiologic studies from North America have estimated the remaining lifetime risk of common fragility fractures among white women aged 50 years to be 17.5% for hip fracture, 15.6% for clinically diagnosed vertebral fracture, and 16% for distal forearm fracture. The corresponding risks among men are 6%, 5%, and 2.5%, respectively. A British study using the General Practice Research Database estimated the lifetime risk of any fracture to be 53.2% at age 50 years among women and 20.7% at the same age among men (Table 1).

Several studies have suggested a wide geographic variation in hip fractures between as well as within countries. In general, people who live in latitudes farther from the equator seem to have a higher incidence of fracture [24]. The highest rates of hip fracture are seen in Caucasians living in northern Europe, especially in Scandinavian countries, where the age-adjusted 1-year cumulative incidence was 903/100,000 for women and 384/100,000 for men in Norway, 1989. The rates are intermediate in the populations of Asia, China, and Kuwait and lowest in black populations [23]. An Australian study concluded that although the increase in hip fracture rates during most of the past century may have ended, the number of admissions for hip fracture is still rising because of an aging population [25]. Many of the lower incidence rates in the developing countries can be partially explained by lower life expectancy; in Latin America only 5.7% of the population is over 65 [26]. Reduced longevity was also the explanation given for the low fracture rates observed in Morocco. Other papers have highlighted the poor appreciation of the role played by osteoporosis in fragility fracture; in one Lebanese study, fewer than 10% of hip fracture patients received any therapy for osteoporosis [27].

There also exists a striking health impact of osteoporotic fracture on mortality. All osteoporotic fractures are associated with significant morbidity, but both hip and vertebral fractures are also associated with excess mortality. Although this may represent complications of the fracture and subsequent surgery for hip fractures, it is likely to reflect comorbidity in persons experiencing vertebral fracture. By 2 years after hip fracture, mortality rates decline back to baseline except in elderly patients and among men. The four main predictors for higher mortality seem to be male sex, increasing age, coexisting illness, and poor functional status before fracture. The 5-year survival seems to be worse for men (72% 5-year survival) than for women (84% 5-

year survival). In women, a risk of death from cardiovascular and pulmonary disease that increases with increasing number of vertebral fractures has been observed [28].

Table 1. Estimated Risk of Fractures at Various Ages

	Current age (yr)	Any fractures (%)	Radius/ ulna (%)	Femur/ hip (%)	Vertebral (%)
Lifetime risk					
Women	50	53.2	16.6	11.4	3.1
	60	45.5	14.0	11.6	2.9
	70	36.9	10.4	12.1	2.6
	80	28.6	6.9	12.3	1.9
Men	50	20.7	2.9	3.1	1.2
	60	14.7	2.0	3.1	1.1
	70	11.4	1.4	3.3	1.0
	80	9.6	1.1	3.7	0.8
10-Year risk					
Women	50	9.8	3.2	0.3	0.3
	60	13.3	4.9	1.1	0.6
	70	17.0	5.6	3.4	1.3
	80	21.7	5.5	8.7	1.6
Men	50	7.1	1.1	0.2	0.3
	60	5.7	0.9	0.4	0.3
	70	6.2	0.9	1.4	0.5
	80	8.0	0.9	2.9	0.7

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1.2 ENVIRONMENTAL FACTORS RELATED TO OSTEOPOROSIS

A detailed understanding of the environmental risk factors for osteoporosis is important for several reasons: first, it may help us understand the pathophysiology of the disorder; Secondly, they will contribute to the clinic treatment of individual patients. Thirdly, they may help in design of preventative strategies against the disease.

According to one report [43], risk factors of osteoporosis can be grouped into different categories: those that influence the risk of falling and responses to trauma; those that influence the accretion and loss of BMD throughout the life course; and those that influence the skeletal strength independent of BMD. Table 2 summarizes the risk factors for osteoporosis and fracture [43].

In addition, race (being Caucasian or Asian), inactive lifestyle, presence of insulin-dependent diabetes also been suggested by many reports as risk factors [1, 108].

1.3 HERITABILITY OF OSTEOPOROSIS

Although environmental factors play a very important role in osteoporosis, genetic influences appear to have a larger effect. Studies in twins and families indicate that genetic factors play an important role in the regulation of BMD and other determinants of osteoporotic fracture risk. The heritability of BMD has been estimated to lie between 50% and 85% in twin studies, with the strongest effects in the axial skeleton). Family-based studies have also yielded strong heritability estimates for BMD, with effects that are maximal in young adults and persist even after adjusting for lifestyle factors that are known to regulate BMD. Other determinants of osteoporotic fracture risk also have a heritable component, including: femoral neck geometry and hip axis length, ultrasound properties of bone, biochemical markers of bone turnover 8), body mass index, muscle strength, age at menarch and age at menopause [46]. Table 3 shows the heritability estimation for some of the bone related traits.

Table 2. Risk Factors for Osteoporosis and Fracture

Age
Female Sex
Body Mass Index
Maternal Family History of Hip Fracture
Prior Fragility Fractures
Low Bone Mineral Density
Low Birth Weight
Genetic Factors
Sex Hormore
Premature Menopause
Primary or Secondary Amenorrhoea
Primary and Secondary Hypogonadism in men
Disease States
Thyrotoxicosis, Cushing's disease, Hyperparathyroidism
Stroke, inflammatory Arthritides
Drugs
Corticosteroids
Anti-convulsants
Heparin
Smoking
Alcohol
Dietary Calcium and Vitamin D deficiency

Heritability of Bone Mass: Studies over the last 30 years in healthy twins consistently demonstrate a large genetic contribution to bone mass even into old age. A number of family studies using healthy parent-children pairs, healthy sister pairs, and parent-child pairs in whom the parent had osteoporosis have corroborated the major role of genes in determining bone mass. Furthermore, heritability of bone mass can be detected during childhood even though the skeleton is undergoing major changes in both skeletal size and mass. In addition, it was found by some scientists that some of the sex differences in bone mass are accounted for by gender-specific genes [47, 48].

Heritability of Bone Size and Structure: Height and other anthropometric variables related to skeletal size have been known for many years to be highly heritable. In the early reports on the heritability of bone mass, variables related to bone size and structure such as forearm width, phalangeal cortical and medullary width were shown to be as highly heritable as bone mass. More recent reports examining structural variables that are important in maintaining bone strength at skeletal sites where osteoporotic fracture is common, highlight the strong heritability of bone structure at both the hip and at the spine. Hip axis length measured from DXA images and femur axis length measured from radiographs are both highly heritable and predict risk for fracture at the hip [49-51].

Heritability of Bone Loss: In contrast to the extensive studies on heritability of bone mass, few studies on heritability of bone loss have been reported. There are no current studies that powerfully address the important question of whether the rate of bone loss is heritable at skeletal sites where osteoporotic fracture is common [2].

Table 3. Heritability Estimation of Bone Related Traits

	Phenotype	Sibling Pairs (n)	H^2
DXA			
	Lumbar Spine BMD	425	0.89
	Femoral Neck BMD	425	0.77
	Hip BMD (Chinese)	1031	0.71
pQCT			
	Trabecular BMD	244	0.73
	Femurs BMD (Female)	986	0.83
	Vertebrae BMD (Female)	986	0.72

Heritability of Bone Turnover: Bone formation and resorption can be assessed from a number of biochemical markers in blood and urine. These vary with age, race, and disease and its treatment. Formation and resorption markers show a strong correlation among themselves. These markers do not measure bone mass or structure but in some circumstances may reflect bone gain or bone loss. In elderly white women, they may also predict hip fracture. Their relationship to bone strength is tenuous. Nevertheless, a number of studies have reported that there is a heritable component to bone turnover markers although with little consistency in the turnover markers across studies and little corroboration among markers within studies [52].

Heritability of Fracture: The heritability of fracture, as expected with such a complex phenotype, is not strong. In a large prospective study of white American women 65 years of age or older, a history of hip fracture in their mother doubled the risk of hip fracture. Although there appears to be a genetic component to fragility fracture, it seems equally likely from these studies that the underlying genes may not relate to bone strength but to the risk factors for falls, which is also highly heritable [53].

Heritability of Falls: The number of falls along with decreased bone strength, age, and previous fracture are major predictors of hip fracture. Falls are a highly complex phenotype with multiple environmental risk factors. Although falls also have a heritable component, the susceptibility genes for fracture resulting from falls are unlikely to relate to the genes underlying bone strength [54, 55].

It should be noted that the residuals heritability estimates cited above may not accurately reflect the actual percentage of the bone traits that is due to genetic factors but, rather, may reflect the proportion of total variance (after removing the effect of measured covariates) in these traits attributable to genetic factors. Moreover, in most of the cases, the assumptions do not take

into account of gene–gene or gene–environment interactions and can result in artificially inflated estimates of a genetic contribution due to greater sharing of environmental influences. Further more, high heritability does not ensure large individual gene effect.

1.4 STUDY APPROACHES FOR OSTEOPOROSIS GENES

Statistical genetic strategies used to identify and characterize genes that are involved in the pathogenesis of polygenic disorders like osteoporosis include heritability estimation, genetic correlation calculation, linkage analysis, candidate gene studies, and experimental crosses in animals. Heritability estimation and genetic correlation, which dissect the genetic impact on total phenotypic variation, are preliminarily exploratory work before any downstream research. In essence, the rest of these approaches involve looking for evidence of an association between a phenotypic characteristic and a series of polymorphic genetic markers. The phenotypic characteristic may be a continuous variable such as BMD or may be a categorical variable such as fracture. More interestingly, we can even use “composite traits” generated by PCA (Principal Component Analysis). The genetic markers used in these studies are polymorphic regions of DNA which are analyzed by PCR-based techniques on DNA extracted from peripheral blood. There are two main types of marker: repeat polymorphisms of variable length (Microsatellites) and single nucleotide polymorphisms (SNPs). Genetic studies involve typing a large number of markers spread at regular intervals throughout the genome (a genome search), or typing markers that are concentrated in specific areas of interest (candidate loci) or specific genes of interest (candidate genes). Regions of chromosomes that contain alleles that influence continuous phenotypic traits such as BMD are termed quantitative trait loci (QTL). The power to detect a

QTL contributing to a multifactorial phenotype like the traits associated with osteoporosis is directly proportional to the magnitude of the specific effects of the QTL. The genetic tools commonly used for QTL identification include: whole genome linkage scans in families, sib pairs and experimental animals. More recently, researchers have suggested that genome-wide linkage disequilibrium mapping with SNPs in unrelated individuals may provide an alternative approach to QTL localization, although the feasibility of this remains unclear (Tenesa *et.al,* 2003*)*. Candidate gene approaches have also been widely used in the search for osteoporosis susceptibility genes. While candidate gene studies are in some respects more powerful than linkage-based approaches for the study of complex diseases, they are also prone to give false positive results due to population admixture, or biased selection of cases and controls. In view of this, associations should be regarded as provisional, pending replication in other populations or confirmation by techniques that use family-based controls such as the transmission disequilibrium test (TDT). TDT is based upon the assumption that if a given allele contributes to disease, then the probability that an affected person has inherited the allele from a heterozygous parent should vary from the expected Mendelian ratio of 50:50. Although the TDT test has been considered as a 'gold standard' for confirming the results of association studies, recent experience indicates that even this approach may yield results that are not reproducible [(46)].

1.4.1 Genetic, Environmental and Phenotypic Correlation

In genetic studies it is necessary to distinguish two causes of correlation between characters, genetic and environmental. The genetic cause of correlation is chiefly pleiotropy. Pleiotropy is simply the property of gene whereby it affects two or more characters, so that if the gene is segregating it causes simultaneous variation in the character it affects. The degree of correlation

arising from pleiotropy expresses the extent to which two characters are influenced by the same genes. But the correlation resulting from pleiotropy is the overall, or net effects of all the segregating genes that affect both characters. Some genes may increase both characters, while others increases one and reduce the other; the former tend to cause a positive correlation; the latter a negative one. So pleiotropy does not necessarily causes a detectable correlation. The environment is a cause in so far as two characters are influenced by the same differences of the environmental conditions. Again, the correlation resulting from the environmental causes is the overall effect of all the environmental factors that vary; some may tend to cause a positive correlation, others a negative one. The association between two characters that can be directly observed is the correlation of phenotypic values, or the phenotypic correlation. This is determined from the measurements of the two characters in a number of individuals of the population. It can be shown that the phenotypic covariance is the sum of the genetic and environmental covariance, and the genetic and environmental causes of correlation combine together to give the phenotypic correlation [(59)].

1.4.2 Linkage Analysis

Linkage analysis dissects the inheritance of the disease or defined phenotype in relation to cosegregation of the polymorphic genetic markers within a pedigree. It looks for co-inheritance of phenotype and/or genotype in related populations. This procedure can be carried out with a series of polymorphic genetic markers. In parametric linkage analysis, the use of the wrong model can lead to false linkage or more likely can miss the linkage signal. On the other hand, Nonparametric (or model-free) linkage methods make fewer assumptions about the trait model. although the assumption that the marker locus model(s) is known without error is still in force. It

is less suitable using the parametric linkage analysis for the study of complex diseases such as osteoporosis where multigeneration families are difficult to come by and the mode of inheritance for the disease is unclear [2].

Several genome-wide linkage scans have been performed in attempts to identify loci that regulate BMD. A variety of study designs and analytical methods have been used, including analysis of families with a history of osteoporosis, families or sibling pairs drawn from a population, and sibpairs who are discordant for BMD values. Some important QTL for BMD identified by genome-wide linkage scan are summarized in Table 4. Few of the genome-wide scans so far performed have identified QTL that meet the criteria for genome-wide significance, and there has been limited replication of QTL between different studies. Moreover, only one gene that regulates susceptibility to osteoporosis has been identified by this approach: the BMP2 gene, which encodes bone morphogenic protein 2 – an important regulator of osteoblast differentiation. Some important findings have emerged from these studies, however, including the realization that QTL for regulation of BMD differ at different skeletal sites, are gender specific, and may also be age group specific. The lack of replication between studies may simply reflect the fact that genes that regulate BMD differ in different populations or that genes that predispose to osteoporosis have modest effects, which are difficult to detect by conventional linkage analysis. Technical advances such as the development of densely spaced panels of single nucleotide polymorphisms for genome-wide scans are likely to improve the chances of detecting genes of modest effect size in the future. There is also a prospect that meta-analysis of genome-wide scans may reveal significant QTL that have not been detected by individual studies [56, 58].

1.4.3 Candidate Gene Association Studies

Candidate gene association studies in osteoporosis have logically tackled the main regulators of bone metabolism, such as calciotrophic hormones, bone matrix proteins, steroid hormones and local regulators of bone metabolism. Relevant information on candidate genes which have been studied so far in relation to the genetics of osteoporosis are summarized in Table 5, together with a more detailed discussion below of specific candidate genes which have been the most extensively investigated.

Vitamin D Receptor (VDR): The active metabolites of vitamin D play an important role in regulating bone cell function and maintenance of serum calcium homeostasis by binding to the VDR and regulating the expression of a number of response genes [62-64].

Type I collagen: Type I collagen is the major structural protein of bone, thus the genes encoding this protein (COLIA1 and COLIA2) are candidates for the genetic regulation of bone mass.

Table 4. Chromosomal Regions Implicated in Genome-Wide Linkage Scans of BMD

Region	Locus	Phenotype(s)	LOD	Ethnicity	Comments	Reference
1p36		Femoral Neck BMD	3.53	Caucasian (US)	42 families (N=254)	Devoto, 2001
1p36		Whole Body BMD	2.40	Caucasian (UK)	1094 female twin pairs (N=2188)	Wilson, 2003
1q		Spine BMD	4.3	Caucasian (US)	938 sisters	Econs, 2004
1q21-23		Spine BMD	3.64	Caucasian (US)	464 sister pairs (N=706)	Koller, 2000
2p23-24		Hip BMD	2.25	Caucasian (US)	7 pedigrees with low BMD (N=149)	Devoto, 1998
2p21-24		Forearm BMD	2.15	Chinese	96 nuclear families (N=218)	Niu, 1999
2p25		Femoral Neck BMD	3.98	Mexican American	29 pedigrees (N=664)	Kammerer, 2003
3p21		Spine BMD	2.1-2.7	Caucasian (UK)	1094 female twin pairs (N=2188)	Wilson, 2003
4p		Forearm BMD	4.33	Mexican American	29 extended pedigrees (N=664)	Kammerer, 2003
4q31		Spine BMD	3.08	Caucasian (US)	53 extended pedigrees (N=630)	Deng, 2002
4q32		Wrist BMD	2.26	Caucasian (US)	53 extended pedigrees (N=630)	Deng, 2002
4q34		Hip BMD	2.95	Caucasian (US)	7 pedigrees with low BMD (N=149)	Devoto, 1998
5q33-35		Femoral Neck BMD	2.23	Caucasian (US)	464 sister pairs (N=706)	Koller, 2000
6p11-12		Spine BMD	2.13	Caucasian (US)	464 sister pairs (N=706)	Koller, 2000
6p21		Femoral Neck BMD	2.93	Caucasian (US)	330 extended pedigrees (N=1557)	Karasik, 2002
8q24		Wards BMD	2.13	Caucasian (US)	330 extended pedigrees (N=1557)	Karasik, 2002
10q21		Femoral Neck BMD ^	4.42	Caucasian (UK)	715 families (N= 3691)	Ralston, 2005
10q26		Hip BMD	2.29	Caucasian (US)	53 extended pedigrees (N=630)	Deng, 2002
11pter-14		Whole Body BMD	2.08	Caucasian (UK)	1094 female twin pairs (N=2188)	Wilson, 2003
11q12-13	*LRP5*	Spine BMD	5.74	Caucasian (US)	Single extended kindred	Johnson, 1997
11q24		Spine BMD	2.08	Caucasian (US)	7 pedigrees with low BMD (N=149)	Devoto, 1998
12q23		Spine BMD	2.08	Caucasian (US)	330 extended pedigrees (N=1557)	Karasik, 2002
12q24		Forearm BMD	2.53	Mexican American	29 extended pedigrees (N=664)	Kammerer, 2003
12q24		Spine BMD	2.96	Caucasian (US)	53 extended pedigrees (N=630)	Deng, 2002
13q14-22		Trochanter BMD	3.46	Mexican American	29 extended pedigrees (N=664)	Kammerer, 2003
13q		Femoral Neck BMD	2.51	Mexican American	29 extended pedigrees (N=664);	Kammerer, 2003
13q33-34		Spine BMD	2.43	Caucasian (US)	53 extended pedigrees (N=630)	Deng, 2002
14q31		Spine BMD	2.08	Caucasian (US)	330 extended pedigrees (N=1557)	Karasik, 2002
14q		Trochanter BMD	3.5	Caucasian (US)	774 sister pairs	Peacock, 2004
15q		Femoral Neck BMD	4.3	Caucasian (US)	774 sister pairs	Peacock, 2004
16p12-q23		Spine BMD	2.11	Caucasian (UK)	1094 female twin pairs (N=2188)	Wilson, 2003
18p11		Spine BMD ^^	2.83	Caucasian (UK)	715 families (N= 3691)	Ralston, 2005
20p12.3	*BMP2*	Spine+Hip BMD	4.93	Icelandic	207 extended pedigrees (N=1323)	Styrkarsdottir, 2003
21q		Trochanter BMD	3.14	Caucasian (US)	330 extended pedigrees (N=1557)	Karasik, 2002
22q12-13		Spine BMD	2.13	Caucasian (US)	464 sister pairs (N=706)	Koller, 2000

*Only QTLs with a LOD score ≥2.0 are shown in the Table.

Indeed, deletions or point mutations in these two genes have been identified as the molecular basis of up to 90% of cases of osteogenesis imperfecta, a hereditary disease characterised by osteoporotic bone and skeletal fracture in early life [65, 66].

Estrogen receptor (ESR): Estrogen, by interacting with its receptors in bone and other tissues plays an important role in regulating skeletal growth and maintenance of bone mass. Knockout mice with null alleles at the ESR locus have reduced BMD compared with wild-type controls. Osteoporosis has also been observed in man with an inactivating mutation of the ESR gene [67, 68].

Transforming growth factor beta (TGF): A rare polymorphism in intron 4 of TGF-1 has been associated with very low BMD and osteoporotic fracture in one study but the effects on TGF function are unclear. Another polymorphism, a C-T transition in a TGF-1 coding region, has been described which causes a leucine-proline substitution at amino acid 10. The C allele was associated with high BMD and a reduced frequency of osteoporotic fractures in two Japanese populations [69].

Androgen receptor (AR): A polymorphic (AGC)n repeat polymorphism has been described in exon 1 of the AR, which codes for a polyglutamine tract in the activation domain of the receptor. Length variations in the polymorphism have previously been associated with differences in receptor function and large expansions of the tract have been found to cause X-linked spino-cerebellar muscular atrophy [70].

Other Genes [71-75]

Osteocalcin: Dohi *et al.* described a C_T transition in the gene promoter of the osteocalcin gene that was related to BMD in a Japanese population but Sowers *et al.* (1999) found no association between this polymorphism and BMD or circulating osteocalcin levels in an American population.

Apolipoprotein E (ApoE): The human ApoE gene is polymorphic, with three common alleles (2, 3, 4) coding for three isoforms (E2, E3, E4) which differ from each other by a single amino acid and in their binding affinity for the four ApoE receptors. Shiraki *et al.* reported that BMD values were significantly reduced in Japanese women who carried the ApoE4 allele and a recent study in American Caucasian women found an association between the presence of at least one ApoE4 allele and hip fracture.

α2-HS-glycoprotein: α2-HS-glycoprotein (AHSG) is a serum-derived protein that binds to bone matrix due to its affinity to hydroxyapatite. Zmuda *et al* studied the relationship between a coding polymorphism of AHSG and osteoporosis in Caucasian women from the USA. The polymorphism (AHSG*1 or AHSG*2) was not significantly related to hip, lumbar spine or calcaneal BMD but, compared with the homozygous AHSG*2 women, calcaneal broadband ultrasound attenuation (BUA) was 13% lower in heterozygous and 16% lower in homozygous AHSG*1 women. Height was also reduced in homozygous AHSG*1 women, intermediate in heterozygous women, and highest among homozygous AHSG*2 subjects. These results suggest that the AHSG polymorphism may contribute to the genetic influence on calcaneal BUA and height.

Interleukin-6 (IL-6): Two studies have looked for evidence of an association between BMD and polymorphisms at the IL-6 locus. An AT-rich minisatellite repeat was associated with lumbar spine BMD in one study and a polymorphic AC-rich minisatellite was reported to be associated with wrist BMD in another. The mechanisms by which these polymorphisms affect IL-6 gene function are unclear, but it is possible that they could be mediated by linkage disequilibrium with a functional polymorphism that is known to be present in the IL-6 promoter.

Table 5. Candidate Genes Studies in Osteoporosis

Candidate	Function	Association	Reference
VDR	Calcium Absorption, Osteoblast-Osteoclast activity	BMD; Cacium absorptionMorrison Serum Osteocalcin Level;Fracture	Morrison et.al 1992 Garnero 2005
ESR	Osteoblast-Osteoclast activity	BMD	Sano 1995, Kobayashi, 1996
AR	Osteoblast Function	BMD	Sowers 1999; Lindberg 2005
PTH	Calcium homeostasis Osteoblast-Osteoclast activity	BMD	Hosoi et.al 1999
Calcitonin Receptor	Osteoclast Function	BMD Vertebral Fracture	Masi 1998, Taboulet, 1998 Munoz-Torres 2004
Peroxisome Proliferators--Activated Receptor Γ	Adipocyte Differentiation	BMD	Ogawa et.al 1999
COLIA1	Matrix Component	BMD; Vertebral Fracture; Hip Fracture	Grant et.al, 1996 Todhunter, 2005
α2-HS-Glycoprotein	Matrix Component	Heel ultrasound	Zmuda et.al, 1998 Liu et.al, 2003
Osteocalcin	Matrix Component	BMD	Dohi et.al, 1998
TGFβ-1	Osteoblast-Osteoclast activity Serum TGFβ level	BMD; Vertebral Fracture;	Grainger et.al, 1999 Mendez-Davila 2004
IL-6	Osteoclast Activity	BMD	Murray 1997, Todhunter, 2005
IL-1RN	Osteoblast-Osteoclast activity	Postmenopausal Bone Loss	keen et.al, 1998
ApoE	Vitamin K Transport	BMD, Hip Fracture	Cauley 1999 Long 2004

Calcitonin receptor: A coding polymorphism causing a proline-leucine substitution at codon 436 of the calcitonin receptor gene has been described. The relationship between this polymorphism and BMD has been studied in French and Italian populations. Masi *et al.* (1998)

reported that individuals homozygous for the leucine substitution had reduced bone mass when compared with heterozygotes and praline homozygotes. Taboulet *et al.* (1998) reported an association between BMD and this polymorphism, and found that heterozygotes had higher BMD and a reduced risk of fracture when compared with homozygotes. The effects of this polymorphism on calcitonin receptor function has not yet been studied.

1.4.4 Experimental Cross Animals

To identify QTLs that affect BMD, investigators have crossed inbred animals from a strain that shows increased susceptibility to the disease. Linkage studies and allele sharing studies can then be performed in the large number of the F2 progeny. Linkage studies in mice have resulted in the identification of several QTL that regulate BMD in mice, and the same approach has been used to localize QTL for other phenotypes relevant to the pathogenesis of osteoporosis such as circulating levels of insulin-like growth factor-1, bone structure, bone shape, and bone strength [(76)]. Most investigators have focused on the detection of QTL for BMD regulation, and such loci have now been identified on almost all mouse chromosomes, with replication of several QTL across different strains. The studies in mice have clearly shown that the genes that regulate BMD have effects that are site specific and gender specific [(77, 78)]. Identifying the genes and genetic variants responsible for these effects is technically challenging because mouse QTL regions are usually large, requiring successive rounds of selective breeding to narrow the region of interest to a manageable size. Even then, identification of the causal variants remains difficult because the predisposing genetic variant is in complete linkage disequilibrium with all adjacent variants on the same chromosomal segment [(79, 80)].

1.5 BONE QUALITY AND ASSESSMENT OF BONE QUALITY

1.5.1 Bone Quality

Bone is a geometrically complex and composite material characterized by an elaborate array of mechanical properties [81]. As such, there is no single property that is adequate to describe "bone strength" [82]. Although areal BMD is a continuous variable, an operational definition of osteoporosis defines the disease as a BMD below 2·5*SD from the mean BMD of young adults for skeletal sites such as lumbar spine or proximal femur as evaluated by dual energy X-ray absorptiometry (DXA). This phenotypic trait is most commonly used in studies evaluating heritability or polymorphic gene markers of osteoporosis. However, in order to understand the genetic basis for decreased bone strength, and ultimately osteoporotic fractures, one might eventually need to assess the inheritance of, and identify the specific genes associated with, a multitude of skeletal and extraskeletal traits, such as bone size, shape and microarchitecture, body weight (the single most influential variable correlated with BMD), muscle strength, biochemical variables of calcium and phosphate homeostasis, ovarian function, etc. The clinical expression of osteoporosis is the skeletal fracture. A fracture is a stochastic event which is determined by both bone-related factors (mass, size, architecture, microarchitecture, intrinsic properties of bone material) and bone independent factors (falls, protecting responses, soft tissue padding, etc.). The latter may have their own heritable and non-heritable components, which increases even further the complexity of the genetic determination of osteoporotic fractures [83].

The operational definition of bone quality is proposed to be the "totality of features and characteristics that influence a bone's ability to resist fracture" [82], that is the set of

characteristics that influence bone strength. The Bone Quality Framework was developed as a means of describing how bone strength is determined by the overall quality of bone (structural and material properties), which is affected by the rate of bone turnover (Figure 1). The structural properties of bone include its geometry (size and shape) as well as its microarchitecture (trabecular architecture and cortical thickness/porosity). The material properties of bone include its mineral and collagen composition as well as the number, size, and localization of micro-damage. The bone turnover rate is a function of the bone renewal process (modeling and remodeling) in which old or damaged bone is resorbed and new bone is created to replace it [(22)].

1.5.2 Structural Properties

Geometry: The size of bone appears to have an effect on overall fragility. Vertebral bone size has been found to be reduced in women with spinal fractures, and 50% of the deficit in bone mineral content is the result of a reduction in bone size. Smaller bone size was also observed when patients with spinal fractures were matched with controls with the same areal BMD. Similarly, femoral strength is partly a function of the hip axis length, which could be used as a marker for the ability of the femur to absorb the impact of a fall. The geometry of bone affects the distribution of bone mass. Changing the distribution of mass can change the ability of bone to resist bending and torsion. This change would not, however, be reflected in a BMD measurement [(84)].

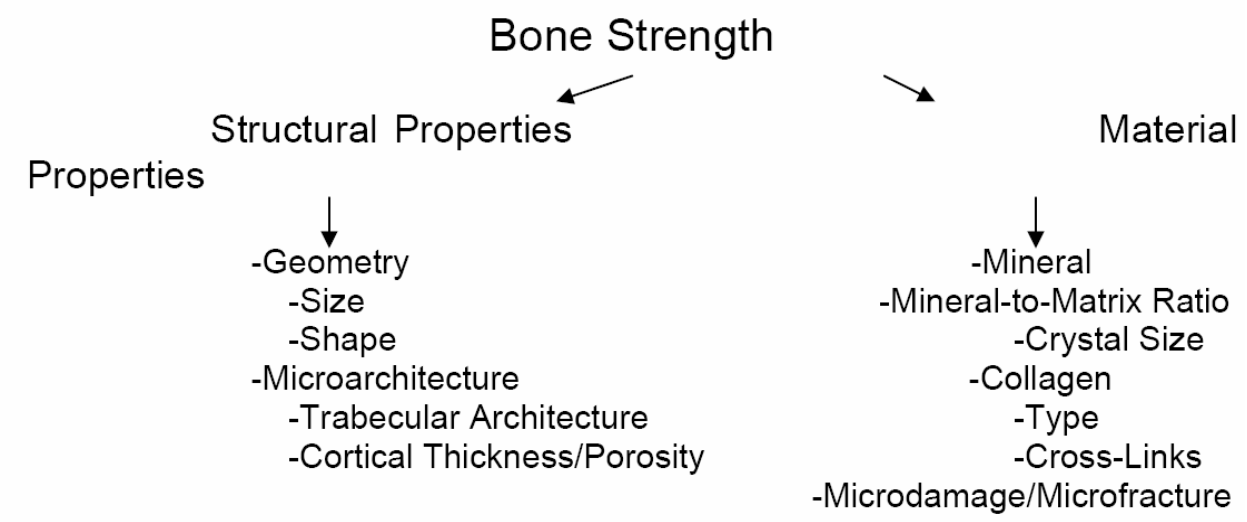

Figure 1. *The Bone Quality Framework*

Microarchitecture: At the tissue level, the microarchitecture of bone is an important structural property. Microarchitecture can be understood in terms of trabecular architecture (which itself can be understood as the orientation, thickness, and spacing of the trabeculae, as well as the extent to which the trabeculae are interconnected) and cortical thickness and integrity. From a mechanical point of view, trabecular failure (buckling and bending) occurs if there has been a reduction in trabecular elements that are perpendicular to the direction of the load. In terms of cancellous architecture, bone that is distributed as widely separated, disconnected thick trabeculae is less competent than an equivalent amount of more numerous, connected and thin trabeculae. The trabecular architecture of bone is particularly important to bone strength. A study that examined the trabecular architecture in osteopenic women and men of similar bone mass, with and without vertebral fracture, found that patients with fracture had four times the number of broken trabeculae as women without fractures. A study that modeled the loss of trabecular bone predicted that the loss of individual trabeculae has a greater impact on bone strength than the same amount of bone loss attributed to trabecular thinning. An intact trabecular network

appears to be vital for maintaining maximum bone strength. The function of trabecular bone is to transfer loads across joints such as the hip, and to resist compression, as in the spine. Bone is made up of both trabecular and cortical areas, and the proportion of trabecular bone varies from site to site. The femoral neck of the hip consists of 25% trabecular bone, whereas in a vertebral body the percentage ranges from 66% to 90%. Most of the bone mass that is lost in postmenopausal women is from the deterioration of trabecular bone. This is most likely the result of the more rapid rate of bone turnover in trabecular bone compared with cortical bone) The negative effect of remodeling is a result of hormonal changes, and the loss of trabecular horizontal links results in an irreversible loss of structural integrity. Because the amount of trabecular bone in the vertebrae is so high, this deterioration is particularly apparent in the spine and manifests as compression fractures.

Bell et al. suggested a novel mechanism for increased cortical porosity in patients with hip fracture, which appears to depend on the presence of giant canals in the femoral neck. These canals are related to clusters of remodeling osteons, building blocks of bone that are composed of a series of concentric rings of bone cells and bone matrix, surrounding a hole filled with blood vessels and nerves. The researchers investigated the relationship between remodeling and bone loss, osteon diameter; wall thickness, and osteoid width in the femoral neck of patients with hip fracture and compared these parameters with age- and gender-matched healthy controls. Composite osteonal systems were nearly twice as prevalent in patients with fractures and had significantly thinner walls. This study suggests that the principal remodeling deficit in hip fracture is specific to composite osteons, which leads to increased porosity of cortical bone [85, 86].

1.5.3 Material Properties

Bone tissue is composed principally of inorganic bone apatite crystals that mineralize an organic type I collagen matrix. The degree of mineralization, the material properties of the collagen matrix, crystal size, and the mineral-to-matrix ratio are all important for bone strength.

Mineral: The degree of mineralization of bone has an important influence on bone strength. Follet and colleagues showed that a greater degree of mineralization of cancellous bone led to greater stiffness and compressive strength. The degree of mineralization depends on the rate of remodeling, which may explain the observation that an increased degree of mineralization increases bone strength, while bone matrix volume and microarchitecture remain unchanged. Bone mineralization density distribution depends on the remodeling activities of bone cells and the time course of mineralization of newly formed bone matrix. Bone densitometry measurements reflect the degree of mineralization of the bone and cannot distinguish whether the drop in density is the result of lost bone mass along with its mineral content, or whether bone turnover is occurring at a higher rate and replacing more mineralized old bone with less mineralized new bone. In other words, BMD and the degree of mineralization are not interchangeable. The collagen matrix of bone is not uniformly mineralized; rather it exhibits a range of mineral concentration, determined by the duration of the secondary mineralization of individual bone packets. This effect has been demonstrated by microradiography and back-scattered electron imaging. In bone, mineral particles are strongly oriented in the direction of collagen fibers as determined by X-ray diffraction. The ultrastructural organization of mineral particles may influence toughness and the elastic modulus or mechanical properties of the whole bone [87-89].

Collagen: One of the most distinctive characteristics of type I collagen, as found in mineralized tissue, is the chemistry of its cross-links. A study by Oxlund and colleagues showed that bone collagen from cancellous vertebral bone taken from deceased individuals with osteoporosis showed increased extractability and a substantial reduction in the concentration of divalent reducible collagen cross-links compared with age and gender matched controls. The extractability of bone collagen depends on molecular packing, noncovalent intermolecular forces, and cross-links between collagen molecules. There was a reduced concentration of known collagen cross-links in bone from individuals with osteoporosis. This change could result in a reduction of the material strength of the bone trabeculae and explain why the individuals with osteoporosis had fractures even though they had a similar amount of trabecular bone as the healthy controls [(90)].

1.5.4 Bone Turnover

Bone is not a static material, but a complex living tissue that undergoes constant renewal to repair the microdamage that occurs on a daily basis. Bone turnover or remodeling, is a crucial process of bone renewal that most likely occurs at an optimal physiologic rate for maximum bone strength in a healthy individual. New bone tissue is created during the growth period of the human skeleton. As the bone grows, the mass of bone inside the periosteal envelope develops into a cortex. Once longitudinal bone growth has stopped and peak bone size and BMD have been reached, bone renewal or remodeling continues on the endosteal surfaces. Osteoclasts resorb damaged bone tissue, which results in a resorptive cavity on the trabecular and endocortical surfaces, or a cutting cone within the cortex. The resorption cavity is subsequently filled with new bone by osteoblasts. The new bone undergoes first a rapid primary and then a

slower secondary phase of mineralization. As long as the rates of bone resorption and bone replacement are balanced, neither a net loss of bone nor an increase in damaged bone occurs [91].

1.5.5 Assessment of Bone Quality

A large body of epidemiologic data indicate that despite its limitations, the current standard for predicting fracture risk is an areal BMD measurement by dual-energy X-ray absorptiometry (DXA) [92, 93]. However, BMD measurements reflect only one aspect of bone quality, the quantity of bone per area. There exists great disparity between the information provided by BMD and that required to improve the diagnosis and treatment of osteoporosis. New imaging modalities and biomarkers capable of assessing various components of bone quality have the potential to provide information required to improve the diagnosis of osteoporosis, prediction of future fracture risk and monitoring of treatment response. Hence, there is a clear need for the development of more sensitive risk assessment tools in addition to BMD. Considerable progress has been made in the development of noninvasive methods to assess the skeleton, so that osteoporosis can be detected early, its progression and response to therapy monitored carefully, and its risk effectively ascertained. The capability now exists to evaluate the peripheral, central, or entire skeleton as well as the trabecular bone or cortical bone envelopes accurately and precisely, with the capacity to determine bone strength and predict fracture risk. A variety of techniques are currently available to assess the skeleton noninvasively: radiographic absorptiometry, single photon and single X-ray absorptiometry, dual photon and dual X-ray absorptiometry, standard quantitative computed tomography (QCT) and peripheral QCT, quantitative ultrasound (QUS), and magnetic resonance microscopy (mMR). Of these

techniques, only the latter three—QCT, QUS, and mMR—have substantial potential to provide information about bone quality and structure beyond bone mineral density (BMD).

DXA

Dual-energy X-ray absorptiometry (DXA), a two-dimensional measurement [(33)], is now one of the most frequently used techniques for body composition measurement as a result of the increasing worldwide availability of these scanners (Figs.2 and 3). The technique is attractive because it is non-invasive, is easily applied for both healthy individuals and patients, and the radiation dose is extremely small. Scanning times, which may have been an impediment to its use in pediatric studies, have decreased substantially with newer technology. A further attractive feature is its ability to provide regional-body composition analysis [10].

Figure 2. *Bone Measurements by DXA*

DEXA is capable of good precision for the measurement of body fat, fat-free mass and bone mineral, and this has been well documented [31, 32]. This attribute makes it potentially a valuable

tool for longitudinal studies in the clinical setting. DEXA accuracy is more difficult to judge, partly because of lacking a technique for direct measurement. Postmortem chemical analysis of animals has been compared with DEXA measurements in a number of studies, with variable results depending on the equipment and software used [34-36].

QCT

The validity of quantitative computed tomography (QCT) for measuring vertebral cancellous bone is widely accepted and it is used worldwide [37]. QCT can determine in three dimensions the true volumetric density (mg/cm^3) of trabecular or cortical bone at any skeletal site. Because of the high responsiveness of trabecular bone and its importance for vertebral strength, QCT has been employed principally to determine trabecular bone density in the vertebral centrum. In this application, QCT has been used to assess vertebral fracture risk [38, 39], measure age-related bone loss [11, 12, 40], and follow the progression of osteoporosis and other metabolic bone diseases [41]. In general, spinal QCT is performed with standard clinical computed tomography (CT) scanners, and an external bone mineral reference phantom is used to calibrate the CT number measurements to bone-equivalent values. Typically, special software is employed to automatically place regions of interest inside the vertebral body.

Special purpose peripheral QCT (pQCT) scanners have been employed for measurements of bone mineral content (BMC) and BMD of peripheral skeleton. pQCT allows for a true volumetric measurement of appendicular cortical and trabecular bone and provides three-dimensional localization of the target volume (Figs.4 and 5). Ease of use, the ability to separately assess cortical and trabecular bone and to measure BMD, BMC, and cross-sectional area of both

bone and muscle make the method an interesting alternative to the technique of single- and dual-energy X-ray absorptiometry (DXA).

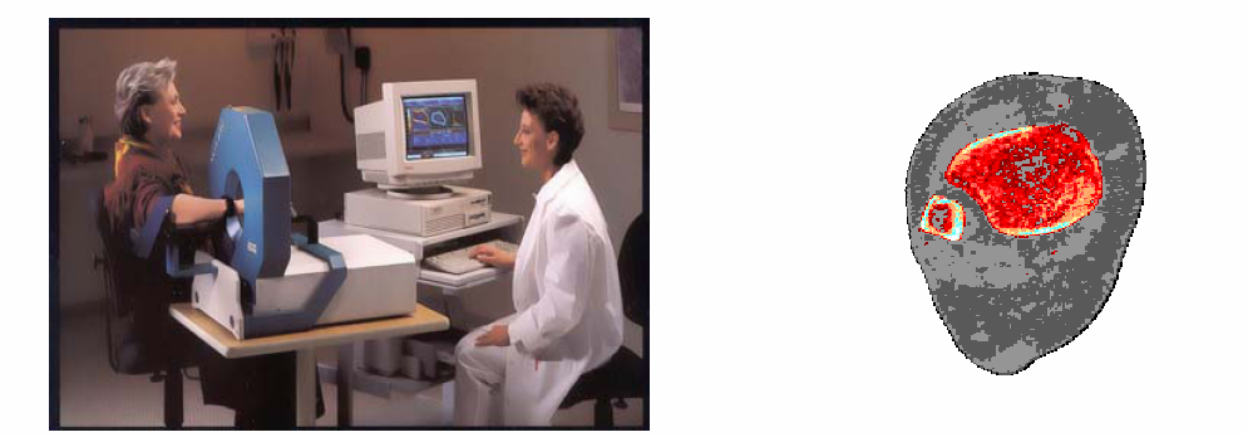

Figure 3. *Bone Measurements by pQCT*

There are many advantages of pQCT measurements over DXA. First, pQCT is capable of three-dimensional imaging, which can provide information on the architecture of bone. It offers an attractive possibility to noninvasively determine bone mechanical properties. This procedure allows assessment of not only the geometric properties (including CSMI- cross-sectional moment of inertia), but also the "true", volumetric mineral density (vCtBMD) of the cortical region of any long bone. Assuming proportionality between elastic modulus in bending and vCtBMD or cortical bone within the experimental range, the latter could be regarded as an indirect indicator of (cortical) bone material quality in mechanical terms. The CSMI, on the other hand, is an architectural indicator (for bending or torsion analysis) by itself. Hence pQCT noninvasively evaluates both the mechanostatic behavior and the mechanical properties of long bones under any experimental condition. In addition, it allows determination of the material property of the bone, by estimation of the bone mass per volume unit. DXA densitometry only measures bone "mass" and does not inform about bone material quality and architectural design [11, 37]. Peripheral

QCT measurements of BMD at the radius have been found to be successful in distinguishing between osteoporotic and nonosteoporotic patients and in monitoring subjects during clinical studies [42].

1.5.6 Biomarkers

Besides the noninvasive imaging technology discussed above, scientists also use biomarkers to assess bone quality [94-96]. Currently available biochemical indices of bone turnover, particularly those of bone resorption, predict fracture risk independently of BMD and have been shown to account for a substantial proportion of the reduction in fracture risk following antiresorptive therapy. It is not clear whether these markers directly reflect aspects of skeletal fragility or whether they indirectly reflect skeletal traits, such as increased cortical porosity, cortical thinning and degradation of the trabecular network, which are consequences of increased resorption.

1.6 TOBAGO POPULATION AND FAMILY STUDY

The country, Trinidad & Tobago (T&T) is an independent democracy and English is the principal language. The two main islands of this country lie at the southern end of the Caribbean archipelago. Tobago (Fig. 6), the smaller island (7 by 29 miles, 1994 estimated population 50,744), lies 60 miles northeast of the island of Trinidad (45 by 45 miles, current estimated population 1,272,385) [97-98].

The population of Tobago was reported to be 92 percent black, 4.5 percent mixed, 2 percent East Indian, 0.4 percent white, 1 percent other in the 1990 census. This distribution is

very different from the 38 percent black, 20 percent mixed, 42 percent East Indian, and 0.3 percent white/other reported in the total Trinidad & Tobago population. The older population of the island appears to be a stable population of descendents of West African forbearers who arrived around 200 years ago. They are thought to share considerable West African Ancestry with African Americans. The Tobago population of non-African descent has been very small throughout this time period, and genetic admixture is estimated to be relatively low [99]. This low admixture rate will allow us to focus more sharply on the genes of West African descent than is possible in most populations outside of West Africa.

As part of a large population-based prostate cancer surveillance project of all age-eligible men on the Caribbean island of Tobago [(18)], in our Tobago family study we recorded the number of living siblings for each participant, as well as the vital status and residence of their parents. These data revealed that the sibship size in Tobago is large, with a median living sibship size of 8. To date, we recruited 471 individuals age 18 and older in eight multigenerational families of the following sizes: 102, 26, 49, 28, 113, 21, 38, and 94, with a mean family size of >50 individuals. These 284 women and 187 men with phenotype data ranged in age from 18-103 years (mean age, 43 years). Among these 471 individuals in eight pedigrees we have thousands of different relationship pairs.

Figure 4. ***Map of Tobago Island***

1.7 INTRODUCTION TO THE METHODOLOGY

The major objective of this PhD project is to disentangle the genetic and environmental components that influence bone strength-related traits. Furthermore, I wanted to identify traits that were significantly heritable, because these traits are usually chosen for subsequent genetic analysis such as linkage or association study. Several different methods can be used to estimate heritability, and all use the theoretical correlations between relatives. As described below, I chose the variance component approach, as implemented in SOLAR, beause it has the capability to handle large, multigenerational pedigrees [100, 101].

In addition to characterizing the influence of genetic and environmental factors on one trait (univariate analyses) , we also know that a common gene or common sets of genes might influence two or more traits, i.e. there might be pleiotropy. The relative magnitude of a pleiotropy effect on two traits can be measured by estimating the genetic correlation (ρ_g) between two traits using bivariate analyses, as described below.

General speaking, under the variance component framework, the level of any phenotype, y, for individual i can be modeled as

$$\mathbf{y = \mu + \Sigma \beta_i X_{ij} + g_i + e_i} \quad (102)$$

where μ is the mean of the trait, X_{ij} is the j-th covariate for the ith individual, β_i is its regression coefficient, and g_i and e_i represent the random deviations from m for individual i that are attributable to additive genetic and residual error effects, respectively. The residual error component includes true random error, measurement error, and any non-additive genetic components. The effects of g_i and e_i are uncorrelated and normally distributed with mean zero and variances σ^2_g and σ^2_e respectively. The σ^2_e term represents the residual environmental variance, after accounting for the effects of measured covariates. Maximum likelihood methods

were used to simultaneously estimate the mean and variances as well as the covariate and genetic effects.

For a simple additive genetic model, the covariance matrix for a pedigree can be written as

$$\mathbf{\Omega = 2\,\Phi\,\sigma^2_g + I\,\sigma^2_e}$$

where Φ is the kinship coefficient matrix and I is an identity matrix. The proportion of the total phenotypic variation in any phenotype that could be attributable to additive genetic effects (σ^2_g / ($\sigma^2_g + \sigma^2_e$)) corresponds to ‘‘narrow’’ sense heritability (h^2) since it reflects the degree of additive genetic variance only.

$$\mathbf{h^2\ (Narrow\ sense\ heritability) = \sigma^2_g / \sigma^2_p = \sigma^2_g / (\sigma^2_g + \sigma^2_e)}$$

This is how we estimated the heritability for all of our bone traits in the subsequent chapters. In fact, in our study we directly estimate residual (h^2r) instead of h^2. Residual heritability represents the proportion of the residual phenotypic variation due to additive genetic effects (after removing the effects due to measured covariates). The relationship between these two terms can be expressed by

$$\mathbf{h^2r = h^2 / (1- R^2)}$$

where R^2 is the proportion of total phenotypic variation explained by the measured covariates.

The extenstion of the univariate analyses described above to the bivariate analyses that we preformed (or more generally, multivariate analyses) is straightforward. The covariance matrix for the pedigree for the multivariate analysis can be written as

$$\mathbf{\Omega = G \otimes 2\,\Phi + E \otimes I_n} \quad (103)$$

where $\otimes$ is the Kronecker product operator, G is the residual additive genetic covariance matrix, and E is the (unmeasured) environmental covariance matrix. For the bivariate analyses that we

performed, we first obtained trait-specific estimates of σ^2_g and $\sigma^2_{e,}$, that is, σ^2_{g1}, σ^2_{g2} and σ^2_{e1}, σ^2_{e2}, We also estimated the additive genetic, ρ_g, and (unmeasured) environmental, ρ_e, correlations between the two traits. We then determined whether any phenotypic covariation between the traits was attributable to genetic and/or environmental factors. In other words, we tested the null hypotheses that there was no genetic correlation (that is, $\rho_g = 0$ versus $\rho_g \neq 0$) and/or no environmental correlation (that is, $\rho_e = 0$ versus $\rho_e \neq 0$). rg ~ 1 means two traits share almost identical genetic determinants and rg ~ 0 means there are unique genes or genetic factors which control these two traits separately. The negative rg has the similar interpretation as positive ones except that the genetic effects might drive the trait on the opposite directions.

1.8 SPECIFIC AIMS

The specific aims of my dissertation proposal are given below. The corresponding analyses and results to these aims/hypothesis were will be presented in subsequent chapters.

Aim 1: Dissect the genetic architecture of bone strength phenotypes in families of African heritage. (Chapter II)

(1-A) For environmental factors: Age, Sex, Smoking, Alcohol Drinking, Physical Activities, female menopause status and together with some other important anthropometric, life-style, medication *etc.* factors will be treated and controlled as a risk factor (including main effect and some of the interaction). Significant covariates and R^2 will be evaluated by stepwise regression in R and SOLAR.

(1-B) For genetic impact: SOLAR, genetic software which can handle multi-generation pedigrees will be used to estimate residual heritability (h2r).

Aim 2: Estimate the extent of possible pleiotropic effects of genes on BMD and bone geometry phenotypes. (Chapter III)

Aim 3: Develop "composite traits" (or "combined phenotypes") by two multivariate analysis methods-- Principal Component Analysis and Factor Analysis. (Chapter V and IV)

(3-A) Compare results from Principal Component Analysis and Factor Analysis applied to simulated family data. **(Chapter V)**

(3-B) Derive composite traits by multivariate analysis, using data on bone quality related phenotypes, from the Tobago Family Health Study. **(Chapter VI)**

Aim 4: Test for association between bone quality related traits (including composite traits) and SNPs from candidate two genes Wnt10b and ENPP1. (Chapter VI)

2.0 GENETIC AND ENVIRONMENTAL DETERMINANTS OF VOLUMETRICAND AREAL BONE DENSITY IN TOBAGO MULTI-GENERATIONAL FAMILIES

In this chapter, we determined the extent to which genes (heredity) and environmental factors contribute to both areal and volumetric BMD phenotypes within families of African ancestry (Tobago Population). In addition, we assessed a number of environmental factors of probable importance to skeletal health, including diet, physical activity, anthropometric characteristics, body composition, medication use and medical status. The analysis was based on 471 individuals in 8 extended multigenerational families of African descent. We used quantitative genetic methods to determine which environmental factors influence 12 BMD traits, as well as the relative influence of genes on these phenotypes. Several interesting findings include: 1) Areal BMD at the femoral neck and hip was highest in the Afro-Caribbean men and women at all ages compared to U.S. black and white population. 2) Trabecular volumetric BMD and cortical volumetric BMD decreased at different pattern across age and gender; 3) Anthropometric, lifestyle, and medical factors accounted for 12-38% of the variation in areal and volumetric BMD. Among them, sex, height and weight accounted for the majority (73% to 99%) of the total variation due to significant covariates, and 4) Residual heritabilities (range 0.29-0.70) were similar to those reported in other ethnic groups. Heritability of cortical BMD was substantially lower than that of areal or trabecular volumetric BMD. All detailed methods, results and discussions associated with this chapter were summarized and published in ***Journal of Bone***

Mineral Research (Wang X, Kammerer CM, Wheeler VW, Patrick AL, Bunker CH, Zmuda JM 22(4):527-536, 2007); please refer to it as below.

Title:

Genetic and Environmental Determinants of Volumetric and Areal Bone Density in Multi-Generational Families of African Ancestry: The Tobago Family Health Study

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Funding Resource:

The study was supported by grants R03-AR050107 and R01-AR049747 from the National Institute of Arthritis and Musculoskeletal Diseases.

2.1 INTRODUCTION

Osteoporosis is a systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone with a consequent increase in bone fragility and susceptibility to fractures[1]. Bone mineral density (BMD) is one of the most important indicators of the mechanical strength of the skeleton and the risk of osteoporosis related fractures. BMD can be assessed by two-dimensional dual energy X-ray absorptiometry (DXA) or by three-dimensional computed tomography. Quantitative computed tomography (QCT) may provide several advantages over DXA for the assessment of BMD. First, QCT is a three-dimensional technique that measures BMD volumetrically (g/cm^3) and is independent of the potential confounding effects of bone size. Bone volume estimates derived from DXA images have been proposed [2], but are thought to be of limited use. Second, QCT directly examines the trabecular and cortical bone compartments separately whereas DXA yields a measure of integral BMD (i.e., cortical and trabecular) that may be confounded by the high prevalence of extra-vertebral mineralization among older adults (e.g., osteophytes)[3-6]. Further, measures of trabecular BMD may confer the highest risk for vertebral fracture [7,8] and are more sensitive to changes in bone metabolism.

Many intrinsic and extrinsic factors have been correlated with areal BMD, including heredity, age, gender, body mass index, sex steroid hormones, physical activity, calcium intake, and diabetes[9-13]. However, most heritability studies have relied solely on DXA measures of integral BMD; none to our knowledge have comprehensively dissected the relative contribution of genetics and environmental factors to the determination of trabecular and cortical volumetric BMD as measured with QCT. Furthermore, although several studies report that populations of African ancestry have substantially higher BMD and lower osteoporotic fracture risk than other

ethnic groups [14-17], there is a paucity of information about the genetic and environmental factors that contribute to skeletal health in populations of African origin. Thus, dissection of the genetic architecture of QCT measures of volumetric BMD, especially in populations of African heritage, is important to advance our understanding of the etiology of osteoporosis.

In the current study, we investigated the genetic and environmental contributions to areal BMD measured by traditional DXA as well as volumetric BMD measured by peripheral QCT in 471 individuals from 8 large, multigenerational Afro-Caribbean families. We also compared our results to those obtained in populations of European descent and African Americans.

2.2 MATERIALS AND METHODS

2.2.1 Study Samples

As part of a large population-based prostate cancer surveillance project of all age-eligible men on the Caribbean island of Tobago [18], we recorded the number of living siblings for each participant, as well as the vital status and residence of their parents. These data revealed that the sibship size in Tobago is large, with a median living sibship size of 8.

Thus, the men in our population-based study served as potential probands for the family study, and were selected without regard to medical history or BMD. To be eligible, a proband had to be a healthy Afro-Caribbean resident, have had a spouse who was willing to participate in the study, and have at least six living offspring and/or siblings aged 18+ years who were residing in Tobago. These potential probands were sorted by family size, and individuals with the largest

family sizes were recruited first. All eight probands for the Tobago Family Health Study were between 52-103 years of age and none of them had previously been diagnosed with prostate or any other cancer. In addition, all first-, second- and third-degree relatives of these probands and their spouses were invited to participate regardless of their medical history or BMD. To date, we recruited 471 individuals age 18 and older in eight multigenerational families of the following sizes: 102, 26, 49, 28, 113, 21, 38, and 94, with a mean family size of >50 individuals. These 283 women and 188 men with phenotype data ranged in age from 18-103 years (mean age, 43 years). Among these 471 individuals in eight pedigrees we have the following relationships: 361 parent-offspring, 495 full siblings, 101 grandparent-grandchildren, 1137 avuncular, 61 half sibs and 1380 cousins. Written informed consent was obtained from every participant, using forms and procedures approved by the Tobago Ministry of Health and Social Services and University of Pittsburgh Institutional Review Boards.

2.2.2 Peripheral Quantitative Computed Tomography (pQCT) Assessment

Single axial tomographic slices of the non-dominant forearm and left tibia were scanned using a Stratec XCT 2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) according to standardized measurement and analysis procedures. Each scan was acquired with a 0.5-mm voxel size, slice thickness of 2.5 mm, and at a speed of 20 mm/s. The precise position of the measurement sites were determined in a 30 mm planar scout view using the medial endplate of the radius and tibia as standard anatomic landmarks and automatically set by the software at 4% (i.e. distal) or 33% (i.e. shaft) of the length of the radius and tibia proximal to the distal endplate. These anatomical sites were chosen in order to assess primarily trabecular and cortical bone,

respectively. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process.

Image processing was performed using the Stratec software package (Version 5.5E). All 4% ultra distal radius and tibia scans were analyzed using identical parameters for contour finding and separation of trabecular and cortical bone (contour mode 2, T=169 mg/cm^3; peel mode 1, area=45%) to determine the volumetric bone mineral density of the total (mg/cm^3) and trabecular (mg/cm^3) bone compartments. All 33% proximal radius and tibia shaft scans were analyzed using identical parameters for contour finding and separation of total and cortical bone (contour mode 2, T=169 mg/cm^3;cortmode 1, T=710 mg/cm^3) to determine the volumetric BMD of the total (mg/cm^3) and cortical (mg/cm^3) bone compartments.

The short-term *in vivo* precision of the separate pQCT measurements for 15 subjects ranged from 0.43% (for total density at the distal tibia) to 6.21% (for total density at distal radius). The test-retest correlations for all six pQCT traits ranged between 0.85 (for total density at the distal radius) and 0.996 (total density at the distal tibia). A phantom was scanned daily to maintain quality assurance.

2.2.3 Dual Energy X-Ray Absorptiometry (DXA)

Areal BMD (g/cm^2) of the lumbar spine (L_1 to L_4) and proximal femur (total hip, femoral neck) was measured by DXA using the array beam mode on a Hologic QDR 4500W scanner (Hologic, Inc.; Bedford, MA). Standardized procedures for participant positioning and scan analysis were followed according to the manufacturer's recommended protocol. Scans were analyzed with QDR software version 8.26a.

The short-term *in vivo* precision of the DXA measurements was assessed in 12 subjects. All CVs were less or equal to 1.16% and all test-retest correlations are above 0.99. A phantom was scanned daily and reviewed by DXA Resource Group (Worcester, MA) to maintain longitudinal quality assurance of the scanner during the course of the study.

2.2.4 Anthropometry

Body weight was measured to the nearest 0.1 kilogram with participants wearing indoor clothing but without shoes using a balance beam scale. Standing height was measured to the nearest 0.1 cm without participants wearing shoes using a wall-mounted stadiometer. The average of two measurements was used. Body mass index was calculated by dividing body weight (kg) by height (m^2). An inelastic tape measure was used to determine the waist circumference (in cm) at the umbilicus.

2.2.5 Other Measurements

Information on demographic characteristics, medical history and lifestyle habits was obtained by questionnaire and interview by trained and certified clinical staff. Race/ethnicity was based on self-declaration and participants provided detailed information on the ethnic origin of their parents and grandparents. Respondents were assigned to an ethnic group if they reported that all four grandparents as belonged to that group. The Tobago population is predominantly of West African origin (97% of the island according to the most recent census data) with low non-African admixture. Previous studies using molecular markers have confirmed a low admixture (6% non-African) in this population. [19]

Subjects were classified as current smokers (yes/no). Participants who had smoked fewer than 100 cigarettes in their lifetime were considered nonsmokers. Information on alcohol consumption was obtained by questionnaire and expressed as drinks per week. Information on calcium and vitamin D supplement use was also obtained (yes/no). Milk consumption at several ages was assessed by questionnaire [20]. We assessed caffeine consumption by assuming one cup of coffee contained 95 mg of caffeine; tea, 55 mg and colas, 45 mg as previously described[21,22]. Subjects were asked if they walk for exercise (yes/no). Physical activity was also assessed as a continuous variable by the number of minutes walked and hours spent watching television per week.

Reproductive characteristics including ever being pregnant, menopausal status, use of hormone therapy and current oral contraceptive use, were recorded (all coded as yes/no). Because only 5/283 (1.8%) women reported ever using post-menopausal hormone therapy, we did not consider this variable as a potential correlate of BMD in subsequent analyses. Women were defined as post-menopausal if they declared that they had no menses for at least 12 months and were greater than 40 years old (n=76) or they had experienced a hysterectomy or ovariectomy (n=9).

Participants were asked to bring prescription and non-prescription medications to the clinic for verification. Current use was defined as use within the preceding 30 days. A study-specific medication dictionary was used to categorize the type of medication from product brand and generic names obtained from the medication containers. Dose or duration of use or specific indication was not queried. Subjects were asked whether a doctor or health care provider had ever told them they had certain medical conditions including arthritis, cancer, or cardiovascular disease. Hypertension was defined as a diastolic blood pressure exceeding 90mmHg or systolic

blood pressure exceeding 140mmHg (n=91) or currently taking blood pressure medication (n=41). Diabetes was defined as fasting glucose level exceeding 126 mg/dl (n=45) or currently taking diabetes medication (n=26).

2.2.6 Statistical Analysis

Prior to statistical analysis, the distributions of all BMD traits were assessed for non-normality. Subsequently, all outliers (± 4 standard deviations) were removed for each BMD trait, and no more than 4 values were removed for a single variable.

The major goal of our analysis was to determine the extent to which genetic and environmental factors contribute to the total phenotypic variation of the areal and volumetric BMD measures. Initially, to identify possible covariates influencing each BMD trait, we first performed combined forward and backward stepwise linear regression analysis, ignoring the non-independence of the subjects, using the R statistical package (Version 2.2.1). We required each variable remaining in the model to be significant at $P \leq 0.10$. We subsequently evaluated each of the potentially significant covariates using a variance component framework that enabled us to take into account the correlations among family members [13]. Briefly, the variance components approach involves partitioning the variance of a quantitative trait into components attributable to individual-specific covariates (e.g., age, BMI, diabetes status, etc.), an additive genetic (polygenic) component, and a residual non-measured environmental component. The additive genetic component is modeled as a random effect from the covariance matrix, which is a function of the coefficient of relatedness between all pairs of individuals. For example, two full sibs share 1/2 of their genes in common on average, and thus have a coefficient of relatedness

equal to 1/2. Thus, the effects of all independent variables on BMD are estimated conditional on the correlations among related individuals. The significance of a particular independent variable (e.g., diabetes status) was assessed by the likelihood ratio test, which compares the likelihood of a full model (e.g., age, BMI, and diabetes status) to that of a nested model (e.g., age and BMI only, with the diabetes status effect constrained to be zero). Similarly, the significance of the residual heritability (h^2r), was determined by comparing the likelihood of a model in which h^2r was estimated, to a nested model in which h^2r was constrained to 0. These analyses were performed using the program SOLAR[23]. In addition to h^2r, which represents the proportion of the residual phenotypic variation due to additive genetic effects, we also estimated h^2, which represents the proportion of total phenotypic variation (including variation due to measured covariates) that is attributable to additive genetic effects. The relationship between these two terms can be expressed by: $h^2r = h^2 / (1- R^2)$, where R^2 is the proportion of total phenotypic variation explained by the measured covariates.

To compare the effects of covariates across all traits, we report the strength of association between covariates and the BMD traits as a percent difference in the BMD trait per unit of the covariate, instead of the non-standardized beta coefficients. Percent differences in each BMD trait for each covariate were calculated as beta coefficient * unit range / mean BMD. For continuous covariates, the unit range was every 5 years for age, every 10 kg for body weight and every 8.5 cm (1 SD) for height. The unit range for dichotomous covariates equaled 1 [3]. The percentage difference between younger and older age groups was calculated as: [(Trait Mean 60+ age group - Trait Mean 18-29 yrs age group) / Trait Mean 18-29 yrs age group] *100% for each gender.

All P-values which tested for gender differences were computed using SOLAR univariate regression analysis, which accounts for the non-independence of the family data.

2.3 RESULTS

2.3.1 Subject Characteristics

Among the 283 women and 188 men, BMI was higher but waist circumference was similar in women than men ($P<0.001$) (Table 1). Although the prevalence of cigarette smoking and alcohol consumption was higher in men than women, the prevalence of hypertension, diabetes, cardiovascular disease and arthritis were comparable in both genders. Mean number of minutes walked or time watching TV per week was comparable in men and women. Current use of supplemental vitamin D and calcium and milk consumption were all significantly higher in women than in men ($P< 0.05$).

2.3.2 Sex Differences in BMD

Mean areal and volumetric BMD in men and women with and without adjustment for height and weight are shown in table 2. All measures of areal BMD were significantly greater in men than in women. Mean differences in unadjusted areal BMD between men and women ranged from 0.07 g/cm^2 or 7% at the lumbar spine and femoral neck to 0.11 g/cm^2 or 10% at the total hip and whole body. The magnitude of this sex difference represents approximately ½ (lumbar spine, femoral neck) to 1 full (whole body) standard deviation. These sex differences in areal BMD

were only slightly attenuated and remained statistically significant after controlling for height and body weight. Sex differences in volumetric BMD were generally greater at the distal than proximal (shaft) regions of the radius and tibia and were greater for trabecular than cortical BMD (Table 2). Trabecular volumetric BMD was 14% greater at the distal radius and 7% greater at the distal tibia among men than women, differences that represent approximately ¾ and ½ standard deviation. Adjusting for height and body weight had little effect on these results. Cortical volumetric BMD, on the other hand, was significantly greater in women than men at the radius and tibia. Although these BMD differences were statistically significant and persisted after adjustment for height and body weight, they were small (~1%).

2.3.3 Age Related Patterns in BMD by Sex and Comparisons with Other Populations

The sex-specific mean BMD values for young participants (age 18 to 29 years) as well as the absolute and percentage difference compared to older aged participants (age 60+ years) are shown in Table 3. Consistent with observations for the entire sample (Table 2), the young adult Afro-Caribbean men generally had greater areal BMD values than women. These relative differences were largely maintained throughout life (e.g., Figure 1 shows femoral neck BMD; other skeletal sites not shown).

Table 6. Characteristics of the Afro-Caribbean Family Members by Gender

(table 1 in this chapter)

Characteristics	***Men (N=188)***	***Women (N=283)***
Age (years)	**42 ± 17**	**42 ± 17**
Anthropometric		
BMI (kg/m^2)	**26.7 ± 4.9**	**29.4 ± 7.0** *
Waist circumference (cm)	**90.1 ± 12.4**	**89.7 ± 17.2**
Height (cm)	**177.0 ± 7.4**	**166.5 ± 6.5** *
Weight (kg)	**83.7 ± 17.3**	**81.3 ± 19.4**
Reproductive		
Current Oral Contraceptive Use (%)	**N/A**	**33.1**
Parity (%)	**N/A**	**76.7**
Post-Menopausal (%)	**N/A**	**30.0**
Life Style and Diet		
Current smoking (%)	**11.4**	**0.7** *
Alcohol consumption (% >1 drink per week)	**28.9**	**2.8** *
Walking (min/week)	**53 ± 73**	**45 ± 110**
Television viewing time (hours/week)	**16.6 ± 8.5**	**15.5 ± 7.7**
Current milk consumption (times/week)	**3.9 ± 2.8**	**4.7 ± 2.9** *
Current caffeine consumption (mg/day)	**90 ± 162**	**79 ± 83**
Current Supplemental Vitamin D use (%)	**9.3**	**18.5** *
Current Supplemental Calcium use (%)	**8.8**	**24.6** *
Medical Conditions		
Hypertension (%)	**30.8**	**27.2**
Diabetes (%)	**12.5**	**17.3**
Cardiovascular Disease (%)	**3.8**	**6.0**
Arthritis (%)	**7.0**	**12.1**

* Indicates the comparison by gender is statistically significant (P<0.05).

Table 7. Mean DXA and pQCT BMD Measures in the Afro-Caribbean Family Members by Gender

(table 2 in this chapter)

	MEN (N=188)	*WOMEN (N=283)*
DXA areal BMD (g/cm^2)		
Whole Body	**1.26 ± 0.11**	**1.15 ± 0.13***
	(1.25) †	**(1.16)***
Lumbar Spine	**1.13 ± 0.15**	**1.06 ± 0.15***
	(1.11)	**(1.06)** *
Total Hip	**1.17 ± 0.14**	**1.06 ± 0.17***
	(1.17)	**(1.05)** *
Femoral Neck	**1.03 ± 0.15**	**0.96 ± 0.16***
	(1.02)	**(0.97)** *
pQCT volumetric BMD (mg/cm^3)		
Distal Radius		
Total	**447 ± 76**	**416 ± 85***
	(448)	**(415)***
Trabecular	**239 ± 40**	**210 ± 40***
	(239)	**(210)** *
Radius Shaft		
Total	**989 ± 73**	**1010 ± 95***
	(990)	**(1009)**
Cortical	**1213 ± 22**	**1222 ± 26***
	(1211)	**(1223)** *
Distal Tibia		
Total	**342 ± 55**	**312 ± 51***
	(344)	**(310)** *
Trabecular	**259 ± 35**	**243 ± 35***
	(260)	**(242)** *
Tibia Shaft		
Total	**851 ± 79**	**826 ± 91***
	(862)	**(818)** *
Cortical	**1181 ± 27**	**1185 ± 31**
	(1178)	**(1187)** *

* Indicates the comparison by gender is statistically significant for both adjusted and unadjusted means ($P<0.05$).
Values are unadjusted means (±SD). † Mean values after adjustment for height and weight are shown in parentheses.

The largest areal BMD difference between young and old Afro-Caribbean men and women was observed at the femoral neck: -11% and -14% over life in men and women, respectively. We also compared the apparent age- and sex-related patterns in BMD at the femoral neck in our Afro-Caribbean family members with published values for non-Hispanic Blacks and non-Hispanic Whites in the U.S. NHANES III study (Figure 1) [24]. As expected, mean areal BMD at the femoral neck decreased in all age and sex groups (Figure 1), and was highest among the Afro-Caribbean men and women throughout life. However, comparison of the slopes of the curves across age groups within each gender indicate that Afro-Caribbean men and women may lose bone at a similar tempo to U.S. white and black men and women. A similar pattern was observed for total hip BMD (data not shown). At the lumbar spine in Afro-Caribbeans, areal BMD increased over life by 14% in men but decreased by 8% in women. Lumbar spine BMD data were not available in the U.S. NHANES III survey for comparison.

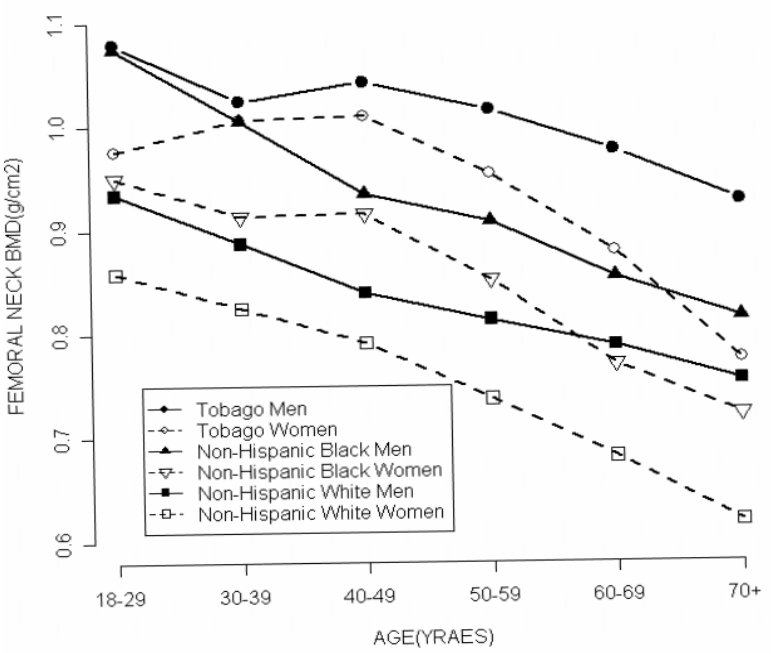

Figure 5. *Mean Femoral Neck Areal BMD by Age and Gender Among Afro-Caribbean, U.S. non-hispanic White and U.S. non-hispanic Blacks (Figure 1 in this chapter)*

Table 8.DXA and pQCT Bone Density Difference by Age Group and Gender

(table 3 in this chapter)

	Men			*Women*		
	Mean ± SD 18-29 years	**Δ between 18-60 years Absolute**	**Percent**	**Mean ± SD 18-29 years**	**Δ between 18-60 years Absolute**	**Percent**
DXA BMD (g/cm^2)						
Whole Body	1.24 ± 0.11	-0.01	-0.8	1.14 ± 0.11	-0.10	-8.8
Lumbar Spine	1.06 ± 0.12	0.15	14.1	1.05 ± 0.12	-0.08	-7.6
Total Hip	1.18 ± 0.16	-0.04	-3.4	1.04 ± 0.12	-0.07	-6.7
Femoral Neck	1.08 ± 0.17	-0.12	-11.1	0.98 ± 0.16	-0.14	-14.3
pQCT BMD (mg/cm^3)						
Distal Radius						
Total	472 ± 75	-54	-11.4	421 ± 67	-73	-17.3
Trabecular	259 ± 36	-38	-14.7	217 ± 35	-26	-11.9
Radius Shaft						
Total	995 ± 76	-14	-1.4	1019 ± 71	-117	-11.5
Cortical	1203 ± 22	11	0.9	1221 ± 16	-33	-2.7
Distal Tibia						
Total	365 ± 53	-44	-12.0	329 ± 50	-60	-18.2
Trabecular	281 ± 34	-35	-12.4	253 ± 35	-27	-10.7
Tibia Shaft						
Total	838 ± 70	8	0.9	825 ± 80	-83	-10.1
Cortical	1177 ± 20	-2	-0.2	1192 ± 20	-47	-3.9

The apparent decline with age in total volumetric BMD was similar in magnitude at the distal radius and distal tibia and somewhat greater in women (-17% to -18%) than men (-11% to -12%) (Table 3). The apparent age-related decline in total volumetric BMD at the radius shaft and tibia shaft was also similar in magnitude. However, the decline with age was much greater in women (-10% to -11%) than in men (0.9% to -1%). Separate measures of trabecular and cortical volumetric BMD at these skeletal sites also revealed different patterns by sex and anatomic region. For instance, age-related declines in volumetric BMD were greater for trabecular than cortical bone, particularly among men. Moreover, the age-related decline in trabecular volumetric BMD tended to be greater in men than in women, whereas the decline in cortical volumetric BMD was greater in women than in men.

We also plotted the age- and sex-specific mean volumetric BMD at the radius and tibia across life for the Afro-Caribbean men and women (Figures 2A and 2B). Mean trabecular volumetric BMD (Figure 2A) was higher in men than women at both skeletal sites over life and decreased with increasing age in both groups and at both sites. In contrast, cortical volumetric BMD at both skeletal sites increased until age 40-49 and was higher in women than men. In subsequent age groups, mean cortical BMD decreased markedly in women, whereas it remained fairly constant with advancing age in men, at least until the oldest age group (70+).

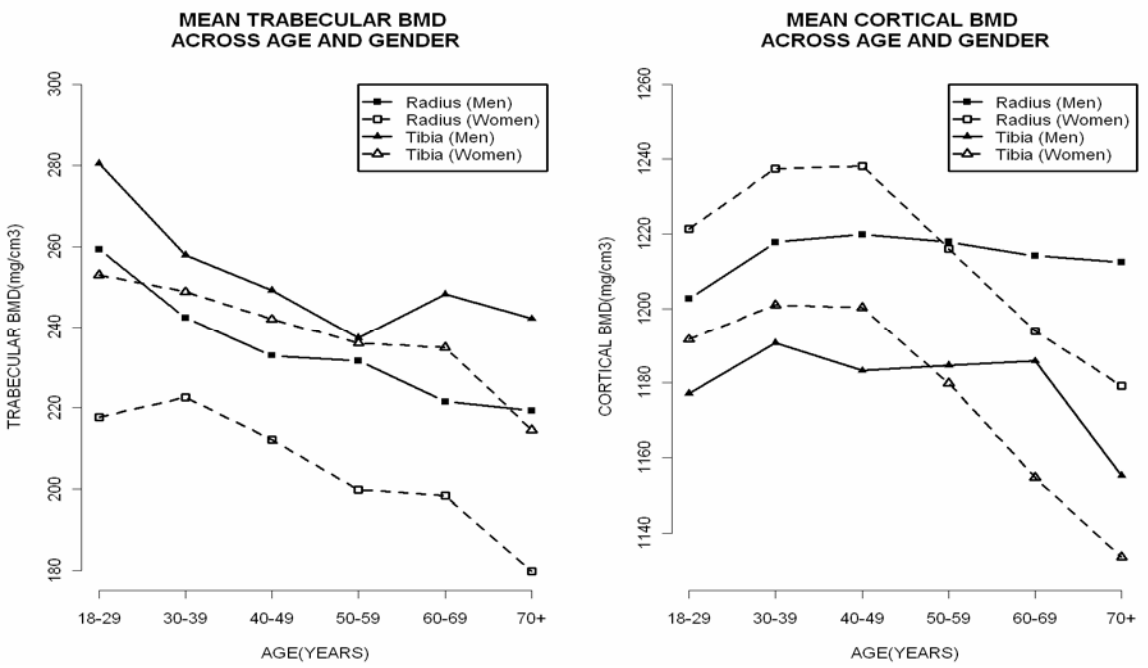

Figure 6. Mean BMD by Age, Gender and Skeletal Site among Afro-Caribbean men and women

(Figure 2 in this chapter)

2.3.4 Anthropometric, Lifestyle, Reproductive and Medical Correlates of BMD

We next investigated the potential correlates of the DXA and pQCT BMD traits. Table 4 describes the percent difference in BMD for the most important correlates. With a few exceptions, areal and volumetric BMD were generally higher in men than in women, decreased with increasing age and height, and increased with increasing body weight. In contrast to the other BMD traits, volumetric cortical BMD at the radius and tibia decreased with increasing body weight. With the exception of total spine and cortical volumetric BMD of the radius and tibia, these four factors (age, gender, height, body weight) accounted for the majority (73% to 99%) of the total variation due to significant covariates (Table 5).

In addition to these four covariates, post-menopausal women had lower measures of BMD than pre-menopausal women. This difference tended to be more prominent for areal BMD at the lumbar spine versus proximal femur and for trabecular versus cortical volumetric BMD. Parity was associated with greater cortical but not trabecular volumetric BMD at both the radius and tibia. Diabetes was associated with an increased BMD for most DXA and pQCT traits.

History of hypertension and cardiovascular diseases showed weaker and inconsistent associations with BMD phenotypes. We were unable to detect strong associations between BMD phenotypes and walking time, TV watching time or supplemental calcium intake.

2.3.5 Heritability of BMD

After incorporating all significant covariates, additive genetic effects (residual heritability), accounted for 55% to 65% of the remaining variation in areal BMD (Table 4). In contrast, residual heritability of trabecular volumetric BMD at the radius and tibia was higher, and

Table 9. Environmental Correlates of BMD Phenotypes in Afro-Caribbean Family Members

(table 4 in this chapter)

	Age	*Gender*	*Weight*	*Height*	*Smoking*	*Menopause Status*	*Parity*	*Diabetes Status*	*Arthritis Status*	*Vitamin D Supplement*	*H2r ±SE*
DXA Areal BMD (g/cm^2)											
Whole Body	- ^	+++ **	++ **			--- **	++ ^^				0.55 ± 0.11
Lumbar Spine	+ **		++ **	++ *		---- **		++ ^^	++ ^^		0.62 ± 0.11
Femoral Neck	-- **	+++ *	++ **			--- *	++ ^				0.55 ± 0.10
Total Hip	- *	++++ **	++ **	-- *		-- *		++ ^^	-- ^		0.65 ± 0.09
pQCT Volumetric BMD (mg/cm^3)											
Distal Radius											
Total	-- **	++++ *	++ **	-- ^^		--- ^^		++ ^			0.63 ± 0.10
Trabecular	-- **	++++ **	++ **	--^^	--- ^	--- ^^		+++ **			0.70 ± 0.10
Radius Shaft											
Total	- *	-- ^		--*		--- **		++ *		++ ^^	0.46 ± 0.11
Cortical		- *	- *			-- **	+ *		- ^^		0.29 ± 0.09
Distal Tibia											
Total	-- **	++++ **	++ **	--**		--- *		++ ^^		++ ^^	0.73 ± 0.10
Trabecular	-- **	++++ **	++ **	--**	--- ^^			+++ **		++ ^	0.69 ± 0.10
Tibia Shaft											
Total	- ^^	++ *	+ ^	--**		--- **		++ *			0.53 ± 0.11
Cortical	- **		- *			-- **	++ **	+ *			0.42 ± 0.10

† Symbols indicate positive (+) and negative (-) correlation. The strength of the correlation is indicated by the number of symbols: one symbol indicates less than 1% difference; two symbols, a 1%-5% difference; three symbols, a 5%-10% difference; and four symbols indicates a 10% or greater difference.

‡ ^p<0.10, ^^p<0.05, *p<0.01, **p<0.001;

§ Only variables which showed a consistent association with BMD phenotypes are shown.

accounted for ~70% of the residual variation at these skeletal sites. Residual heritability of cortical volumetric BMD was lower than for trabecular volumetric BMD and ranged from 29% to 42% of the total residual variation. Residual heritability of total volumetric BMD at the radius and tibia ranged from 46% to 73% of the total residual variation with sites comprised predominantly of trabecular bone (distal radius and tibia) having higher residual heritability than sites comprised predominantly of cortical bone (radial and tibial shaft).

Altogether, significant measured covariates explained from 12% to 38% of the total phenotypic variation in areal and volumetric BMD (Table 5). Furthermore, the proportion of total variation due to additive genetic factors accounted for as much as or more of the total phenotypic variation than did measured covariates. Heritability of trabecular BMD was higher

than heritability of cortical BMD (e.g., 0.52 versus 0.23 for the radius), although measured covariates accounted for a similar proportion (21% to 26%) of the total phenotypic variation for each of these traits.

Table 10. Proportion of Total Phenotypic Variation attributable to Genetic and Measured Environmental Factors

(table 5 in this chapter)

	*Covariates in Base Model **	*All significant Covariates †*	*Genetic*	*Unmeasured environment + error*
DXA BMD (g/cm^2)				
Whole Body	0.33	0.38	0.34	0.28
Lumbar Spine	0.12	0.21	0.49	0.30
Total Hip	0.32	0.33	0.44	0.23
Femoral Neck	0.32	0.32	0.37	0.31
pQCT BMD (mg/cm^3)				
Distal Radius				
Total	0.19	0.19	0.51	0.30
Trabecular	0.25	0.26	0.52	0.22
Radius Shaft				
Total	0.11	0.16	0.39	0.45
Cortical	0.07	0.21	0.23	0.56
Distal Tibia				
Total	0.30	0.30	0.50	0.20
Trabecular	0.26	0.27	0.51	0.22
Tibia Shaft				
Total	0.10	0.12	0.46	0.42
Cortical	0.13	0.23	0.32	0.55

* Covariates included in base model: Age, Gender, Weight and Height.
† All significant ($p< 0.05$) covariates: refer to table 4.

2.4 DISCUSSION

To our knowledge, the Tobago Family Health Study is the first comprehensive analysis to dissect the genetic and environmental factors influencing bone mineral density (BMD) measured by both DXA and QCT in families, and it is the largest family study of individuals of African

descent to date. Clinical data, including BMD phenotypes and intrinsic and extrinsic environmental covariates, were collected on 471 members of 8 multigenerational families with a mean family size >50 individuals and with nearly 3,500 relative pairs. Each family had at least 3 to 4 generations with phenotypic data. These large families combined with QCT measurements enabled us to begin to disentangle the relative contributions of genes (heredity) and environment to the determination of integral volumetric BMD as well as volumetric BMD in the cortical and trabecular compartments, and also compare our results with those from other populations. Our analyses revealed substantial overall heritability of areal and volumetric BMD with heterogeneity by skeletal site and bone compartment.

Sex-specific areal BMD at the femoral neck was higher at all ages among Afro-Caribbeans than in non-Hispanic blacks or whites in the U.S. NHANES III cohort (Figure 1). Except for the youngest and oldest age groups, mean areal BMD at the femoral neck among Afro-Caribbean women was almost as high as or higher than BMD of U.S. black men. The higher BMD in Afro-Caribbeans than in non-Hispanic blacks may reflect lower admixture in the Afro-Caribbeans compared to U.S. black, 6% versus 20%, respectively [19],[25]. Although areal BMD at the femoral neck in U.S. black men is almost as high as that in our Afro-Caribbean family members at age 18-29 years, it appears to decrease with age to a larger extent among U.S. black men. Between the 18 to 29 and 60+ age groups, mean femoral neck BMD decreased 11.1% in Afro-Caribbean men, 15.6% in U.S. white men, and 20.5% in U.S. black men. Although the total percent difference in femoral neck areal BMD also was lowest in Afro-Caribbean women (14.3%), the percent decline for U.S. black women (18.9%) was slightly less than that for U.S. white women (20.6% femoral neck) [24].

Lumbar spine areal BMD increased across the lifespan among Afro-Caribbean men. Similar age-related increases in lumbar spine areal BMD have been noted in other studies [3]. Manifestations of spinal degenerative disease such as disc space narrowing, vertebral endplate sclerosis, and osteophytes at the spinous processes and facet joints are prevalent among the elderly and may be more common among older men than women [4,26]. Such degenerative changes are likely to increase the apparent BMD measured in the posterior-anterior position [8,27,28].

Skeletal site-specific differences in the decline in volumetric BMD over life may be attributable to differences in bone composition (e.g., the relative composition of trabecular and cortical bone), mechanical loading (e.g., weight-bearing versus non-weight-bearing), or other factors [29]. In men and women of European ancestry, volumetric trabecular BMD at both the central and peripheral skeleton decreases with increasing age, and this decrease begins before mid-life in both sexes. In contrast, volumetric cortical BMD at both the central and peripheral skeleton does not appear to change in either sex before mid-life, after which there is a dramatic decrease, and this decrease is greater in women than in men [30]. The overall age patterns in volumetric BMD measured at the radius and tibia in our Afro-Caribbrean families are similar to those reported by Riggs et al. (2004) in U.S. whites: men had higher mean trabecular BMD than women at both bone sites, and mean trabecular BMD decreased earlier and across all age groups. Furthermore, similar to a previous report (Riggs et al., 2004), men had lower peak cortical BMD at both skeletal sites, and mean cortical BMD did not decrease until after mid-life in either men or women. The percent difference between the youngest and oldest age groups was almost twice as high among U.S. whites as Afro-Caribbeans, respectively, for trabecular BMD at the radius (~29% vs 16%) and the tibia (~24% vs 12%). Similarly, for cortical BMD, the overall loss

between the youngest and oldest age groups was <4% for both Afro-Caribbean men and women at the radius and tibia, whereas U.S. men lost ~17% and U.S. women lost ~27% at these sites. [30]. The reasons for these apparent differences in loss of areal and volumetric BMD across the lifespan among these ethnic groups are unknown, but they could be due in part to differences in environmental factors (for example, prevalence of smoking and alcohol use are lower among these Afro-Caribbeans than among US whites and blacks), genetic variation, or genotype by environment interactions.

We also investigated the heritability of areal and volumetric BMD in our Afro-Caribbean families. Unlike several previous family studies which considered only age, gender and BMI when estimating heritability, but similar to a study of areal BMD in Mexican Americans [13], we examined a large number of potential covariates for BMD. In general, four covariates, age, sex, height, and weight, accounted for most (73% to 99%) of the variation in areal and volumetric BMD due to measured covariates. For three traits, areal BMD at the lumbar spine and cortical BMD at the radius and tibia, inclusion of other covariates, especially menopausal status, dramatically increased the proportion of total phenotypic variation due to measured covariates. Although menopausal status is significantly associated with almost all of the areal and volumetric BMD traits, the strongest and most significant associations were with lumbar spine BMD and cortical BMD at the radius and tibia.

We found that smoking was associated with decreased trabecular, but not areal BMD or total or cortical volumetric BMD. Several cross-sectional studies [31-33] have found lower BMD among current compared with never smokers. Male smokers had 0.3 standard deviations lower femoral neck BMD compared with never smokers in a meta-analysis of 5 published studies, an effect size that was similar to that observed among women [31]. Compared with never smokers,

past smokers had 5% or 0.3 standard deviations lower calcaneal BMD independent of important covariates [34]. Men aged 20-29 years old who smoked had 10% lower hip BMD compared with non-smokers, suggesting that smoking may also reduce peak BMD [35]. The lower BMD among current smokers persists after adjusting for important covariates including age, body weight, alcohol intake, and physical activity. However, our sample size was too small to look for sex by smoking effects as reported by Deng and colleagues [36].

Similar to some investigators [37,38], but not others [39], we observed the slightly counterintuitive result that ever-pregnant status (i.e. parity) was associated with increased areal BMD. Although pregnancy is known to be associated with transient decreases in BMD, the observation that increased parity in associated with increased BMD is not well understood, although it may be due to increases in body weight, intestinal calcium absorption, and later age at menopause [26]. Further investigation in our study indicated that ever-pregnant status was associated with increased cortical, but not trabecular BMD at both skeletal sites.

As reported by others [(40,41)], we found that diabetes was associated with increased areal and volumetric BMD. The diabetes association was stronger for trabecular than cortical BMD at both the radius and tibia. Furthermore, as described above, we observed that smoking was associated with decreased trabecular but not cortical BMD, and parity was associated with increased cortical, but not trabecular BMD at both skeletal sites. Thus, unlike analyses of areal BMD, in which conflicting results are often obtained, analyses of volumetric BMD may reveal that different bone compartments are being influenced by specific environmental factors and this knowledge may lead to a better understanding of the possible mechanisms involved.

Except for cortical volumetric BMD, residual heritabilities of areal and volumetric BMD in our Afro-Caribbean families ranged from 0.55 (for femoral neck) to 0.70 (for radius trabecular

BMD). Similar to family studies in other population groups [13,42], genes accounted for a larger proportion of the total variation than did measured covariates in our Afro-Caribbean families. We do not know why heritability of cortical volumetric BMD at the radius and tibia was considerably lower than that for trabecular volumetric BMD or areal BMD. The proportion of variation due to measured covariates was similar across all volumetric and areal BMD traits, indicating that perhaps unmeasured covariates may have a larger effect – or perhaps that we are not adequately powered to model the effects of some covariates. However, we did not observe any difference in the magnitude of the effect of measured covariates on loaded (tibia) versus unloaded (radius) bones, indicating that the potential effects of unmeasured covariates may not be large.

In conclusion, our study provides the first comprehensive genetic epidemiologic analysis of volumetric BMD measured by QCT, and the first analysis of these traits in extended families of African descent. Our findings reveal that genes account for as much or more of the total variation in areal and volumetric BMD than do environmental factors, but also that the magnitude of the effect of genetic and environmental factors differs between trabecular and cortical bone. Identification of the genetic and environmental determinants of both trabecular and cortical bone mass could reveal novel insights into the etiology of osteoporosis.

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3.0 PLEIOTROPY AND HETEROGENEITY AMONG BONE STRENGTH RELATED PHENOTYPES IN EXTENDED PEDIGREES

In this chapter, we utilized quantitative genetic methods by SOLAR to estimate the extent of the possible genetic correlations (ρ_G, i.e., pleiotropy) among selected phenotypes within families of African descent. In particular, we describe the extent to which a common set of genes simultaneously affects variation within pQCT measured BMD traits; within traits by bone geometry measurements; and between BMD and bone size traits from what we mentioned above. When estimating ρ_G, the common environmental correlation (ρ_E) as well as the total phenotypic correlation (ρ_P) were automatically estimated (ρ_E) or derived (ρ_P) under the variance component framework. The common environmental correlation represents the extent to which the co-variation of two phenotypes affected by shared unmeasured environmental factors. And total phenotypic correlation describes the extent of how two phenotypes correlated to each other overall in families. Several interesting findings include: 1) Strong positive genetic correlations were observed for trabecular or cortical BMD measured at the tibia and radius ($\boldsymbol{\rho_G}$ >0.82, P<0.01); 2) There was no significant correlations observed between trabecular and cortical BMD measured within the same anatomical site; 3) Genetic correlations between volumetric BMD and bone length and circumference were also not statistically significant. All detailed methods, results and discussions associated with this chapter were summarized in the manuscript (in press) for ***Journal of Bone Mineral Research***; please refer to it as below.

Title:

Pleiotropy and Heterogeneity among Bone Strength Related Phenotypes in Extended Pedigrees

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Funding Resource:

The study was supported by grants R03-AR050107 and R01-AR049747 from the National Institute of Arthritis and Musculoskeletal Diseases.

3.1 INTRODUCTION

Osteoporosis is a heritable disorder characterized by decreased bone strength and increased risk of fracture. Bone mineral density (BMD) is a major determinant of bone strength and osteoporotic risk, but factors other than BMD also contribute to bone strength. In particular, bone size has important effects on the biomechanical properties of bone independent of BMD. For example, the external diameter of long bones is exponentially related to its strength such that even small increments in external bone dimensions have a large influence on bone strength. For the same amount of bone mass, long bones with larger periosteal circumference will have greater bending strength compared to bones with smaller periosteal circumference. Part of the known heritable component to bone strength and fracture risk may thus be due to familial influences on bone dimensional properties and periosteal circumference. In contrast to our understanding of the familial and genetic influences on areal BMD, measured by two-dimensional dual-energy X-ray absorptiometry (DXA), considerably less is known about the genetic architecture of bone size determined by three-dimensional imaging methods such as quantitative computed tomography (QCT).

Because QCT measures of volumetric BMD are not confounded by bone size, analyses of these traits, separately and together, could reveal whether different or similar sets of genes and environmental factors affect these two components of bone strength. Furthermore, the effects of genes and environmental factors on bone strength and osteoporosis may be skeletal site-specific. For example, individuals with low bone mass at one anatomical region often do not have low bone mass at a different anatomical region [1-3]. Moreover, murine models indicate that bone strength and trabecular and cortical BMD are highly heritable and genetically regulated in a

skeletal-site specific manner [4-7], and a better understanding of these observations could reveal fundamental mechanisms underlying bone strength.

In the current study, we used quantitative genetic analysis methods to investigate the contribution of genes, shared genes, and shared environments to phenotypic variation in bone strength related phenotypes measured by peripheral QCT in extended, multigenerational families including measures of volumetric BMD, bone length and periosteal circumference. We tested the hypothesis that bone size and BMD are largely regulated by unique (non-shared) sets of genes. We also investigated if trabecular and cortical volumetric BMD are largely regulated by different genes.

3.2 MATERIALS AND METHODS

3.2.1 Study Sample

As part of a large population-based prostate cancer surveillance project of all age-eligible men on the Caribbean island of Tobago [8], we recorded the number of living siblings for each participant, as well as the vital status and residence of their parents. The probands were selected without regard to medical history or BMD. All eight probands for the Tobago Family Health Study were between 52-103 years of age and none had previously been diagnosed with prostate or any other cancer. In addition, all first-, second- and third-degree relatives of these probands and their spouses were invited to participate regardless of their medical history or BMD. To date, we recruited 471 individuals age 18 and older in eight multigenerational families with a mean family size of >50 individuals. These 284 women and 187 men with phenotype data ranged in

age from 18-103 years (mean age, 43 years). A total of 3,535 different relationship pairs were ascertained [9]. An example of the pedigree structure is shown in Figure 1. Written informed consent was obtained from every participant, using forms and procedures approved by the Tobago Division of Health and Social Services and University of Pittsburgh Institutional Review Boards.

3.2.2 Peripheral Quantitative Computed Tomography (pQCT) Assessment

Single axial tomographic slices of the non-dominant forearm and left tibia were scanned using a Stratec XCT 2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) according to standardized measurement and analysis procedures. Each scan was acquired with a 0.5-mm voxel size, slice thickness of 2.5 mm, and at a speed of 20 mm/s. The precise position of the measurement sites were determined in a 30 mm planar scout view using the medial endplate of the radius and tibia as standard anatomic landmarks and automatically set by the software at 4% or 33% of the length of the radius and tibia proximal to the distal endplate. These anatomical sites were chosen in order to assess primarily trabecular and cortical bone, respectively. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process.

Image processing was performed using the Stratec software package (Version 5.5E). All 4% ultra distal radius and tibia scans were analyzed using identical parameters for contour finding trabecular (mg/cm^3) bone compartments. All 33% proximal radius and tibia shaft scans were analyzed using identical parameters to determine the volumetric BMD of cortical (mg/cm^3) bone compartments. Periosteal circumference (PC) was measured using a scan through the diaphysis (at 33% of the bone length in the proximal direction of the distal end of the bone) of the

radius and tibia using the circular ring model. The short-term *in vivo* precision of the pQCT measurements was evaluated in 30 subjects. All CVs for measures of pQCT BMD and periosteal circumference were ≤2.1 %.

3.2.3 Anthropometry

Body weight was measured to the nearest 0.1 kilogram with participants wearing indoor clothing but without shoes using a balance beam scale. Standing height was measured to the nearest 0.1 cm without participants wearing shoes using a wall-mounted stadiometer. The average of two measurements was used in analysis. Seated height was measured to the nearest centimeter with the participant seated on a stool against a wall-mounted stadiometer. Trunk length (cm) was estimated as the seated height minus the height of the stool. Leg length (cm) was estimated as the difference between standing height and trunk length. Body mass index was calculated by dividing body weight (kg) by height (m^2).

3.2.4 Measurements of Covariates

Information on demographic characteristics, medical history and lifestyle habits was obtained by questionnaire administered by trained and certified clinical staff. Our choice of potential correlates for phenotypes was based on our previous studies and from the literature [10-12]. A detailed description of all of the covariates was reported previously [9]. Briefly, subjects were classified as current smokers (yes/no) and participants who had smoked fewer than 100 cigarettes in their lifetime were considered nonsmokers. Information on alcohol consumption was obtained by questionnaire and expressed as drinks per week. Subjects were asked whether a

doctor or health care provider had ever told them they had certain medical conditions including arthritis, diabetes, hypertension or cardiovascular disease. Hypertension was defined as a diastolic blood pressure exceeding 90 mm Hg or systolic blood pressure exceeding 140 mm Hg or currently taking blood pressure medication (n=41). Diabetes was defined as fasting glucose level exceeding 126 mg/dl (n=45) or currently taking diabetes medication (n=26).

3.2.5 Statistical Analysis

We first assessed the distributions of all the traits and, if necessary, performed transformations (log, square root *etc*.) to reduce non-normality. Subsequently, all outliers (± 4 standard deviations) were removed for each trait, and no more than 3 values were removed for a single variable.

Prior to our estimation of heritability, we performed combined forward and reverse stepwise linear regression analysis on all data, using the R statistical package (Version 2.4.0). We required each variable remaining in the model to be significant at a less stringent P value ($P \leq 0.10$) initially. This step was performed ignoring the non-independence of the subjects. However, we subsequently evaluated each of the potentially significant covariates using a variance component framework that enabled us to take into account the correlations among family members. Covariates were treated as significant if the corresponding P value was less or equal to 0.05.

Heritabilities were estimated under the variance component framework using SOLAR (Sequential Oligogenic Linkage Analysis Routines) [13], which partitions the variance of a quantitative trait into components attributable to individual-specific covariates (e.g., age, BMI,

diabetes status, etc.), an additive genetic (polygenic) component, and a residual non-measured environmental component.

Genetic correlations were assessed using bivariate maximum likelihood methods as implemented in SOLAR. In this analysis, the total phenotypic correlation (ρ_P) between two traits was decomposed into the components due to a gene or common sets of genes and shared environmental effects. In order to estimate the genetic (ρ_G) and environmental correlations (ρ_E) for pairs of traits, the matrix of kinship coefficients was generated conditioning on all the related individuals within each pedigree. Using standard quantitative genetic methods, the phenotypic variance-covariance matrix and its genetic and environmental components were then obtained. From these matrices, the genetic correlation ρ_G was estimated directly. Likelihood ratio statistics were used to test the significance of ρ_G between any pair of traits [(14)] .

In bivariate analysis, the genetic cause of correlation (ρ_G) is chiefly pleiotropy, in which a common gene/genes affects two or more phenotypes. The degree of correlation arising from pleiotropy describes the extent to which two phenotypes are co-influenced by the same gene or common set of genes. On the other hand, the environment correlation (ρ_E) describes how two phenotypes are co-regulated by the same unmeasured (or unadjusted) environmental factors. Both ρ_G and ρ_E reflect the overall genetic and environmental factors which affect the covariation of the two traits.

The residual phenotypic correlation (ρ_P) between two traits is the sum of both residual genetic and unmeasured environmental components and is estimated as follows [(15)]:

$$\rho_P = (\text{sqrt}(h^2 r_1) * \text{sqrt}(h^2 r_2) * \rho_G) + \text{sqrt}(1 - h^2 r_1) * \text{sqrt}(1 - h^2 r_2) * \rho_E$$

in which h^2r_1 and h^2r_2 are residual heritabilities for the two traits; ρ_G and ρ_E represent genetic and environmental correlation between two traits after adjusting for measured covariates. We also estimated the proportion of ρ_P due to shared genetic contributions as [(sqrt(h^2r_1)* sqrt (h^2r_2)* ρ_G)] / ρ_P *100%; the relative proportion of phenotypic correlation due to common unmeasured environmental factors was calculated similarly.

In this study, we estimated heritabilities, as well as genetic and environmental correlations using two different models. In the base model, we incorporated the effects of age, gender and body weight only. In the full model, we incorporated the effects of all potential significant covariates reported by previous studies [9-12, 16]. Because the results were similar for both models, we report only the results from the base model (Tables 3 to 5).

3.3 RESULTS

3.3.1 Subject Characteristics

Among our 471 participants, BMI was higher (P<0.001) but body weight was similar in women and men (Table 1). Prevalence of cigarette smoking and alcohol consumption was higher in men than women; the prevalence of hypertension, diabetes, cardiovascular disease and arthritis were comparable in both genders.

Men had significantly greater trabecular volumetric BMD than women at both the distal radius and tibia. However, women had greater cortical volumetric BMD at the radius shaft. There was no gender difference in cortical volumetric BMD at the distal tibia. All measures of bone size were significantly greater in men than women.

Table 11. Characteristics of Study Subjects

(Table 1 in this chapter)

Characteristics	***Men (N=187)***	***Women (N=284)***
Age (years)	42 ± 17	42 ± 17
Anthropometric		
BMI (kg/m^2)	26.7 ± 4.9	29.4 ± 7.0 *
Height (cm)	177.0 ± 7.4	166.5 ± 6.5 *
Weight (kg)	83.7 ± 17.3	81.3 ± 19.4
Life Style		
Current smoking (%)	11.4	0.7 *
Alcohol consumption (% >1 drink per week)	28.9	2.8 *
Medical Conditions		
Hypertension (%)	30.8	27.2
Diabetes (%)	12.5	17.3
Cardiovascular Disease (%)	3.8	6.0
Arthritis (%)	7.0	12.1
BMD (mg/cm3)		
Radius		
Trabecular	239 ± 40	210 ± 40 *
Cortical	1213 ± 22	1222 ± 26*
Tibia		
Trabecular	259 ± 35	243 ± 35 *
Cortical	1181 ± 27	1185 ± 31
Bone Size related Phenotypes		
Trunk Length (cm)	88.3± 3.9	84.0± 4.0 *
Leg Length (cm)	88.7± 5.3	82.5± 4.3 *
Radius		
Length (mm)	305± 18	282± 19 *
PC † (mm)	43.2± 3.3	36.4± 2.9 *
Tibia		
Length (mm)	398± 40	375± 37 *
PC (mm)	79.4± 6.2	71.3± 5.4 *

* Indicates the comparison by gender is statistically significant (P<0.05).
† PC indicates Periosteal Circumference.

3.3.2 Heritability Estimation

In Table 2, we present the proportions of total phenotypic variation due to measured covariates and residual heritabilities for both the base and full models. For both models, the estimates for residual heritabilities (h^2r) of trabecular BMD were substantially higher than those for cortical BMD. For bone size related traits, the residual heritabilities at the weight bearing skeletal sites (tibia length and PC) were higher than the corresponding measures at non-weight bearing skeletal sites (radius length and PC).

3.3.3 Correlations between Trabecular and Cortical BMD

We also examined the genetic correlation between cortical and trabecular volumetric BMD measures to determine the extent to which a common set of genes may influence these distinct bone compartments (Table 3). We found that the genetic correlations between the two trabecular and two cortical BMD measurements were significantly positive (0.87±0.05 and 0.83±0.12, respectively; $P<0.01$). Moreover, the common genetic correlations were much higher than their corresponding shared (unmeasured) environmental correlations (0.48±0.13 and 0.66±0.05, respectively). In stark contrast, the genetic and environmental correlations between cortical and trabecular BMD measurements within an anatomical region were low and not statistically significant.

Table 12. Proportion of Total Phenotypic Variation attributable to Genetic and Measured Environmental Factors

(Table 2 in this chapter)

	Environment (base model) *	*Genetic † (base model)*	*Environment (Full model) ‡*	*Genetic (Full model)*	*Significant Covariates (Full model) §*
Volumetric BMD (mg/cm3)					
Radius					
Trabecular	0.25	0.66 ± 0.10	0.26	0.69 ± 0.09	AG, SX, WT, SM, DB
Cortical	0.07	0.17 ± 0.08	0.20	0.28 ± 0.08	SX, WT, MP, AR
Tibia					
Trabecular	0.24	0.67 ± 0.10	0.26	0.73 ± 0.11	AG, SX, WT, SM, DB, VD
Cortical	0.13	0.32 ± 0.09	0.22	0.41 ± 0.08	AG, WT, MP, PG
Bone Size related Phenotypes					
Trunk Length (cm)	0.42	0.49 ± 0.11	0.43	0.44 ± 0.12	AG, SX, WT, DB
Leg Length (cm)	0.35	0.51 ± 0.09	0.35	0.51 ± 0.09	AG, SX, WT
Radius					
Length (mm)	0.37	0.32 ± 0.10	0.37	0.33 ± 0.10	SX, WT
PC (mm)	0.65	0.60 ± 0.10	0.66	0.59 ± 0.09	AG, SX, WT, MP, DB, VD
Tibia					
Length (mm)	0.11	0.64 ± 0.09	0.12	0.65 ± 0.10	AG, SX, WT, AR
PC (mm)	0.50	0.74 ± 0.09	0.50	0.78 ± 0.09	AG, SX,WT, MP, VD

* Adjusted covariates in base model: Age, Gender and Weight

† Residual heritability (mean ± SE), all $P<0.01$.

‡ Full Model: Adjusted for all potential covariates

§ Abbreviations for covariates name: AG: Age, SX: Sex, WT: Weight, SM: Current Smoking Status, AC: Current Alcohol Drinking Status, MP: Menopause Status, PG: Ever Pregnant, DB: Diabetes Status, AR: Arthritis Status, VD: Vitamin D Supplement

3.3.4 Correlations between BMD and Bone Size Phenotypes

Bone size is a determinant of bone strength independent of BMD. Previous studies in mice suggest that genes which contribute to variation in bone size may differ from those that contribute to variation in BMD.[17, 18] Thus, we also examined whether a common set of genes might have pleiotropic effects on bone size and BMD in our collection of extended families (Table 4). Except for the correlation between periosteal circumference (PC) and cortical BMD at the radius shaft, all other correlations were generally low and only marginally ($0.10>P>0.05$) or not statistically significant. The moderate genetic correlation between periosteal circumference and cortical BMD at the radius shaft ($\rho_g = -0.44 \pm 0.20$, $P<0.05$), indicates that about 20% of the covariation between these traits may be attributable to common genetic determinants. Overall,

these results indicate that the majority of genes that influence bone size and BMD may be unique for each skeletal phenotype.

Table 13. Correlations between Trabecular and Cortical BMD at the Radius and Tibia

(Table 3 in this chapter)

Traits	$\rho_G \pm SE$	$\rho_E \pm SE$	ρ_p
Trabecular, Radius *vs.* Cortical, Radius	0.25±0.23	0.03±0.12	0.12
Trabecular, Radius *vs.* Trabecular, Tibia	0.87±0.05 ###	0.48±0.13 #	0.68
Trabecular, Radius *vs.* Cortical, Tibia	0.25±0.18	-0.03±0.14	0.12
Cortical, Radius *vs.* Trabecular, Tibia	0.32±0.23	-0.24±0.14	0.02
Cortical, Radius *vs.* Cortical, Tibia	0.83±0.12 ###	0.66±0.05 ###	0.69
Trabecular, Tibia *vs.* Cortical, Tibia	0.30±0.18	-0.13±0.14	0.10

* P-value: ###:P<0.001, ##: P<0.01, #: P<0.05,
† Adjusted for Age, Gender and Weight
ρG indicates genetic correlation; ρE, environmental correlation; ρp, phenotypic correlation; PC, Periosteal Circumference

3.3.5 Correlations among Bone Size related Phenotypes

Finally, we also investigated the relationship among the different measures of bone size (Table 5). The genetic correlation among the different traits ranged from 0.49± 0.11 (tibia length vs.

Table 14. Correlations between Bone Size amd BMD Phenotypes

(Table 4 in this chapter)

Traits	$\rho_G \pm SE$	$\rho_E \pm SE$	ρ_p
Radius Length *vs.* Cortical BMD, Radius	0.10±0.28	-0.08±0.09	-0.05
PC, Radius *vs.* Cortical BMD, Radius	-0.44±0.20 #	-0.27±0.10 #	-0.30
Tibia Length *vs.* Cortical BMD, Tibia	-0.33±0.19	0.12±0.12	-0.07
PC, Tibia *vs.* Cortical BMD, Tibia	-0.28±0.16	-0.35±0.14 #	-0.32

* P-value: ###:P<0.001, ##: P<0.01, #: P<0.05
† Adjusted for Age, Gender and Weight
ρG indicates genetic correlation; ρE, environmental correlation; ρp, phenotypic correlation; PC, Periosteal Circumference

tibia PC) to 0.82 ± 0.06 (radius PC vs tibia PC) (all P-values < 0.01). Thus, approximately 25-70% of the covariation between measures of bone length or width within and between anatomical regions may be attributable to the pleiotropic effects of a common set of genes. The corresponding total phenotypic correlations are moderate (-0.09 to 0.43) and common unmeasured environmental correlations are not significantly different from zero (All $\rho_E \leq 0.29 \pm 0.16$; P>0.1). In addition, we assessed the relationship between trunk length and leg length and found that the genetic and unmeasured environmental correlations were high, but in opposite directions (ρ_G =0.77 ± 0.11 and ρ_E = -0.44 ± 0.17; P<0.05). Thus, the phenotypic correlation between these two traits was close to zero.

Table 15. Correlations between Different Bone Size Measures

(Table 5 in this chapter)

Traits	$\rho_G \pm SE$	$\rho_E \pm SE$	ρ_p
Radius Length *vs.* Tibia Length	0.77±0.13 ###	0.03±0.12	0.28
PC, Radius *vs.* PC, Tibia	0.82±0.06 ###	0.29±0.16	0.43
Radius Length *vs.* PC, Radius	0.53±0.14 ##	0.15±0.12	0.23
Tibia Length *vs.* PC, Tibia	0.49±0.11 ###	-0.02±0.18	0.21
Trunk Length *vs.* Leg Length	0.70±0.11 ###	-0.44±0.17 #	-0.09

* P-value: ###:P<0.001, ##: P<0.01, #: P<0.05
† Adjusted for Age, Gender and Weight
ρG indicates genetic correlation; ρE, environmental correlation; ρp, phenotypic correlation; PC, Periosteal Circumference

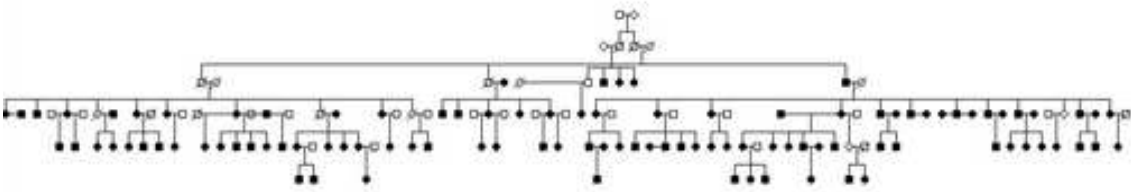

Figure 7. *Example of Pedigree in the Tobago Family Health Study (Figure 1 in this chapter)*

4 generation family including 142 family members; 102 filled symbols indicate family members with phenotype data.

3.4 DISCUSSION

The Tobago Family Health Study provided an excellent and unique opportunity to characterize the genetic architecture of several bone strength related traits in a population of West African heritage, a racial group that has high bone strength but has heretofore been largely under-represented in osteoporosis research. Tobago is a relatively small island, and the population is predominantly of West African origin (97%). [19] Family size is large thus facilitating recruitment of extended multi-generational families suitable for genetic studies. The present study also provided the first test of the hypothesis that trabecular and cortical volumetric BMD are conjointly inherited in humans (i.e., share common genes).

We found that the positive phenotypic association between volumetric BMD measured at the tibia and radius is due in large part both to the effects of shared genes and to shared environments. Our results show that both cortical and trabecular BMD are heritable polygenic traits and that the shared genetic correlation is substantially higher than the common environment correlation. Although ρ_G was similar for cortical and trabecular BMD, we also estimated the relative proportion of the total phenotypic correlation that was attributable to genes (as described in Methods). The relative genetic contribution to the total phenotypic correlation was higher for trabecular BMD (78%) than it was for cortical BMD (28%) mostly because of the low residual heritability of cortical BMD.

On the other hand, our findings suggest that the genes influencing phenotypic variation in cortical BMD are largely distinct from those that control trabecular BMD within a given anatomical site. Thus, the majority of the phenotypic variation in cortical and trabecular volumetric BMD within a skeletal region may be largely due to unique genes for each bone type.

These results appear to be inconsistent with studies by Livshits and colleagues [20, 21], who reported that a single major locus with pleiotropic effects may affect both phalangeal trabecular and cortical areal BMD (measured by hand radiographs). They also concluded that a single major locus (and polygenes) with pleiotropic effects influence measures of areal BMD at the femoral neck and lumbar spine.[20] Possible reasons for the apparent discrepancy between our study results include differences in the study populations (Caucasian versus African ancestry), skeletal sites examined (hands, hip and spine versus arm and leg) and the different methods used to measure BMD (x-rays and areal BMD versus pQCT and volumetric BMD). In addition, although bivariate segregation analyses can be powerful, they may also be misleading [22, 23]. However, our findings are consistent with several studies reporting that loci (both single genes and polygenes) may regulate areal BMD in a site-specific manner [14, 24-26] Furthermore, our results in humans are consistent with findings from inbred strains of mice where the distribution of bone mineral into the trabecular and cortical compartments was shown to be regulated genetically and by distinct loci.[4] Localizing and identifying the genes that contribute uniquely to the density and strength of trabecular and cortical bone in humans and animal models may lead to fundamental insight on the complex nature of osteoporosis.

The heritability of body size in humans has been assessed in numerous past studies, but has largely been based on measures of total body height. Body size can also be divided into components of size such as trunk length, long bone length and bone circumference. These aspects of body size have different developmental patterns [27] and might also be affected by distinct sets of genes. However, the genetic architecture of specific components of body size has not been well defined in humans. Thus, we comprehensively evaluated the heritabilities and genetic relationships among different measures of body and bone size. After adjusting for age,

sex and weight, we found evidence for a strong genetic influence on all measures of body and bone size with residual heritabilities ranging from 0.32±0.10 (radius) to 0.64±0.09 (tibia) for measures of bone length and 0.60±0.09 to 0.74±0.09 for measures of periosteal circumference at the tibia and radius shaft, respectively. We also found a high genetic correlation between measures of bone length ($\rho G = 0.77$) and periosteal circumference ($\rho G=0.82$) at the tibia and radius (Table 5). These observations suggest that, compared to unmeasured environmental factors, common genes have greater impact on the phenotypic covariation in long bone length and circumference at different anatomical regions.

On the other hand, the genetic correlations between measures of bone length and circumference, although significantly different from zero, were moderate ($\rho G=0.49$ to 0.53). Thus, most of the covariation in long bone length and circumference is not attributable to pleiotropic effects. This result may not be surprising because long bone growth and geometry are governed by two distinct biological mechanisms of bone formation.[28] Longitudinal bone growth occurs by endochondral ossification at the epiphyseal growth plates by chondrocytes whereas radial bone growth at the diaphysis occurs by appositional deposition of cortical bone at the periosteum by osteoblasts. Our results are also consistent with analyses of inbred mice which have identified distinct genetic loci for femur length and width.[29]

In addition, we observed low and mostly non-significant genetic correlations between the measures of bone size and BMD measured at several different skeletal sites (Table 4). With exception of the moderate genetic correlation between periosteal circumference and cortical volumetric BMD at the radius, the majority of the phenotypic variation in bone size and BMD appears to be due largely to different genes. These observations are consistent with findings from inbred strains of mice where different quantitative trait loci have been identified for

periosteal circumference [18] or cross-sectional area [17] versus BMD. Thus, identifying the genes contributing to bone strength and osteoporosis will require careful dissection of the genetic architecture of both bone size and BMD related traits. Moreover, the identification of specific genes for bone size could provide important insight on bone biology and osteoporosis.

We assessed the relative contributions of genes and environmental factors using two different models: a base model which incorporated age, sex, and body weight and a full model which incorporated all significant covariates. Our results were similar for both models, most likely because age, sex, body weight and height account for the majority (73%-99%) of total phenotypic variation due to significant covariates in this population [9]. Although the sample sizes are small, we also estimated sex specific heritabilities and genetic correlations. In general, the sex-specific estimates were similar in magnitude to each other and to those obtained on the overall population, except the standard errors were larger (results not shown). Therefore, we have no strong evidence for sex-specific differences. However, because our male and female sample sizes are small, we have little power to detect such differences in our current families.

The results of our multivariate analysis not only provide insight into the complex genetic architecture of bone strength related traits, but may also have practical implications. Large genetic correlations in multivariate quantitative genetic analyses can help guide genetic mapping efforts. For instance, using a multivariate bone phenotype characterized by cortical BMD at different skeletal regions may be advantageous if the pleiotropy was due largely to a few loci.[15] Thus, our findings provide a rationale for future multivariate linkage analyses to identify novel genetic loci with pleiotropic effects on bone related traits. The present study may also have implications for understanding the determinants of bone strength and the etiology of osteoporosis. For example, the high polygenic heritabilities and low genetic correlations that we

observed between cortical and trabecular BMD within a given anatomic site is consistent with osteoporosis being a heterogeneous disorder.

In conclusion, our results suggest that the density of the skeleton is a highly heritable polygenic trait that is under compartment (bone type) specific genetic regulation. Moreover, the majority of the phenotypic variation in bone size and density appears to be strongly influenced by non-shared genes. Identifying the genes for each of these bone strength related traits may lead to a better understanding of the complex nature of osteoporosis and could also impact on the molecular diagnosis, prevention and treatment of this skeletal disorder.

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4.0 COMPARISON AND EVALUATION OF MULTIVARIATE ANALYSIS STRATEGIES FOR UNCOVING PLEITROPIC FACTORS ON FAMILY DATA

Evidence for linkage with bone related phenotypes has been reported for multiple chromosomal regions. Despite many studies suggesting that bone phenotypes (especially BMD) variation at various skeletal sites is governed by shared genetic factors, most previous studies revealed site-specific peaks, suggesting that minimal genetic pleiotropy (shared genetic determinants) exists between these traits. These observations indicate that a relatively small number of genes may contribute to an underlying clustering of bone related phenotypes. Identification of these common genes and elucidation of their molecular basis will contribute to a better understanding and possible treatment for osteoporosis which is a systemic disease.

Multivariate analysis (MA) methods were widely used since 2001 in an attempt to dissect the pleiotropic genetic and environmental basis for complex diseases, such as osteoporosis, metabolic syndrome, and asthma. However, many statistical issues remain unaddressed by these reports. First**,** the selection of either PCA or FA seems arbitrary; none of the groups justified why they chose one instead the other. We decided to evaluate the performance of these two approaches. In particular, we wanted to assess which method is better able to detect the underlying environmental or genetic factors. Second, most reports used raw traits as input variable, but a few used residuals after regressing out some important environmental factors. Does analysis of residuals significantly improve the ability of PCA or FA methods to detect

underlying genetic components? No direct comparisons to answer this question have been reported. Third, many groups have compared the heritability of composite traits (obtained from PCA or FA) with the original phenotypes. Does higher heritability of the composite trait compared to the original phenotype necessarily imply that the composite trait better reflects the underlying genetic components and thus increase the chance for detecting underlying genes? No literature that we are aware of has addressed this question.

Hence, the goal of this chapter is to explore the answers to the above three questions using simulated data on nuclear families. Several interesting findings include: 1) Both PCA and FA behaved qualitatively similar in most cases, although FA performed better to detect predominant signals from an underlying trait; 2) Using residuals in the PCA or FA analyses greatly increased the probability that PCs or factors detect common genetic components instead of common environmental factors, except if there is statistical interaction between genetic and environmental factors; 3) There was no predictable relationship between heritabilities obtained from composite phenotypes versus original complex traits, however composite trait heritability generally reflected the genetic characteristics of the detectable underlying components. All detailed methods, results and discussions associated with this chapter were summarized and contributed recently to ***Genetic Epidemiology,*** titled "A Comparison of Principle Component Analysis and Factor Analysis Strategies for Uncovering Pleiotropic Factors**"**. Please refer to it as below.

Title:

A Comparison of Principle Component Analysis and Factor Analysis Strategies for Uncovering Pleiotropic Factors

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4.1 INTRODUCTION

Numerous studies over the past several decades indicate that genes contribute to the development of complex diseases such as osteoporosis, obesity, and diabetes. Many risk factors for these diseases (such as bone mineral density, body fat, glucose levels) have been shown to be moderately to highly heritable. In recent years, many studies have suggested that a majority of these highly heritable traits (risk factors) are governed by a set of common genes (i.e. pleiotropy, defined as when two or more phenotypes are co-regulated by a common gene or a common sets of genes) [Deng, et al. 2006; Hegele 1997; Li, et al. 2002; Mitchell, et al. 1996]. One piece of evidence in support of the above hypothesis is that bivariate linkage analyses of some of these traits revealed stronger linkage signals than were obtained from univariate linkage analysis of each trait separately [Devoto, et al. 2005; Li, et al. 2006; Livshits, et al. 2004; Martin, et al. 2004].

Conventional measurements of these complex disease-related phenotypes produce many intercorrelated phenotypes. For example, bone mineral density (BMD) can be measured by peripheral Quantitative Computed Tomography (pQCT) at distal and shaft sites for both radius and tibia. High phenotypic and genetic correlations are observed from these bone phenotypes due to the common contributions from trabecular and cortical components. Therefore it is possible that there might be a relatively small number of factors (both genetic and environmental) involved in certain metabolic pathways that contribute to variation in an underlying cluster of phenotypes. Identification of these common factors and elucidation of their molecular basis should contribute to a better understanding of and possible treatment for some complex diseases.

It is well-known that bivariate and tri-variate genetic analyses are computationally intensive. And genetic analyses of more than three traits are beyond our current computational

ability. Therefore, multivariate analysis might be an alternative yet effective solution to identify common genetic and environmental factors that affect multiple traits. Principal component analysis (PCA) and factor analysis (FA) both involve a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated (PCA) or correlated (FA) variables called principal components or factors. During the PCA/FA extraction, the shared variance of a variable is partitioned from its unique variance and error variance to reveal the underlying factor / PC structure. Only the shared variance appears in the solution. So it is reasonable to believe that these two methods have the potential to classify phenotypic variation into independent / dependent components that may amplify or purify genetic signals and hence be used to dissect genetic networks regulating complex biological systems.

Since 2001, ten groups of investigators that we are aware of have published articles that used multivariate analysis methods in an attempt to dissect the genetic and environmental basis for complex diseases, such as osteoporosis, metabolic syndrome, and asthma. Seven of these groups applied PCA [Chase, et al. 2002; Guo, et al. 2005; Hakulinen, et al. 2006; Karasik, et al. 2004; Lin, et al. 2005; Musani, et al. 2006; Peacock, et al. 2004], while the other three used FA [Austin, et al. 2004; Holberg, et al. 2001; Lee, et al. 2004]. In addition, 7 groups used raw phenotypes directly as the input variables, one group used raw traits but performed analysis by gender and generation [Karasik, et al. 2004], and the last two groups used residuals (after adjustment for significant covariates) [Austin, et al. 2004; Lin, et al. 2005]. The goals of the 10 groups also differed: one group used multivariate analysis for phenotype clustering/classification, by which it developed composite index scores summarizing characteristics of raw traits from different skeletal sites [Lee, et al. 2004]. The remaining nine groups all focused on exploring the underlying genetic/environmental basis of composite traits

(that is, principal components or factors) derived from PCA or FA. Among these nine groups, two reported genetic or environmental correlations between composite traits and some well-defined real (observed) phenotypes [Guo, et al. 2005; Hakulinen, et al. 2006]; two reports focused exclusively on heritability estimation for composite and real traits [Austin, et al. 2004; Lin, et al. 2005]; and three reports concentrated on the association (or linkage) between these composite traits and QTLs (Quantitative Trait Loci) [Holberg, et al. 2001; Musani, et al. 2006; Peacock, et al. 2004]; The final two papers did both heritability estimation and association/linkage analysis for composite phenotypes [Chase, et al. 2002; Karasik, et al. 2004].

However, many statistical issues remain unaddressed by these reports. First**,** the selection of either PCA or FA seems arbitrary; none of the groups justified why they chose one instead the other. Consequently we decided to evaluate the performance of these two approaches. In particular, we wanted to assess which method is better able to detect underlying environmental or genetic factors. Second, most reports used raw traits as input variables, but a few used residuals after regressing out some important environmental factors. Does analysis of residuals significantly improve the ability of PCA or FA methods to detect underlying genetic components? No direct comparisons to answer this question have been reported. Third, many groups have compared the heritability of composite traits (obtained from PCA or FA) with the original phenotypes. Does higher heritability of the composite trait compared to the original phenotype necessarily imply that the composite trait better reflects the underlying genetic components and thus increase the chance for detecting underlying genes? No literature that we are aware of has addressed this question. Hence, the goal of this paper is to explore the answers to the above three questions using simulated data on nuclear families.

4.2 MATERIALS AND METHODS

4.2.1 Study Design

Our overall study design is illustrated in Figure 1. Three datasets of underlying (unobserved) traits were generated by simulation; seven underlying traits (E_1, E_2, G_1, G_2, G_3, S_1 and S_2) were involved in making these three datasets. The differences among these three datasets are the variances of the underlying environmental traits and the inclusion or exclusion of S_2 (a gene by sex interaction phenotype). For each of these three datasets of underlying traits, two sets of complex phenotypes were created using arbitrary algebraic functions of the underlying traits. There are 50 complex traits in each of the two function sets. Set 1 involves somewhat simpler algebraic combinations of traits than set 2 (details below). The seven underlying traits represent the unobserved environmental or/and genetic determinants that influence population variation of real traits, which are in turn represented by the sets of 50 complex traits. Using these complex traits, we created three different inputs for further multivariate analysis: raw traits, residuals model 1 (after regressing out E_1 and E_2); and residuals model 2 (after regressing out E_1, E_2 and sex). Finally, we performed both PCA and FA on each dataset × function set × residual combination, for a total of 36 analyses (Figure 1). Each aspect of the study design is described in more detail below.

We evaluated three aspects of the outcomes: 1) the ability to detect the underlying genetic/environmental components; 2) whether the methods worked better when applied to raw traits or to residuals (that is, after regressing out potentially significant environmental covariates); and 3) heritabilities of composite traits (principal component or factor) compared to 50 complex traits or 7 underlying traits.

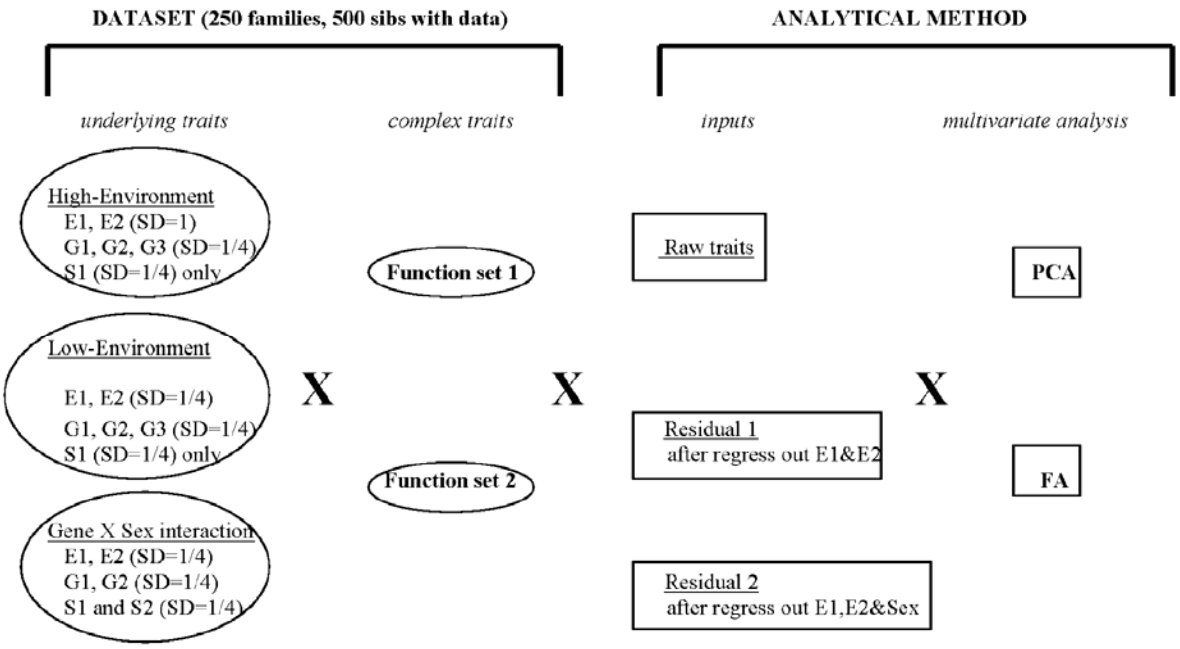

Figure 8. *Blueprint for study design (Figure 1 in this chapter)*

4.2.2 Simulations

We first simulated 250 nuclear families with two parents and two offspring within each family. We then simulated seven underlying original phenotypes (with corresponding genotypes): E_1, E_2, G_1, G_2, G_3, S_1, S_2, (Table I) for the offspring only, for a total of 500 individuals. All of these underlying traits were assumed to be normally distributed conditional on genotype. The simulated "genotypes" for E_1 and E_2 were not used in the model; these two traits were designed as an environmental model (pure environmental effect, no mean differences between people with different genotypes). Because some environmental factors are likely to be similar between siblings, we also allowed for the effect of a shared common environment for E_1 and E_2 by simulating these two traits based on a bivariate normal distribution with means all equal 1,

standard deviation equals 1 or ½ for different datasets and covariance between two sibs equals to 0.2 for E_1 and 0.1 for E_2. Another three traits (G_1, G_2 and G_3) are standard simple genetic models in which means differences among genotypes. As can be seen in Table I, the genotypic means and error variances for, G_1, G_2 and G_3 are identical (mean 1.5, 2.5 and 3.5 for genotype aa, Aa and AA respectively and all standard deviations = ½); only the allele frequencies of these traits differ. The trait S_1 has different means for males and females, but no interaction between sex and genotype. The trait S_2 incorporates sex by genotype interaction.

Table 16. Simulation parameters for 7 Underlying Phenotypes

(Table 1 in this Chapter)

Parameter	Sex-Specific Genotype	E_1	E_2	G_1	G_2	G_3	S_1	S_2
Mean	♂-*aa*	1	1	1.5	1.5	1.5	2	1.5
	♂-*Aa*	1	1	2.5	2.5	2.5	3	2.5
	♂-*AA*	1	1	3.5	3.5	3.5	4	3.5
	♀-*aa*	1	1	1.5	1.5	1.5	1	1
	♀-*Aa*	1	1	2.5	2.5	2.5	2	1
	♀-*AA*	1	1	3.5	3.5	3.5	3	1
SD		1 or 1/4	1 or 1/4	1/4	1/4	1/4	1/4	1/4
Allele Frequency	P(a)	0.8	0.5	0.8	0.9	0.95	0.8	0.7
	P(A)	0.2	0.5	0.2	0.1	0.05	0.2	0.3

4.2.3 Complex Traits

Based on the above underlying traits, we created the first set of 50 complex traits, each of which is an algebraic combination of a subset of the 7 unobserved traits plus the error term, which is normally distributed with mean 1 and standard deviation 1 (table II). Our objective in choosing these functions was to reflect the current genetic/epidemiological assumptions about complex

traits regarding the effects of underlying immeasurable genetic / environment factors. For example, we used additive and multiplicative effects as well as their combinations within and/or between underlying genetic and environmental traits. In addition, we included several very complicated models to assess if PCA and FA could recover underlying traits even from extremely complex traits. In order to assess even more complex models, we then created another set of 50 complex traits, in which we removed some of the algebraically simpler combinations and substituted more complex ones. These new 50 functions were similar in format to the more complicated ones in the first set of functions (Table III). When devising our 50 complex traits for each set, we required that each underlying trait have a similar representation across all 50 complex traits. Based on our function summary file for dataset 1 and 2 (Tables II and III), the percent of times a specific underlying variable (e.g. G_1) was included in the definition of a complex trait across all complex traits was as follows: 60% for E_1, 54% for E_2, 58% for G_1, 54% for G_2, 56% for G_3 and 58% for S_1. In dataset 3, we simply substituted S_1 for G_3 and S_2 for S_1, so the proportions are the same.

These complex traits represent phenotypes that we could observe or directly measure in reality, such as bone mineral density (BMD), body mass index (BMI), glucose level, and blood pressure. The seven original traits represent underlying genetic or environmental components; they contribute to the true variation of the measured (complex) traits but are not actually observed or measured.

4.2.4 Datasets

For each set of functions (Table II and III), we created three different datasets of underlying

Table 17. First Set of 50 Complex Traits

(Table 2 in this Chapter)

Addition
$C1 = E_1 + E_2 + error$ *
$C2 = G_1 + G_3 + error$
$C3 = (G_1 + G_2 + G_3) / 3 + error$
$C4 = G_2 + S_1 + error$
$C5 = E_2 + G_3 + S_1 + error$
$C6 = (G_1 + G_2 + G_3 + E_1 + E_2 + S_1) / 7 + error$
$C7 = (0.5)E_1 + E_2 + error$
$C8 = E_1 + (3.5)G_3 + error$
$C9 = 2G_2 + (0.6)G_3 + error$
$C10 = (1/3)G_1 + G_2 + 2G_3 + error$
$C11 = 2E_1 + (1.4)G_2 + S_1 + error$
$C12 = E_2 + G_3 + (3.2)S_1 + error$
Multiplication and Division
$C13 = E_1E_2 + error$
$C14 = (E_2 + 1) / (E_1 + 1) + error$
$C15 = G_2G_1 + error$
$C16 = (G_1 + 1) / (G_3 + 1) + error$
$C17 = G_3G_2 + error$
$C18 = S_1E_2 + error$
$C19 = (S_1 + 1) / (G_2 + 1) + error$
$C20 = E_1G_3 + error$
$C21 = E_2G_1S_1 + error$
$C22 = E_1G_2S_1 + error$
Combination of addition, subtraction, multiplication and division
$C23 = (E_1 + 1) / (G_3S_1 + 1) + error$
$C24 = (G_2 + 1) / (E_2S_1 + 1) + error$
$C25 = E_1E_2 + G_2 + error$
$C26 = (G_3 + 1) / (G_1 + 1) + E_2 + error$
$C27 = (E_2 + 1) / (E_1 + 1) + S_1 + error$
$C28 = G_3G_2 + (3.2)S_1 + error$
$C29 = E_2S_1 + E_1 + error$
$C30 = (S_1 + 1) / (G_2 + 1) - G_3 + error$
$C31 = E_1G_1 + (0.5)S_1 + error$
$C32 = 3(G_3 + 1) / (S_1 + 1) - E_2 + error$
$C33 = E_1G_3 - (S_1 + 1) / (E_2 + 1) + error$
$C34 = (S_1 + 1) / (G_2 + 1) + (E_2 + 1) / (G_3 + 1) - (0.7)G_1 + error$
Power, exponentiation, logarithm
$C35 = (G_1 + error)^2$
$C36 = (S_1 + error)^3$
$C37 = e^{(G3 + error)}$

Table 17 continued

C38 = $\sqrt{S_1 + \text{error} + 2}$	
C39 = log (G_1 + error + 2)	
Combination of all forms	
C40 = $G_2^{\,2}$ + (G_1 + 1) / (S_1 + 1) + error	
C41 = 1 / (G_3 + 2)^3 – (0.7)G_2 + error	
C42 = $\sqrt{G_3 + 2}$ - S_1E_1 + error	
C43 = log (G_1G_2 + 2) - $\sqrt{G_3 + 2}$ + error	
C44 = (G_2 + S_1 + 1) / ($G_1^{\,2}$ + 1) + error	
C45 = (G_1 + 1) / ($G_2^{\,2/3}$ + 1) + error	
C46 = (G_3 - G_1)S_1 + log (E_2 + 2) + error	
C47 = $S_1^{\,2}$ + (G_1 + G_2)(2E_1 - E_2) + error	
C48 = (E_1 + 2E_2 + 1) / ($G_1^{\,2}$ + $\sqrt{G_3 + 2}$ + 1) + error	
C49 = 1 / [log (2S_1 + 2G_3 - E_1 + 2) + 1] + error	
C50 = ($\sqrt{S_1 + G_2^{\,2} + E_1^{\,3} + 2}$ + 3) / [E_2 – $S_1^{\,3}$ + (0.5)G_1G_3 + 1] + error	

*: error: error function which followings normal distribution with mean1 and SD 1.

traits by simulation to evaluate the performance of the multivariate analysis methods. Datasets 1 and 2 use only 6 out of 7 underlying traits: E_1, E_2, G_1, G_2, G_3, and S_1 (see Tables II, III and Figure 1). The only differences between these two datasets are is the standard deviations of E_1 and E_2 as is described above. For the third dataset, we substituted underlying traits S_1 for G_3 and S_2 for S_1. However, we kept the functions the same and set the standard deviation equal ½ for E_1 and E_2. Take trait C49 in the second set of functions as an example. We used

4.2 / [log (2S_1 + 2G_3 - E_1 + 2) -3] + error

for dataset 1 and 2, and

4.2 / [log (2S_2 + 2 S_1 - E_1 + 2) -3] + error

for dataset 3.

Table 18. Second Set of 50 Complex Traits

(Table 3 in this Chapter)

All 50 traits are Combination of all forms

C1 = $\log (E_1 + 5)E_2 - (S_1 / G_3 + 2)^2$ + error

C2 = $(4.4)G_1 / \sqrt{G_2 - E_1 + 3}$ + error

C3 = $(G_1 + E_2 + G_3) / 3$ + error

C4 = $E_2^{\,2} + (S_1 + 1) / (G_2 + 1)$ + error

C5 = $[(1.2)E_1 + G_2 - 3] / (G_3^{\,2} + \sqrt{G_1 + 2} - 2)$ + error

C6 = $[(1.1)G_1 + (2.7)G_2 + (3/5)G_3 + E_1 - (1.4)E_2 + 2S_1) / 7$ + error

C7 = $\log (E_2 + 1.2) - \exp^{(G1 + \text{error})}$

C8 = $\log (G_1G_2 + 2) - \sqrt{S_1 + 2}$ + error

C9 = $2G_2 + (S_1 + 2E_2 + 1) / (E_2^{\,2} + \sqrt{G_2 + 2} + 1)$ + error

C10 = $(2.3)G_2 + E_1 + 2G_3$ + error

C11 = $(0.2)E_2 + (1.4)G_3 + S_1$ + error

C12 = $[G_1 / (S_1 - 3)] / (E_2^{\,2/3} + 1)$ + error

C13 = $(E_1^{\,2} - 3) [\log (G_1 + 3)]$ + error

C14 = $\{G_2 + [(1.2)G_2 - 2]^3\} / (S_1 + 1)$ + error

C15 = $\sqrt{G_2 + 2 + \text{error}} + G_1^{\,2}E_1$

C16 = $(S_1 + 1) / (G_3 + 1) - E_1$ + error

C17 = $S_1E_2 + 3.3e^{G3 + \text{error}}$

C18 = $\sqrt{G_2 + 2} - G_1E_1$ + error

C19 = $[S_1 + (2.2)G_3] / [(1.7)G_2 - 1.2]$ + error

C20 = $S_1G_2 - 2 / (G_1 + 4)^3 - (0.7)G_3 / 3$ + error

C21 = $S_1G_2E_2$ + error

C22 = $G_1 / (2.2 - G_3)(E_2S_1)$ + error

C23 = $(E_1 + 1) / (G_1G_3 + 1)$ + error

C24 = $[(2.2)G_2 - S_1] / (E_2 E_1 + 1)$ + error

C25 = $(\sqrt{E_1 + G_2^{\,2} + G_3^{\,3} + 1} + 4) / [S_1 - E_2^{\,2} + (0.5)G_1G_2 + 1]$ + error

C26 = $(S_1 + 3) / (G_3 + 1) - (2.1)E_2$ + error

C27 = $(E_2 + 2.1) / (E_1 + 1.2) + G_3$ + error

C28 = $(G_3\text{-}1)G_2 + (2.2)E_1$ + error

C29 = $E_2E_1 + G_1$ + error

C30 = $(S_1 + 1) / (G_2 + 1) - G_1$ + error

C31 = $E_1G_1 + (0.5)E_2$ + error

C32 = $3(G_3 + 1) / (S_1 + 1.7) - 2E_1$ + error

Table 18 continued

$C33 = G_1E_2 - (S_1 + 1) / (G_2 + 1) + \text{error}$

$C34 = (S_1 + 1) / (G_2 + 2) + (E_2 + 1) / (G_3 + 1) - (0.7)G_1 + \text{error}$

$C35 = (G_1 + \text{error})^2 + G_1 / (E_1S_1)$

$C36 = (S_1 + \text{error})^3 - (2.4)[\log (E_2 + \text{error} + 2)]$

$C37 = (3.3)\,[e^{(G3 + \text{error})}] + (1.4)\,(E_1 + \text{error})^2$

$C38 = \sqrt{S_1 + 2 + \text{error}} + G_1{}^2E_2$

$C39 = \log (G_1 + \text{error} + 2) - e^{(E1 + \text{error})}$

$C40 = G_3{}^2 + (G_2 + 1)/(E_1 + 1) + \text{error}$

$C41 = 2 / (E_1 + 5)^3 - (0.7)G_3 + \text{error}$

$C42 = \sqrt{E_2 + 2} - S_1G_1 + \text{error}$

$C43 = \log (G_2E_1 + 2) - \sqrt{G_3 + 2} + \text{error}$

$C44 = (G_1 + E_2 + 1) / (G_3{}^2 + 1) + \text{error}$

$C45 = (G_1 + 1) / (S_1{}^{2/3} + 1) + \text{error}$

$C46 = (G_1 - E_2)G_3 + \log (E_1 + 2) + \text{error}$

$C47 = E_1{}^2 + (G_2 + G_3)\,(2S_1 - E_2) + \text{error}$

$C48 = (S_1 + 2G_2 + 1) / (E_1{}^2 + \sqrt{G_3 + 2} + 1) + \text{error}$

$C49 = (4.2) / [\log (2S_1 + 2G_3 - E_1 + 2) - 3] + \text{error}$

$C50 = (\sqrt{E_2 + E_1{}^2 + G_3{}^3 + 2} + 1) / [G_1 - E_1{}^3 + (0.5)G_1G_2 + 1] + \text{error}$

We designed these three datasets to perform the following comparisons. 1) By comparing analysis results from datasets 1 and 2, we could assess the behavior of the two multivariate analysis methods behave when trait variation due to environment decreases; or in other words, when the proportion of total phenotypic variance due to genetics increases. 2) By comparing analysis results from datasets 2 and 3, we could evaluate the behavior of the analysis methods with and without the presence of sex by genotype interaction. (Figure 1) For simplicity, we will refer to datasets 1, 2 and 3 in the subsequent text as the high-environment dataset, the low-environment dataset, and the gene by sex interaction dataset, respectively.

4.2.5 Statistical Analysis

The input variables for the multivariate analyses were either 50 complex traits in their original form (raw traits) or residuals of these traits (after removing the linear effect of covariates). Two types of residuals were analyzed: 1) residuals after adjusting for E_1 and E_2; or 2) residuals after adjusting for E_1, E_2 and sex. Both sets of residuals were derived from each of the 50 continuous traits by multiple regression after the incorporation of corresponding covariates. To mimic analysis methods that would be used in a real study, we only considered the linear form of covariates in the multiple regression, although quadratic and other non-linear effects of E_1 and E_2 are included in our arbitrary functions.

The Pearson pairwise correlations among all 50 complex traits (or residuals) were estimated using the R statistical package (V2.4.0 for windows) [Guo, et al. 2005]. Principal component analysis and factor analysis were both performed in R using its standard default procedure (varimax rotation, correlation matrix use Pearson) with all default options (Commands: *princomp* and *factanal)*. For simplicity, we performed comparisons using only the first PC or factor, which explains the largest proportion of variation across 50 complex traits. The derived PCs or factors will be referred to as composite traits below.

4.2.6 Evaluation

Two evaluation strategies were applied. First, we evaluated the ability of each method to detect common underlying environment or genetic components. We performed univariate regression analyses and regressed every underlying trait on the first composite trait (PC or factor). Correlations (R^2) between each composite trait and each underlying original trait were estimated.

Second, for each trial, we estimated heritabilities for all phenotypes, including the 7 underlying traits, the 50 complex traits and the two composite traits (first principal component and first factor). Box plots were used to show how heritabilities of 50 complex traits spread. And the heritability estimate for each composite trait was marked on the corresponding box plot. The estimated heritability of a trait using data on full-sibs was calculated as: $H^2 = 2 \times$ (trait correlation between sibs) [Beck, et al. 1998].

4.3 RESULTS

4.3.1 Analysis of Correlations

Tables IV and V summarize all correlations (R^2 from univariate regression) between composite traits and each underlying phenotype. Although we generated three independent replicates for each of our 36 dataset/analysis combinations, the results were similar. Thus we report here the results from one replicate (Tables IV and V).

Overall, both multivariate analysis methods (FA and PCA) show similar correlation patterns between composite trait and underlying trait with very few exceptions. When the trait models are more complicated (traits derived from the function set 2) and analyses are performed on residuals, these two methods appear to detect different underlying traits. For example, FA was most highly correlated with underlying trait S_1, whereas PCA was correlated with trait G_1 in the analyses of the second function set, low environment dataset, and using residuals after regressing out E_1, E_2 or E_1, E_2 and Sex (Table V).

Table 19. Correlations between Composite Trait and Underlying Phenotype in Function set 1

(Table 4 in this Chapter)

		Raw Traits		Residual 1 -regress out E1 and E2		Residual 2 -regress out E1, E2&Sex	
		Correlation -Factor *	Correlation - PC	Correlation -Factor	Correlation -PC	Correlation -Factor	Correlation - PC
High Environment Dataset	E_1	0.90	0.45	~ 0	~ 0	~ 0	~ 0
	E_2	~ 0	0.23	~ 0	~ 0	~ 0	~ 0
	G_1	~ 0	~ 0	~ 0	0.02	~ 0	~ 0
	G_2	~ 0	0.03	~ 0	0.02	0.28	0.22
	G_3	~ 0	0.05	0.05	0.10	0.26	0.17
	S_1	0.09	0.22	0.88	0.68	0.24	0.34
Low Environment Dataset	E_1	0.57	0.24	~ 0	~ 0	~ 0	~ 0
	E_2	~ 0	0.10	~ 0	~ 0	~ 0	~ 0
	G_1	~ 0	~ 0	~ 0	0.03	~ 0	~ 0
	G_2	0.08	0.18	0.06	0.16	0.34	0.28
	G_3	0.02	0.09	0.02	0.07	0.21	0.12
	S_1	0.36	0.43	0.90	0.75	0.25	0.32
Gene by Sex Interaction Dataset	E_1	0.02	0.08	~ 0	~ 0	~ 0	~ 0
	E_2	0.02	0.05	~ 0	~ 0	~ 0	~ 0
	G_1	~ 0	~ 0	~ 0	~ 0	~ 0	0.05
	G_2	0.06	0.09	0.04	0.07	0.23	0.22
	S_1	0.49	0.41	0.41	0.48	0.25	0.14
	S_2	0.82	0.70	0.90	0.82	0.12	0.15

However, even in those cases in which both methods display qualitatively similar results, we think factor analysis demonstrates a stronger ability to detect predominant signals from underlying traits than PCA, by which it may benefit the downstream QTL analysis. We found that when composite traits from both methods show significant correlations to a specific underlying trait, the correlation coefficient (R^2) between the first factor and that underlying trait is substantially higher than the corresponding correlation of the first principal component and the underlying trait. For example, in the first set of functions, high environment dataset, and residuals after adjustment of E1 and E2 model, correlations between S_1 and the first factor from

FA and the correlation between S_1 and the first principal component from PCA are 0.88 and 0.68 respectively.

Table 20. Correlations between Composite Trait and Underlying Phenotype in Function set 2

(Table 5 in this Chapter)

		Raw Traits		Residual 1 -regress out E1 and E2		Residual 2 -regress out E1, E2&Sex	
		Correlation -Factor *	Correlation - PC	Correlation -Factor	Correlation -PC	Correlation -Factor	Correlation - PC
High Environment Dataset	E_1	0.83	0.76	~ 0	~ 0	~ 0	~ 0
	E_2	~ 0	0.03	~ 0	~ 0	~ 0	~ 0
	G_1	0.10	0.12	0.89	0.77	0.02	0.84
	G_2	~ 0	~ 0	~ 0	0.03	0.29	0.05
	G_3	~ 0	~ 0	0.03	0.08	~ 0	~ 0
	S_1	~ 0	0.02	~ 0	0.05	0.51	0.04
Low Environment Dataset	E_1	0.48	0.32	~ 0	~ 0	~ 0	~ 0
	E_2	~ 0	0.04	~ 0	~ 0	~ 0	~ 0
	G_1	0.40	0.50	~ 0	0.85	~ 0	0.88
	G_2	~ 0	0.02	0.27	0.05	0.27	~ 0
	G_3	~ 0	0.02	0.02	0.04	0.03	~ 0
	S_1	~ 0	0.03	0.70	~ 0	0.45	~ 0
Gene by Sex Interaction Dataset	E_1	~ 0	~ 0	~ 0	~ 0	~ 0	~ 0
	E_2	0.03	~ 0	~ 0	~ 0	~ 0	~ 0
	G_1	~ 0	~ 0	~ 0	~ 0	~ 0	0.56
	G_2	~ 0	~ 0	~ 0	~ 0	0.09	~ 0
	S_1	0.33	0.58	0.32	0.54	0.58	0.21
	S_2	0.93	0.78	0.94	0.80	~ 0	~ 0

We also compared results of multivariate analyses performed using raw complex traits versus residuals of the complex traits. As can be seen (Tables IV and V), PCA or FA analysis of residuals greatly improved detection of common genetic components instead of common environmental factors. For example, instead of picking up E_1 for both high and low environment datasets when using raw traits from either function set 1 or 2, factors or PCs detected one of the underlying genetic components. Both PCA and FA obtained the highest correlation with underlying trait S_1 for both datasets using residuals after regressing out E_1 and E_2. Furthermore,

the correlation between the environmental traits (E_1 and E_2) and the composite traits derived from the residuals is zero. As stated in the methods, we only regressed out the linear effects of E_1 and E_2 on the complex traits, even though the complex traits are in fact non-linear functions of E_1 and E_2. Our limited results suggest that performing a linear regression of environmental factors may be effective in removing some of the non-linear effects from environmental correlates. However, these results might depend on the specific set of non-linear functions we used and thus further evaluation is needed.

Finally, our results indicate that removing the effects of a covariate (i.e., sex, in our example) that has an interaction effect with the genotype on an underlying trait (i.e., trait S_2), substantially decreases the potency of PCA or FA for detecting this underlying trait. This is indicated in the second residual model (after adjusting for E_1, E_2 and sex) for both sets of functions in Tables IV and V.

4.3.2 Analyses of Heritabilities

We next compared the heritability of the underlying (unobserved) traits, the complex (observed) traits, and the first principal components from PCA and the first factor from FA. Figure 2 shows the boxplot of heritabilities for 50 complex traits compared with estimated heritabilities for the composite traits. Table VI lists mean heritabilities (or twice the sibling resemblance for non-genetic traits like E_1 and E_2) and the corresponding ranges for underlying traits. All mean heritabilities were calculated after taking the average of heritabilities from three replicates and the range shows the variations of heritabilities among replicates. As indicated by Figure 2, there is no predictable relationship between the heritability of the composite traits and the heritability of 50 complex traits. In other words, in contrast to our expectations, the heritabilities of the

composite traits are not necessarily higher or lower than those of the original traits. We expected that the heritabilities of composite traits would be higher than those of the 50 complex traits, because multivariate analysis would incorporate co-variations for multiple traits due to shared genetic factors (pleiotropy), especially after removing environmental factors via regression analysis.

However, further comparisons of the heritabilities for composite traits and underlying phenotypes (Table VI), indicates that heritabilities of PCs and factors FA and PCA were consistent with the underlying model. In other words, the genetic and environmental information embedded in the composite trait reflects the genetic and environmental signals from underlying traits which had the highest correlations with the composite traits. For example, in function set 1, high environment dataset, using the raw trait model, the FA composite trait seems to be exclusively coming from E_1 (correlation = 0.90) (Table IV). The heritability (or in this case, twice the sibling correlation) for this composite trait and heritability of E_1 are comparable (0.454 vs. 0.47). For the same function and dataset, but using the first residual model (adjusting for E_1 and E_2), the FA composite trait captured information mostly from S_1. The corresponding heritabilities of first factor and S_1 are also comparable (0.265 vs. 0.30).

4.4 DISCUSSION

There are several interesting and useful conclusions based on our study. The seven underlying traits that we simulated are intended to be representative of the unobserved environmental or/and genetic determinants which influence population variation of real traits. Likewise, the sets of 50 complex traits derived from these 7 original phenotypes reflect potentially real phenotypes that

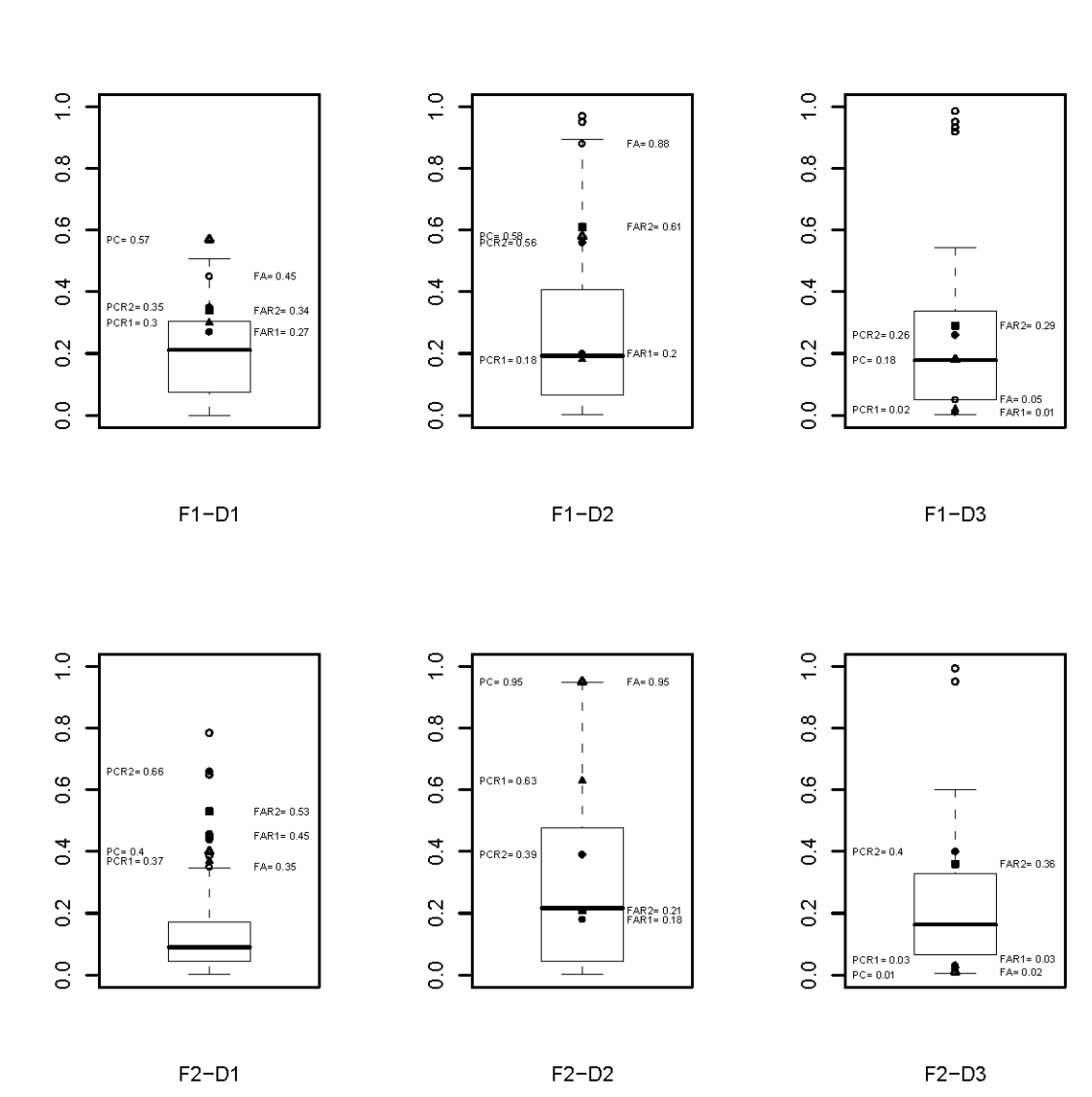

Figure 9. *Heritability Estimation of Composite and 50 Complex Traits (Figure 2 in this chapter)*

Footnote for Figure 9

* F1-D1: first set of functions, dataset 1; F1-D2: first set of functions, dataset 2; F1-D3: first set of functions, dataset 3; F2-D1: second set of functions, dataset 1; F2-D2: second set of functions, dataset 2; F2-D3: second set of functions, dataset 3.

PC: first principal component from PCA with raw trait model; PCR1: first principal component from PCA with residual model 1 (after regressing out E_1 and E_2); PCR2: first principal component from PCA with residual model 2 (after regressing out E_1, E_2 and Sex)

FA: first principal component from Factor Analysis with raw trait model; FA1: first principal component from Factor Analysis with residual model 1 (after regressing out E_1 and E_2); FA2: first principal component from Factor Analysis with residual model 2 (after regressing out E_1, E_2 and Sex)

could be directly measured. Thus, any statistical analysis that can successfully identify variation attributable to any underlying original trait should theoretically have better power to detect genes when used in a genetic linkage or association analysis. As indicated by our results, factor analysis seems to perform better than PC analysis. This conclusion is based on the higher correlation between factors and the most significant underlying traits(whichever shows the highest correlation coefficient) compared to that of PCs and the underlying traits. In a real world analysis, a higher correlation between a composite trait and an underlying phenotype (if it is due to genetics), should increase the probability of detecting and identifying the underlying genes. Hence, we would recommend factor analysis rather than principal component analysis. Another reason we prefer FA, although not discussed in the results, is that PCA assumes an orthogonal relationship between its PCs, while FA does not. The assumption of independent extracted components may conflict with the true genetic model. For example, bone scientists hypothesize

Table 21. Mean Heritability (Sibling Resemblance) Estimation for Underlying Traits

(Table 6 in this Chapter)

	E_1-1**	E_2-1	E_1-2	E_2-2	G_1	G_2	G_3	S_1	S_2
Heritability (H2)	0.47	0.14	0.97	0.80	0.53	0.36	0.18	0.30	0.06
H2r Range	0.44-0.50	0.11-0.22	0.94-0.99	0.74-0.98	0.36-0.69	0.11-0.53	0.10-0.53	0.15-0.40	0.05-0.10

* *: numbers in the table indicates the mean heritabilities and its range for each underlying trait from all repeats;

**: E1-1/E2-1: heritability of E1/E2 in high environment dataset (SD=1); E1-2/E2-2: heritability of E1/E2 in low environment and gene by sex interaction dataset (SD=1/4)

that genes influencing bone size may differ from genes influencing for bone mineral density (BMD) (82,83). However these two sets of genes might interact with each other. If we put

several bone size and BMD traits together into PCA, it is almost impossible to generate two independent PCs which represent both the set of the bone size genes and BMD genes respectively.

Another conclusion from these analyses concerns the use of residuals versus raw trait values in multivariate analysis. Our results indicate that regressing out potentially significant environmental covariates should greatly increase the chances for detecting genetic components using both FA and PCA, but that if the underlying trait exhibits a genotype by environment interaction (see the results of our analyses with sex), removing the linear effects of such environmental covariates, in some cases, could decrease or even remove the genetic signal from the composite trait. This result has two different practical implications for real studies. First, any covariate that is not observed obviously cannot be regressed out, so one must be aware when applying FA or PCA that the factors/components that are identified could represent underlying environmental factors rather than underlying genetic factors. Second, our result actually suggests that for observed covariates, regressing them out might not be the best strategy. If known covariates are not regressed out, derived factors or components may be highly correlated with them, but precisely because the covariates are observed, we can check our results for such correlations and ignore those factors. For example, if one were to perform PCA or FA on a set of traits that reflect physical size (e.g. height, weight, various bone lengths) without regressing out sex, one would undoubtedly discover that the first component was very highly correlated with sex. One could then ignore that component and go on to look at the second one, secure (one would hope) in the knowledge that no genetic interactions with sex had been inadvertently regressed out.

Our conclusions regarding heritability are somewhat counter to the conventional assumption that using multivariate analysis can and should increase trait heritabilities. As shown in the results, the heritability for composite traits is not necessarily higher than that of the original complex traits. However, high trait heritability does not necessarily predict successful detection of genes by linkage or association analysis, because what really matters is the heritability attributable to any single locus. Thus the success in detecting relevant genes depends not only on the number of loci influencing a trait, but also on the relative contribution of each locus. In our analysis, we observed some examples of this phenomenon. For example, in the first set of functions, low environment dataset, using residuals adjusting for E_1 and E_2, the heritabilities for the traits derived using both FA and PCA are relatively low (0.20 and 0.18 respectively). However, both composite traits are highly correlated with underlying trait S_1, which also has a heritability of 0.18. These composite traits should be more useful for downstream gene hunting than any of the individual complex traits precisely because their heritability only reflects a single underlying locus and is not inflated by contributions from other loci or environmental correlation.

Certain limitations of this study need to be acknowledged. One of the most important is that in a real study one would want to identify more than just one factor or principal component, so the performance of the methods to detect more than one meaningful component should be tested in the future. Another critical issue is that in order to compare methods more conclusively it would be desirable to simulate genetic markers and conduct linkage and/or association studies so that the bottom-line success rates of the methods can be quantified. Finally, the effect of sample size on our results should be studied. We simulated 250 families, or 500 sibs with phenotypic data, which is comparable to real datasets to which these methods have been applied.

It is clear from our results that the relatively small sample size does not produce very stable estimates of heritabilities, because we got widely varying heritability estimates across our three replicates of each dataset (Table VI). However, our bottom-line results – correlations between the underlying traits and the composite traits – were quite stable across replicates, giving us confidence in the robustness of the methods.

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5.0 MULTIVARIATE ANALYSIS OF BONE QUALITY RELATED PHENOTYPES FROM THE TOBAGO FAMILY STIDY

5.1 INTRODUCTION

Studies over the past several decades have well documented the major genetic contributions to osteoporosis as well as the bone strength related traits. Most of these bone strength related traits are shown to be moderately to highly heritable [(1)], which were reflected by the higher heritabilies compared to other complex disease phenotypes such as blood pressure, glucose level. Despite the fact that evidence for linkage with some of the bone phenotypes was observed in the recent years, many studies suggested that some of the bone strength related traits are governed by common sets of genes (e.g. pleiotropy) [(104-111)]. The evidence in favor of the above hypothesis is that bivariate linkage analyses revealed new or stronger linkage signals were found for many of the bone traits compared with the univariate linkage results [(112)]. Because conventional measurements of the bone strength phenotypes produce many intercorrelated phenotypes, it is possible that there might be a relatively small number of factors (both genetic and environmental) involved in bone and mineral metabolism that contribute to an underlying clustering of bone strength phenotypes. Identification of these common factors and elucidation of their molecular basis will contribute to a better understanding and possible treatment for osteoporosis.

Multivariate analysis (PCA /Factor analysis) might be one of the effective solutions to the above questions. PCA / Factor analysis (FA) involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated (PCA) or correlated (Factor) variables called principal components or factors. During the factor / PC extraction, the shared variance of a variable is partitioned from its unique variance and error variance to reveal the underlying factor / PC structure. Only the shared variance appears in the solution. So it is reasonable to believe that these two methods have potential to classify phenotypic variation into independent / dependent components that may amplify the genetic signal and hence be used to dissect genetic networks regulating complex biological systems.

The Tobago family study comprises data on both phenotypic (different bone measurement by DXA and pQCT) and environmental factors (including life style, diet, diseases status etc.) on 471 individuals aged 18 and older (mean, 43 years) from 8 large, multigenerational Afro-Caribbean families with a mean family size >50 individuals (range from 21 to 112). Using the up-to-date technology (Dual X-ray Absorptiometry, DXA and Peripheral Quantitative Computed Tomography, pQCT), we measured more than 100 different bone strength related phenotypes from our participants. Among those traits, more than 50 phenotypes are widely studied and well accepted in the bone research field.

All these phenotypes were measured quantitatively (continuous traits). Most of traits are normally distributed; although a few traits are highly skewed (for example, endo-cortical thickness at radius shaft) and other traits (some bone stress strain index traits) demonstrate a bimodal distribution due to the significant gender differences. Many of the phenotypes are correlated each other due to underlying biologtical influences or mathematical construction. For

example: BMD (Bone Mineral Density) traits were calculated from the corresponding BMC (Bone Mineral Content) traits (BMD =BMC / area) and total density is approximately the sum of cortical and trabecular density. Composite traits like SSI (Stress Strain Index) are derived from bone area, bone circumference and BMD, so it may reflect the co-distribution of those raw traits.

In this study, we develop composite traits by PCA or FA, which subsequently could be used in the downstream association studies. Initially, 12 different BMD phenotypes measured by either DXA or QCT were used to further evaluate these two multivariate methods. We were particularly interested to see how these 12 traits would be classified / clustered by multivariate analysis, that is, did the clustering of the original phenotypes correspond with known biological expectations. In addition, as described in the next chapter, we perform association studies using these composite traits to determine whether the composite traits reveal stronger genetic signals (reflecting a true positive association between SNPs and trait) with more power when compared to a "one trait at a time" strategy.

5.2 MATERIALS AND METHODS

5.2.1 Study Phenotypes

Twelve BMD related phenotypes were analyzed in this section: 1) four traits measured by 2-dimensional DXA, including: total BMD at whole body (WBTOTBMD), total Spine BMD (STOTBMD), femoral neck BMD (NECKBMD) and total hip BMD (HTOTBMD); and 2) eight traits measured by 3-dimensional pQCT, including: total density at distal radius and tibia (TOTDENr4 and TOTDENt4), trabecular density at distal radius and tibia (TRABDENr4 and

TRABDENt4), total density at radius and tibia shaft (TOTDENr33 and TOTDENt33), cortical density at radius and tibia shaft (CRTDENr33 and CRTDENt33).

5.2.2 Statistical Analysis

Prior to performing multivariate analyses, we assessed the distributions of all traits, and transformed traits if necessary to reduce non-normality (state which ones you transformed and why). In addition, outliers (observations beyond ±4SD) were removed for each BMD trait and no more than 4 four values were removed for a single variable. For the subsequent multivariate analyses, we analyzed both (1) the 12 original raw BMD traits, and (2) residuals of the 12 traits, after regressing out the significant covariates (refer to Chapter II, table 4). Due to the different measurement scaling units used by DXA and pQCT, we then standardized the distribution for each (raw or residual) trait, i.e., all traits had mean = 0 and SD = 1. PCA and factor analysis were performed using the default option (varimax rotation, correlation matrix use Pearson for commands *princomp* and *factanal*) in the R statistical package R (V 2.4.1) [113]. We also ignored the family relationship among individuals within each pedigree when deriving the composite PCA and FA traits. For simplicity, we will refer in the following text those composite traits which were derived by multivariate analysis using 12 original BMD traits as composite (raw) and similarly those using residuals as composite (residual).

After performing multivariate analysis, we estimated the residual heritabilities (H2r) of all 38 composite traits (12 PCs and 7 factors in both raw and residual model), using SOLAR [114] and compared these results with the H2r of the 12 original BMD traits. Residual heritabilities of the 12 original BMD traits and composite(raw) traits , were estimated after including age, sex,

weight and height as covariates. Heritability of the composite (residual) traits had already been adjusted for sex, weight, and height, and there were no additional effects of these covariates.

5.3 RESULTS

5.3.1 Multivariate Analysis

The proportion of variance and PCA loadings for PC (raw) and PC (residual) traits are summarized in tables 1 and 2, respectively. In most cases, the characteristics of PCs derived from raw or residual traits are qualitatively similar. For example, PC1(raw) and PC1(residual) accounted for approximately half of total phenotypic variation from 12 original BMD traits, and the first 4 PCs (raw or residual) accounted for ~80% of total phenotypic variation. More importantly, the loadings (table 2) of each PC for raw and residual groups are comparable to each other. As can be seen, PC2(raw) and PC2(residual) contain higher loadings for 4 predominantly cortical traits (TOTDENr33, CRTDENr33, TOTDENt33, CRTDENt33) which may indicate a common environment or/and shared genetic impact on these traits. Similarly, higher loadings of 3 or 4 predominantly trabecular traits (TOTDENr4, TRABDENr4, TOTDENt4，TRABDENt4) were observed in PC8(raw) and PC8(residual) for both groups. Finally, both PC10 (raw) and PC10 (residual) had higher loadings for most of the pQCT measured phenotypes plus the DXA whole body BMD.

Table 22. Proportion of Variance of Principal Components in Raw and Residual Models

(Table 1 in this Chapter)

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Raw Trait Model												
Proportion of Variance	0.51	0.19	0.08	0.05	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.01
Cumulative Proportion	0.51	0.70	0.78	0.83	0.87	0.90	0.93	0.95	0.97	0.98	0.99	1.00
Residual Trait Model												
Proportion of Variance	0.47	0.17	0.09	0.06	0.05	0.04	0.04	0.03	0.02	0.01	0.01	0.01
Cumulative Proportion	0.47	0.64	0.73	0.79	0.84	0.88	0.92	0.95	0.97	0.98	0.99	1.00

The factors derived from raw or residual traits differed more than the PC described above. First, the proportions of variances attributable to each factor (raw) differed from that for each factor (residual). For example, the first 3 factors (raw) accounted for ~60% of original total phenotypic variation of 12 raw traits, of the first 3 factors (residual) explained ~40%. Second, the factor (raw) and factor (residual) loadings appear to reflect different aspects of underlying skeletal biology. For example, factor2 (raw) comprised higher loadings for 4 DXA BMD traits. However, factor2 (residual) is comprised of traits related to predominantly cortical bone (TOTDENr33, CRTDENr33, TOTDENt33, and CRTDENt33). Third, from table 5, total spine BMD, trabecular BMD at distal radius, and total density at radius shaft were poorly represented (uniqueness >20%) by factors (raw), whereas total spine BMD, total density at both distal radius and tibia plus total density at radius shaft were poorly represented by factors (residual).

When we compared results across both multivariate methods, we observed two major differences. First, compared to PCs, each of factors accounted for substantially smaller proportion of original phenotypic variation from 12 BMD phenotypes (see Tables 1 and 3). Second, the loadings of PC (raw) and PC (residual) did not differ, but factor (raw) and (factor

(residual) did. In addition to these two major differences, there is another subtle, though important, difference between PCA and FA. Based on our current knowledge of the underlying

Table 23. PCA Loadings in Raw and Residual Model

(Table 2 in this Chapter)

		WB TOT BMD *	SP TOT BMD	FEM NECK BMD	HP TOT BMD	TOT BMD D-Rad.	TRAB BMD D-Rad.	TOT BMD Rad-S	CRT BMD Rad-S	TOT BMD D-Tib	TRAB BMD D-Tib	TOT BMD Tib-S	CRT BMD Tib-S
PC1 †	*Raw* ‡	-0.33	-0.28	-0.34	-0.35	-0.28	-0.32	-0.20	-0.14	-0.35	-0.33	-0.27	-0.18
	Res	-0.33	-0.29	-0.34	-0.35	-0.25	-0.31	-0.22	-0.18	-0.34	-0.32	-0.27	-0.19
PC2	*Raw*	-0.08	-0.05	-0.16	-0.18	0.04	-0.19	0.48	0.56	-0.13	-0.22	0.25	0.47
	Res	-0.02	-0.06	-0.18	-0.15	0.06	-0.20	0.47	0.55	-0.18	-0.31	0.24	0.44
PC3	*Raw*	0.37	0.57	0.20	0.22	-0.39	-0.26	-0.17	0.13	-0.28	-0.29	-0.14	0.09
	Res	0.33	0.44	0.28	0.24	-0.54	-0.34	-0.20	0.06	-0.21	-0.21	-0.11	0.16
PC4	*Raw*	0.04	0.30	-0.16	-0.16	0.56	0.27	0.08	0.20	-0.17	-0.10	-0.62	-0.10
	Res	-0.16	-0.43	0.12	0.12	-0.38	-0.27	-0.08	-0.21	0.25	0.14	0.61	0.17
PC5	*Raw*	0.13	0.38	-0.38	-0.21	-0.17	0.13	0.35	-0.04	0.02	0.00	0.37	-0.59
	Res	-0.15	-0.31	0.29	0.21	0.38	-0.05	-0.40	-0.01	-0.09	-0.06	-0.31	0.58
PC6	*Raw*	-0.12	-0.03	0.07	-0.11	-0.58	0.14	0.22	0.33	0.22	0.44	-0.47	-0.03
	Res	0.29	0.29	-0.41	-0.38	0.12	0.12	-0.36	-0.31	-0.04	0.05	0.30	0.41
PC7	*Raw*	0.09	0.30	-0.40	-0.40	-0.10	0.20	-0.37	-0.19	0.07	0.24	0.10	0.54
	Res	-0.06	-0.07	-0.16	-0.24	-0.46	0.14	0.00	0.29	0.28	0.50	-0.42	0.30
PC8	*Raw*	-0.37	0.40	0.05	-0.08	0.23	-0.67	-0.01	-0.02	0.38	0.21	-0.04	-0.06
	Res	0.20	0.06	-0.12	-0.10	0.32	-0.71	-0.10	0.11	0.51	0.06	-0.13	-0.14
PC9	*Raw*	0.70	-0.33	-0.21	-0.16	0.09	-0.34	-0.14	0.19	0.35	0.01	-0.12	-0.12
	Res	0.75	-0.59	-0.08	0.02	-0.06	0.15	-0.06	0.11	-0.05	-0.13	-0.06	-0.12
PC10	*Raw*	0.25	-0.04	0.00	0.00	-0.01	-0.21	0.59	-0.62	-0.17	0.14	-0.23	0.24
	Res	0.17	-0.06	-0.01	0.04	0.03	-0.23	0.60	-0.6	-0.17	0.21	-0.25	0.23
PC11	*Raw*	0.06	0.03	0.61	-0.67	-0.03	0.12	0.05	-0.12	0.20	-0.32	0.04	-0.02
	Res	0.05	-0.01	0.61	-0.66	-0.04	0.11	0.10	-0.12	0.25	-0.30	-0.03	0.04
PC12	*Raw*	0.12	-0.05	0.25	-0.27	0.14	-0.17	-0.12	0.17	-0.61	0.58	0.18	-0.14
	Res	0.07	-0.04	0.30	-0.30	0.14	-0.22	-0.11	0.20	-0.55	0.58	0.17	-0.16

* Abbreviations of Bone Traits: WB: Whole Body; SP: Spine; FEM: Femoral; TOT: Total; TRAB: Trabecluar; CRT: Cortical; D-Rad: Distal Radius; Rad-S: Radius Shaft; D-Tib: Distal Tibia; Tib-S: Tibia Shaft

† PC1-PC12: 12 composite phenotypes generated by PCA

‡ Raw: PCA model with raw traits input; Res: PCA model with residuals input (after regress out age, sex, weight and height)

biology of the 12 original traits, we believe factor analysis is more successful in grouping/clustering the traits into composite traits than is PCA. For example, factor analysis, factor2 (raw) was more correlated to four DXA phenotypes (higher loadings for these traits); factors 3, 5 and 7 (raw) represented several cortical phenotypes; and factor4 (raw) was more representative of trabecular traits. A similar pattern is evident in the factors (residual) (table 4). However, in PCA analysis, the composition loadings for most of PCs did not cluster based on our current understanding of bone biology (table 2).

5.3.2 Heritability Estimation of Composite Traits

Table 6 shows the residual heritability (after adjustment for age, sex, height and weight) for 12 original BMD traits. Table 7 summarizes the same residual heritability for PCs and factors for both raw and residual trait groups. Several conclusions can be made from these results. First, similar to our simulation results (Chapter IV using simulation data [115]), there was no predictable relationship between the heritabilities

Table 24. Proportion of Variance for Factors in Raw and Residual Model

(Table 3 in this Chapter)

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Raw Trait Model							
Proportion of Variance	0.22	0.20	0.17	0.09	0.08	0.07	0.06
Cumulative Proportion	0.22	0.42	0.59	0.68	0.76	0.83	0.89
Residual Trait Model							
Proportion of Variance	0.16	0.14	0.13	0.12	0.12	0.08	0.08
Cumulative Proportion	0.16	0.30	0.43	0.55	0.67	0.75	0.83

of composite traits and heritabilities of original phenotypes. In other words, the heritabilities of composite traits were not necessarily higher, equal or lower than the original phenotypes. In addition, the residual heritability estimates of composite traits were close to each other if they represented (had higher loadings for) similar underlying original traits. For example, PC8 (raw and residual) (table 2) were both more associated (higher loadings) with some or all of the original traits reflecting trabecular density (trabecular density and total density at distal radius / tibia). The H2r for these two composite traits were 0.47±0.11 to 0.47±0.12 respectively. Similarly factor3 (residual) and factor4 (raw) both reflect trabecular density, and H2r is 0.46±0.14 and 0.43±0.11 respectively). In addition, factor 2 (residual) and factor 3 (raw) both

reflect cortical density traits, and H2r equals 0.27±0.09 and 0.15±0.10 respectively. Finally, we reported in our

Table 25. Factor Loadings in Raw and Residual Model

(Table 4 in this Chapter)

		WB TOT BMD *	SP TOT BMD	FEM NECK BMD	HP TOT BMD	TOT BMD D-Rad.	TRAB BMD D-Rad.	TOT BMD Rad-S	CRT BMD Rad-S	TOT BMD D-Tib	TRAB BMD D-Tib	TOT BMD Tib-S	CRT BMD Tib-S
	Raw ‡	0.32	0.23	0.44	0.42	0.37	0.65	0.15	-0.07	0.76	0.93	0.25	0.03
Factor 1 †	*Res*	0.31	0.37	0.87	0.76	0.16	0.23	0.05	0.07	0.37	0.30	0.17	0.31
	Raw	0.85	0.70	0.55	0.62	0.20	0.40	0.07	0.13	0.34	0.28	0.20	0.12
Factor 2	*Res*	0.16	0.16	0.05	0.09	0.28	0.05	0.79	0.84	0.12	-0.05	0.30	0.16
	Raw	0.11	0.14	0.05	0.01	0.21	0.02	0.74	0.97	0.11	-0.01	0.34	0.54
Factor 3	*Res*	0.20	0.18	0.26	0.29	0.19	0.32	0.09	-0.06	0.67	0.85	0.17	0.20
	Raw	0.13	0.08	0.13	0.16	0.86	0.32	0.17	0.03	0.22	0.12	0.10	0.09
Factor 4	*Res*	0.87	0.52	0.27	0.33	0.11	0.22	0.09	0.13	0.24	0.20	0.14	0.87
	Raw	0.15	0.05	0.09	0.18	0.11	0.09	0.29	0.04	0.24	0.10	0.86	0.18
Factor 5	*Res*	0.17	0.19	0.19	0.22	0.47	0.88	0.16	0.04	0.27	0.37	0.13	0.17
	Raw	0.10	0.11	0.68	0.49	0.10	0.10	0.01	0.02	0.20	0.14	0.09	0.06
Factor 6	*Res*	0.15	0.04	0.09	0.16	0.11	0.08	0.26	0.05	0.23	0.08	0.88	0.15
	Raw	0.08	0.05	0.09	0.03	0.08	-0.01	0.11	0.18	0.02	0.05	0.15	0.81
Factor 7	*Res*	0.12	0.06	0.09	0.07	0.10	-0.02	0.09	0.28	0.00	0.02	0.17	0.12

* Abbreviations of Bone Traits: WB: Whole Body; SP: Spine; FEM: Femoral; TOT: Total; TRAB: Trabecluar; CRT: Cortical; D-Rad: Distal Radius; Rad-S: Radius Shaft; D-Tib: Distal Tibia; Tib-S: Tibia Shaft

† PC1-PC12: 12 composite phenotypes generated by PCA

‡ Raw: PCA model with raw traits input; Res: PCA model with residuals input (after regress out age, sex, weight and height)

previous study that the H2r for trabecular BMD traits are higher than those of cortical BMD traits [(1)]. And we also observe a similar result for the composite traits. The residual heritability for composite traits with high loadings for the original trabecular BMD traits, e.g., PC8 (raw and residual), factor (raw), factor3 (residual)

Table 26. Uniqueness of Variance for Factors in Raw and Residual Model

(Table 5 in this Chapter)

	WBTOT BMD	STOT BMD	NECK BMD	HTOT BMD	TOT DENr4	TRAB DENr4	TOT DENr33	CRT DENr33	TOT DENt4	TRAB DENt4	TOT DENt33	CRT DENt33
Raw Model **Uniqueness**	0.11	0.41	0.05	0.13	0.01	0.29	0.29	0.01	0.15	0.01	0.01	0.01
Residual Model **Uniqueness**	0.01	0.50	0.06	0.15	0.61	0.01	0.26	0.19	0.22	0.01	0.01	0.01

and factor5 (residual), ranges from 0.43±0.11 to 0.64±0.12. On the other hand, heritabilities for composite traits corresponding to cortical bone phenotypes, e.g. PC2 (raw and residual), PC10 (raw and residual), factor3 (raw), factor7 (raw) and factor2 (residual), range from 0.15±0.10 to 0.34±0.10.

Table 27. Heritability Estimation for 12 original BMD phenotypes

(Table 6 in this Chapter)

	WBTOT BMD	STOT BMD	NECK BMD	HTOT BMD	TOT DENr4	TRAB DENr4	TOT DENr33	CRT DENr33	TOT DENt4	TRAB DENt4	TOT DENt33	CRT DENt33
H2r±SE	0.50±0.10	0.53±0.11	0.55±0.10	0.58±0.09	0.58±0.10	0.65±0.10	0.29±0.10	0.17±0.08	0.71±0.10	0.65±0.10	0.45±0.11	0.32±0.09

* All P value <0.01

† After adjusted for Age, Sex, Weight and Height

Table 28. Residual Heritability Estimation for Composite Traits (Raw and Residual)

(Table 7 in this Chapter)

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
H2r±SE (Raw)	0.64±0.11	0.23±0.09	0.54±0.11	0.20±0.10	0.56±0.10	0.34±0.11	0.13±0.08	0.47±0.11	0.63±0.11	0.32±0.11	0.22±0.11	0.20±0.13
H2r±SE (Residual)	0.74±0.11	0.34±0.10	0.71±0.13	0.27±0.11	0.54±0.10	0.28±0.09	0.36±0.11	0.47±0.12	0.52±0.11	0.25±0.12	0.28±0.14	0.16±0.14

* All P value <0.01 except for PC7 (P=0.03), PC12 (P=0.045) in raw model and PC12 (P=0.09) in residual model.

† After adjusted for Age, Sex, Weight and Height

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
H2r±SE (Raw)	0.54±0.11	0.44±0.10	0.15±0.09	0.43±0.11	0.33±0.12	0.46±0.10	0.30±0.10
H2r±SE (Residual)	0.49±0.10	0.27±0.10	0.46±0.14	0.57±0.12	0.64±0.12	0.48±0.13	0.39±0.11

* All P value <0.01 except for Factor 3 (P=0.03) in raw model.

† After adjusted for Age, Sex, Weight and Height

5.4 CONCLUSION

In summary, we derived composite traits from data on 12 bone-strength related phenotypes using two multivariate analysis methods: principal component analysis and factor analysis. PCs and factors were derived using both raw phenotype data, as well as residual phenotypes obtained after adjusting for significant covariates. We found that PCA and Factor analysis behave qualitatively similar for both raw and residual models, although the similarity in results is higher for PCA than FA. We believe factor analysis is more successful in grouping/clustering original BMD phenotypes into composite traits than PCA, because the individual factors comprise clusters that more accurately reflect our current knowledge of bone biology. We also observed there was no predictable relationship between the heritabilities of composite traits and heritabilities of original phenotypes, most likely because the composite traits were comprised of multiple underlying traits, each of them with its own different heritability. However, if the composite traits reflected similar underlying traits, their heritabilities were similar. More important, similar to our previous report, the residual heritabilities for more trabecular related composite traits are substantially higher than those of more cortical related composite traits.

We note that the multivariate analyses were performed ignoring the non-independence of our sample observations, however, we do not expect this violation to seriously affect our results. First, our results are comparable to those of other investigators, because investigators usually ignore family relationship when performing PCA or FA, most likely because methods that incorporate dependencies are not available. Second, we do not think a violation of this

assumption will cause significant deviations from true model. For example, our conclusion agrees perfectly with our previous findings that the residual heritabilities of trabecular related traits were substantially higher than those for cortical ones, even though here we talked about composite phenotypes instead of original traits.

Finally, we note that the current evaluation of two multivariate methods in Chapter IV and V are based on quantitative genetic results and our current assumptions regarding bone biology. We have not assessed whether use of these methods will facilitate detection and identification of specific polymorphisms that influence bone traits. However, we will further assess the PCA and FA methods as part of our association analyses described in Chapter VI.

6.0 ASSOCIATION OF BONE QUALITY PHENOTYPES WITH WNT10B AND ENPP1 SNPS: THE TOBAGO FAMILY HEALTH STUDY

6.1 INTRODUCTION

Genetic susceptibility plays a predominant but poorly understood role in the etiology of osteoporosis and its associated fractures. [116-119] A genetic component to osteoporosis is supported by the strong heritability (60-80%) of bone mineral density [120] and bone structural geometry [121-123] and the increased risk of osteoporotic fractures among first-degree relatives with a positive family history of fracture. [9] Identifying the genetic factors underlying normal variation in BMD and skeletal geometry may therefore provide important insight on bone biology and osteoporosis.

Several groups have conducted genome-wide scans to search for quantitative trait loci (QTL) influencing BMD [125-127] and bone geometry. [128-130] These studies have implicated QTLs on several chromosomes, but the specific genes at these chromosomal sites have not been identified.

More recent studies have drawn our attentions to two genes: Wnt10b (one of 19 genes in wingless-type (WNT) family) and ENPP1 (ectonucleotide pyrophosphatase / phosphodiesterase 1). Wnt10b has been shown to stimulate osteoblastogenesis thereby increasing bone mass in vitro. [131-134] Transgenic mice over-expressing Wnt10b have 4 times greater bone mass and are

protected from bone loss due to estrogen-deficiency. In contrast, Wnt10b knock-out mice have decreased trabecular bone compared to wildtype mice. [132] All these studies collectively implicate the Wnt signaling pathway as having a major role in skeletal biology and bone health. The ENPP1 enzyme regulates soft-tissue calcification and bone mineralization by generating inorganic pyrophosphate (PPi), a solute that triggers cell differentiation and serves as an essential physiological inhibitor of hydroxyapatite deposition. [135, 136] The phenotypic consequences of ENPP1 mutations in men and mice suggest that genetic variability of ENPP1 activity may contribute to common forms of articular disorders, such as osteoarthritis. [137]

In this section, we present our genetic association results from Tobago family study between polymorphisms in the Wnt10b and ENPP1 loci and several bone phenotypes include bone mineral density (areal and volumetric BMD) and skeletal geometry traits. We also compare results of single trait analyses versus analyses of composite traits derived from PCA and FA to determine whether multivariate analyses resulted in more and stronger association signals.

6.2 MATERIALS AND METHODS

6.2.1 Study Samples

SNP associations for *WNT10b and ENPP1* in Tobago Family analysis used DNA samples and phenotypic data collected in the Tobago Family Health Study [5]. To be eligible, a proband must have been Afro-Caribbean, have a spouse who was willing to participate in the study, and have at least six living offspring and/or siblings aged 18+ years residing on the island of Tobago. Potential probands were sorted by family size, and individuals with the largest family sizes were

recruited first. Probands were recruited regardless of health status. All eight probands were between 52-103 years of age. In addition, all first-, second- and third-degree relatives of these probands and their spouses were invited to participate regardless of their medical history or BMD. To date, we have recruited 471 individuals age 18 and older in eight multigenerational families with a mean family size of >50 individuals. These 284 women and 187 men with phenotype data ranged in age from 18-103 years (mean age, 43 years). There are a total of more than 3,535 different relationship pairs ascertained. Written informed consent was obtained using forms approved by the Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health.

6.2.2 Skeletal Measurements

BMD phenotypes: Total body DXA was used to assess bone mineral density at the whole body, total hip, femoral neck, lumbar spine using the array beam mode on a Hologic QDR 4500W scanner (Hologic, Bedford, MA, USA). All pQCT measures were performed using a Stratec XCT 2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) on the nondominant forearm and left tibia according to standardized measurement and analysis procedures.[120] Measures of volumetric BMD included: total BMD and trabecular BMD at distal radius and tibia; total BMD and cortical BMD at radius and tibia shaft.

Bone geometry phenotypes: A variety of bone geometry traits were also obtained using the array beam mode on a Hologic QDR 4500W scanner. Each scan was acquired with a 0.5-mm voxel size, slice thickness of 2.5 mm, and at a speed of 20 mm/s. The precise position of the measurement sites were determined in a 30 mm planar scout view using the medial endplate of the radius and tibia as standard anatomic landmarks and automatically set by the software at 33%

(i.e. shaft) of the length of the radius and tibia proximal to the distal endplate. These anatomical sites were chosen in order to assess primarily cortical bone. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process. Image processing was performed using the Stratec software package (Version 5.5E). All 33% proximal radius and tibia shaft scans were analyzed using identical parameters for contour finding and separation of total and cortical bone (contour mode 2, T=169 mg/cm^3;cortmode 1, T=710 mg/cm^3). Endosteal and periosteal circumference (ENDOC and PERIC), and cortical thickness (CRTTHKC, mm) were measured using a scan through the diaphysis (at 33% of the bone length in the proximal direction of the distal end of the bone) of the radius and tibia using the circular ring model [138, 139].

6.2.3 Selection of SNPs

My colleagues found previously in the Tobago population project (genotyped 1653 men, age 40 and above, mean age =59 yrs) that there were significant associations between the polymorphisms of several SNPs in these two genes (WNT10b and ENPP1) and some of the bone phenotypes (Laura Yerges, unpublished results). Thus, we were interested to see if we could replicate these signals in our Tobago family dataset.

In our Tobago family study, there are only 4 SNPs available for both genes: 3 SNPs were genotyped for WNT10b and 1 SNP for ENPP1. Among those 3 WNT10b SNPS, two of them (WNT_1886, rs1051886 and WNT_1627, rs3741627) were predicted to tag each other in the previous population study by HCLUST [140]. Additional one SNP (WNT_5902, rs10875902) was included on the 3' end of the gene in order to better define the association signal. We genotyped only one SNP (ENPP1_4988, rs1044498) in the family study. This SNP is a missense

polymorphism, responsible for Lys121Gln substitution), which was well reported previously that the Gln121 is associated with insulin resistance, type 2 diabetes and both cardiovascular and nephrovascular complications in diabetes patients. [141]

6.2.4 Statistical Analysis

We assessed the skewness, kurtosis, and distributions for all traits. No transformation was needed for traits we used in association analysis. Outliers (observations beyond ±4SD) were removed for each BMD trait and no more than 4 four values were removed for a single variable.

Single SNP association analyses were performed using, maximum likelihood methods (SOLAR (V2.1.4), which allowed the incorporation of familial relationships.

We tested whether the SNP genotypes displayed a linear (additive) effect on the traits (code SNP genotypes as 0, 1, 2 and they stand for common homozygotes, heterozygotes and rare homozygotes respectively). We also tested for dominant or recessive effect on traits (code SNP using two dummy variables). We performed association studies between four available SNPs and more than thirty different bone phenotypes (including DXA and pQCT measured BMD and bone size related traits). Age, sex, height and weight, the four most important predictors for bone phenotypes reported by previous studies [120], were included in our analysis model as covariates. We compared our results from Tobago family analyses with the previous findings from Tobago population based project and MrOS data (Osteoporotic Fractures in Men)**.** Because the main purpose of these analyses was to try to replicate results from the population study, we report nominal P-values in table 1 and table 2 without multiple comparison adjustment.

6.3 RESULTS

Based on 88 unrelated individuals in our family data, the linkage disequilibrium (LD) between WNT1886 and WNT1627 is very high (r2 = 0.9), whereas LD between either of these two SNPs and WNT5902 is comparatively lower (r2=0.82). And this is true in Tobago population data (Figure 1, unpublished results from S. Moffett). Thus, we decided not to include association results for WNT1627 below, because the genetic information in WNT1886 and WNT1627 is almost identical.

We also estimated the allele frequencies for each SNP in family data. The minor allele frequencies for WNT1886, WNT5902 and ENPP1_4498 are 0.14, 0.14 and 0.12 respectively. There were no obvious departures from HWE observed based on 88 unrelated individuals in our Tobago family data.

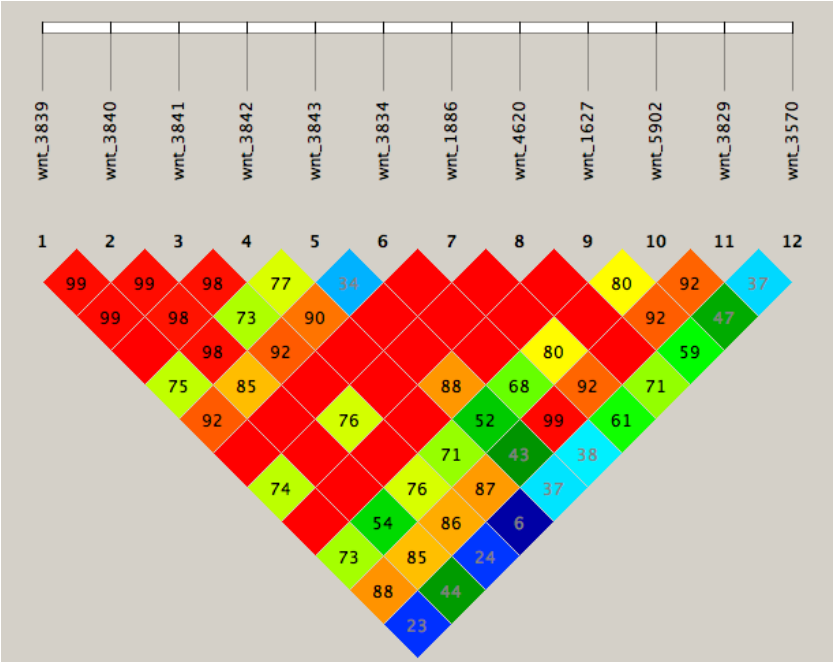

Figure 10. LD for genotyped SNPs in Wnt10B in Tobago Population Study using Haploview GOLD plot *(Figure 1 in this chapter)*

6.3.1 Association with Areal and Volumetric BMD and Bone Size Traits

Tables 1 and 2 summarized the positive association signals from the Tobago family study between bone phenotypes and each of the SNPs. For each SNP, the adjusted genotype-specific mean, standard error, sample size, and nominal p-value in family study. We also provided the corresponding nominal P value from the population-based analyses (for table 1 only. S. Moffet and L. Yerges, unpublished results).

Table 29. Genotype Specific Means and P value for association study for WNT 5902, WNT1886 and ENPP1 4498
(Table 1 in this chapter)

		QCT					
		BMD related phenotypes			*Bone size related phenotypes*		
		Trabecular Density (distal tibia)	Cortical Density (radius shaft)	Cortical Density (tibia shaft)	Periosteal Circumference (radius shaft)	Cortical Thickness (tibia shaft)	Cortical Area (radius shaft)
WNT 5902	GG (n=303)	254 (2.1) *	1216 (1.6)	1183 (1.8)	39.9 (0.2)	5.9 (0.04)	99.8 (0.6)
	GA (n=96)	250 (3.7)	1217 (2.8)	1184 (3.2)	39.2 (0.3)	5.2 (0.08)	99.4 (1.2)
	AA (n=9)	243(11.7)	1218 (9.3)	1179 (10.1)	39.9 (0.9)	4.7 (0.22)	98.0 (3.6)
	P-value † (Family)	0.30	0.52	0.92	**0.03**	**0.01**	**0.02**
	P-value ‡ (Population)	0.63	0.67	0.34	0.21	0.24	**0.07**
WNT 1886	GG (n=303)	255 (2.0) *	1216 (1.6)	1183 (1.7)	39.7 (0.2)	5.2 (0.04)	99.6 (0.6)
	GA (n=96)	248 (3.7)	1216 (2.8)	1184 (3.1)	39.2 (0.3)	5.2 (0.08)	99.2 (1.3)
	AA (n=9)	243(11.0)	1215 (8.8)	1180 (9.4)	39.8 (0.8)	4.8 (0.21)	98.2 (3.8)
	P-value (Family)	0.45	0.67	0.86	**0.05**	**0.05**	0.16
	P-value (Population)	0.66	0.69	0.23	**0.007**	0.28	**0.005**
ENPP1 4498	GG (n=303)	253 (1.9) *	1215 (1.6)	1180 (1.8)	39.8 (0.2)	5.2 (0.04)	99.6 (0.7)
	GA (n=96)	246 (3.8)	1222 (3.0)	1189 (3.6)	39.5 (0.3)	5.1 (0.08)	98.3 (1.3)
	AA (n=9)	227 (12.5)	1206 (10.3)	1177 (11.8)	39.6 (1.1)	5.3 (0.28)	100.3 (4.4)
	P-value † (Family)	**0.03**	**0.04**	**0.06**	0.12	0.52	0.55
	P-value (Population)	0.22	0.17	**0.002**	0.57	0.87	0.26

* Adjust means (SE): Covariates include Age, Sex, Height and Weight
† Nominal P-value for SNP polymorphism in Tobago family study after adjustment of Age, Sex, Height and Weight;
‡ Nominal P-value for SNP polymorphism in Tobago population Men study after adjustment of Age.

As can be seen in table 1, analyses of the two WNT10b SNPs gave very similar results and were associated in common with several bone geometry traits, including: periosteal circumference at radius shaft and cortical thickness at tibia shaft. Radius shaft cortical area was significantly associated with WNT 5902 only. There were no associations with volumetric BMD measures. The discrepancies between family and population data based results could possibly be explained by gender differences. As is well known that sex is one of the most important confounders for studying bones, in Tobago family data, we included both males and females whereas we studies in the population data we exclusively studied men.

Also in table 1, the last 5 rows, strong associations were observed between ENPP1 genotypes and volumetric BMD traits, but not bone geometry traits. The presence of two copies of "A" allele of ENPP1 4498 was correlated with decreased trabecular and cortical density at radius and tibia. The signal between this SNP and cortical density at tibia shaft was confirmed by the Tobago population study. It might be possible that our findings are due to the pure chance if you considered about the small sample size for rare homozygotes. However, our preliminary results were supported by other two indirect study evidences in Caucasians from Mr. OS data (population base, sample size ~ 880 people). First, the same SNP (rs1044498) was shown to be specifically correlated with femoral neck integral and cortical BMD, but nothing else (L. Yerges, unpublished data); Second, another ENPP1 SNP (rs6916495), which demonstrates high LD with ENPP1 4498 (R^2=0.75), were found by our colleagues showing association driven by decreased femoral shaft BMC but not bone size (L. Yerges, abstract for European calcified tissue society, 2007).

It was very interesting to find out in our association results that for WNT10b SNPs, none of the pQCT measured BMD traits come out to be significant in both family and population based association study. Very similar, but with reverse pattern, we observed only pQCT measured trabecular or cortical density phenotypes, but not bone size traits, were associated with SNP ENPP1 4498. We previously concluded (see chapter III) that pQCT measured BMD traits shared few common genes with bone size traits; the genetic correlation was low between these two categories of bone traits. Our observations that WNT10b is associated with bone geometry traits but not volumetric BMD, whereas ENPP1 is associated with volumetric BMD, but not bone geometry, is consistent with our previous observation and provides molecular evidence that specific genes may affect the variations of BMD and bone size independently.

6.3.2 Association with Composite Phenotypes from Multivariate Analysis

Finally, in table 2, we present the association analysis results between composite phenotypes derived from raw and residual traits and three SNPs. As we can see here, the empirical P values are similar to those nominal ones in most cases. Several interesting patterns were observed. First, we observed more significant associations between the ENPP1 SNP genotypes and the composite traits compared to the two WNT10b SNPs. This is true for both nominal and empirical P-values. The above result is sensible because our composite traits were constructed from 12 BMD related traits, and not bone geometry traits. As reported above (table 1), the ENPP1 SNP was associated with volumetric BMD traits, whereas, WNT10B SNPs were associated with bone size related traits. Second, we reported (in chapter V), that the loadings, the proportion of variances, and even the residual heritability are very similar for PCs derived from raw and residual underlying traits. Therefore, we would expect similar association results for both types

of composite traits. However, from table 2, we found the discrepancy rate is 56% (5 out of 9 cases). Among 4 of these 5 cases, we observed the signals in PC (raw) but not in PC (residual). Thus, based on previous results (Chapter 4), it is possible there are interactions between any of our pre-adjusted covariates (age, sex, height and weight) and the SNPs.

Finally, the association analyses of based on the composite traits are more and stronger than those using individual traits. For example, 6 out of 12 PC traits showed a nominally significant result with ENPP1, where as 3 out of 12 original traits gave a nominally significant result. Besides, instead of getting more border line nominal P values by raw raws (P values are between 0.03-0.06), many of highly significant P values ($P \leq 0.01$) were observed for composite phenotypes. Given the other strengths of multivariate analysis such as reduction of multiple testing and ability for exploring the pleiotropy effect, we might recommend the multivariate method in our further association analysis in Tobago project.

Table 30. Nominal P value for association between composite traits and SNPs for WNT and ENPP1

	WNT 5902		WNT 1886		ENPP1 4988	
	Raw	Residual	Raw	Residual	Raw	Residual
PC1	0.048 *	0.01		0.02	0.037	
PC2						0.002
PC3						
PC4	0.04					
PC5					0.037	
PC6						
PC7					0.016	0.04
PC8					0.023	0.004
PC9						
PC10					0.038	
PC11						
PC12					0.037	
Factor1		0.037			0.03	
Factor2				0.03		0.049
Factor3						0.001
Factor4						
Factor5	0.01		0.047			
Factor6		0.01		0.05		
Factor7						

* Nominal P-value without multiple comparison correction

6.4 CONCLUSIONS

We tested for association between the polymorphisms of three SNPs (two WNT10b SNPs and one ENPP1 SNP) and more than thirty different skeletal measurements, which include (areal and volumetric) BMD traits and bone size traits. We also reported the association results between the same three SNPs and composite phenotypes derived by multivariate analysis from 12 original BMD traits.

We found nominally significant association signals between the two WNT10b SNPs and bone size related traits, but none of pQCT measured BMD traits. In contrast, association between SNPs in ENPP1 and bone phenotypes were observed exclusively on pQCT measured trabecular and cortical BMD, but not bone size. The above results may suggest that there were specific genetic determinants affecting the variations of BMD and bone size separately, which agrees with the previous conclusions of a low genetic correlation between BMD and bone size related traits.

In addition, we also detected many nominally-significant associations between the SNP ENPP1 4498 and BMD derived composite phenotypes, but fewer associations with the two WNT SNPs. Because the composite traits were derived from BMD traits, and not bone geometry traits, this result is consistent with the individual trait analyses. In addition, because of substantial discrepancies of association signals for raw and residual composite traits, we suggest that gene-environment interactions may be present between the three SNPs genotypes and the bone traits. Finally, we believe using the multivariate methods in association analyses in our study may benefit our research because we observed more and stronger association signals with composite phenotypes than for the original bone traits.

7.0 CONCLUSIONS

As is well-known, populations of African ancestry have greater bone strength and consequently lower osteoporotic fracture risk than other ethnic groups, such as Caucasians or Asians. However, despite their lower osteoporotic risk, the number of affected blacks is expected to increase dramatically throughout the next half century due to increases in longevity and the number of older adults at risk. In sharp contrast to our understanding of the etiology and prevention of osteoporosis in Caucasians, there is a paucity of information about skeletal health in non-white populations, and particularly those of African heritage. As part of this dissertation, I investigated the complex genetic and environmental architecture of bone strength-related phenotypes in large, extended multigenerational families of Tobago, an African origin population.

Specifically I

(1) identified environmental covariates that influenced bone strength-related traits and determined what proportion of the phenotypic variation was attributable to genetic and environmental factors;
(2) examined the relationship between trabecular and cortical bone density traits: How these two types of bone density traits genetically and environmentally correlated to each other;
(3) confirmed the hypothesis that there is little shared genetic and environmental determinants which controlled for the co-variations BMD and bone size traits;

(4) tested the possibility in our data if the weight bearing traits and non-weight bearing traits were influenced by different sets of unique genes separately;

(5) explored the application of multivariate analysis for studying bones: using the composite phenotypes derived from PCA and FA analysis from both simulation and real data, we evaluated the performance of these two methods for uncovering underlying genetic components.

Summary of results

First of all, through the cross sectional study, we found Tobago population has similar peak total hip and femoral neck areal BMD compared to African American, but higher than Caucasians. However, the bone loss rate in the Tobago population is the lowest among all these three populations. Besides, we also observed the gender difference among these bone traits. All measures of areal BMD and most of volumetric BMD at radius and tibia (except for cortical BMD) are significantly greater in men than in women. We identified several environment covariates that explain 12% - 38% of original variation of BMD traits. These significant environment factors include but are not limited to age, sex, weight, height, menopause, current smoking and drinking alcohol status, parity and diabetes *etc*. Among them, sex, height and weight accounted for the majority (73% to 99%) of the total variation due to these significant covariates. On the other hand, the proportion of total variation caused by additive genetic factors accounted for as much as or more of the total phenotypic variation than did measured covariates. The heritabilities of both areal and volumetric BMD traits mentioned above range from 23%-52%.

Many of our results support the hypothesis that genetic and environmental influences on trabecular and cortical BMD phenotypes differ. As examples of environmental differences, we observed that smoking seems to affect only trabecular traits, whereas parity (ever pregnant) influences only cortical BMD, and current diabetes status has a much stronger impact on trabecular versus cortical BMD. In addition, the estimated environmental correlations between two trabecular or two cortical traits were high (rhoE equal to 0.48±0.13 to 0.66±0.05, $P<0.05$), but were low between cortical and trabecular traits. This result implies that in addition to measured factors such as age, sex, weight and height etc., there exists important unmeasured common environmental covariates affecting the shared variance of these two types of traits. The first evidence of possible genetic differences between cortical and trabecular bones arises from the heritability estimation. We found significantly higher residual heritabilities for trabecular than cortical traits at both radius (0.70±0.10 *vs.* 0.29±0.09) and tibia (0.69±0.10 *vs*. 0.42±0.10). In addition, of the estimated genetic correlations between two trabecular or two cortical sites were very high (rhoG equal to 0.82±0.12 to 0.87±0.05, $P<0.001$), but the rhoGs between trabecular and cortical BMD did not differ from 0. Furthermore, multivariate analysis revealed that many of composite phenotypes had predominantly higher correlations (higher loadings) for either trabecular or cortical BMD traits, but seldom both. The exciting results above may explain previous inconsistent findings for DXA measured total BMD phenotypes (i.e. the combinations of trabecular and cortical BMD) in which some investigators detected significant covariates or candidate genes whereas others did not.

Results of this project also indicate that determinants of bone size are independent of BMD, which is consistent with results from previous studies in mice. We observed that the genetic and environmental correlations between bone size and BMD traits were generally low and non-significant. Moreover, genotypes at two WNT10B SNPs were associated with bone size related traits, but not BMD traits. In contrast, genotypes at the ENPP1 SNP were associated trabecular and cortical BMD traits, but not bone size. This latter results seems to conflict with our previous conclusion that trabecular and cortical BMD were largely (but not completely) regulated by separate sets of genes. However, the proportion of variance of trabecular and cortical traits due to the ENPP1 SNP are between 0.006 and 0.007. Therefore, this minor value will not affect any of our above conclusions at all.

Although investigators report evidence that weight bearing versus non-weight bearing bone sites may be influenced by different genetic and environmental factors, our analyses failed to demonstrate any significant differences from results of univariate (significant environment correlates and heritabilities), bivariate (genetic and environment correlations) or multivariate analysis (loadings of composite traits). Some possible reasons for the discrepancies between our results and the previous studies are as follows: (1) the original hypothesis is incorrect, (2) the Tobago population is different, (3) the smaller sample size in our study was not powerful enough to detect a difference. Although we saw no differences for most of BMD measures at the tibia and radius, however, for two bone size related traits (tibia length and periosteal circumference), the residual heritabilities at the weight bearing skeletal sites were higher than the corresponding measures at non-weight bearing skeletal sites.

Finally, we addressed several important issues of multivariate analysis by both simulated and real family data, although we also realized that there are certain limitations need to be acknowledged, such as violation of independence assumption, smaller sample size, limited focus only on first composite trait and arbitrary functions in simulation study. First, results of our simulation study demonstrated that both PCA and FA behaved qualitatively similar, although FA appeared to be better at detecting predominant genetic signals from an underlying trait. In addition, we determined that using residuals in the PCA or FA analyses greatly increased the probability that PCs or factors detect common genetic components instead of common environmental factors, except if there is statistical interaction between genetic and environmental factors. We also observed no predictable relationship between heritabilities obtained from composite phenotypes versus original complex traits, although the composite trait heritability generally reflected the genetic characteristics of the detectable underlying components. The latter two conclusions (using residual strategy and heritability patterns of composite traits) are supported in our analyses of real data assessment. Most importantly, in real data analysis, composite phenotypes from multivariate analysis exhibited more and stronger association signals with SNP data than those for original bone traits, indicating that they were able to capture the underlying genetic signals.

Significance of this dissertation

Results of this study provide the first comprehensive genetic epidemiologic analysis of volumetric BMD measured by QCT, and the first analysis of these traits in extended families of African descent. The Tobago Family Health Study provided an excellent and unique opportunity

to characterize the genetic architecture of several bone strength related traits in a population of West African heritage, a group that has been largely under-represented in osteoporosis research. Our results will contribute to the better understanding of the genetic and environmental risk factors of osteoporosis and may lead to better methods of treatment and prevention of the disease.

Future directions

Results of my project suggest several different avenues for additional research in three general categories: (1) measuring additional bone traits and (2) performing genome wide linkage and association (GWL and GLA) studies, and (3) perform gender-specific analyses. First, as I observed in my study, analyzing bone compartments, such as trabecular and cortical bone, separately revealed that these two compartments were differentially influenced by both genetic and environmental factors. Information on fancier phenotypes measured by latest advanced technology (High Definition QCT, Magnetic Resonance Imaging) might be even better surrogates for bone strength and provide greater insights regarding the mechanisms involved in regulating bone strength. In addition, obtaining bone measurements by central CT at different skeletal sites would enable comparisons between QCT and DXA measured phenotypes, as well as allow us to determine whether environmental and genetic factors have systemic or localized effects on bone. Second, because of the strong evidence that different genes influence specific bone compartments, obtaining genotype data on markers across the genome would enable the eventual identification of these genes, and perhaps gene by environment interactions, using univariate and multivariate GWL and GWA methods. Last but not the least, we need to keep

increasing our sample size to generate higher power, especially for gender specific analysis of the different bone compartments.

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