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A Meta-Analysis of the Transferability of Bone Mineral Density Genetic Loci Associations From European to African Ancestry Populations

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Abstract

Genetic studies of bone mineral density (BMD) largely have been conducted in European populations. We therefore conducted a meta-analysis of six independent African ancestry cohorts to determine whether previously reported BMD loci identified in European populations were transferable to African ancestry populations. We included nearly 5000 individuals with both genetic data and assessments of BMD. Genotype imputation was conducted using the 1000G reference panel. We assessed single-nucleotide polymorphism (SNP) associations with femoral neck and lumbar spine BMD in each cohort separately, then combined results in fixed effects (or random effects if study heterogeneity was high, I^2 index >60) inverse variance weighted meta-analyses. In secondary analyses, we conducted locus-based analyses of rare variants using SKAT-O. Mean age ranged from 12 to 68 years. One cohort included only men and another cohort included only women; the proportion of women in the other four cohorts ranged from 52% to 63%. Of 56 BMD loci tested, one locus, 6q25 (*C6orf97*, $p = 8.87 \times 10^{-4}$), was associated with lumbar spine BMD and two loci, 7q21 (*SLC25A13*, $p = 2.84 \times 10^{-4}$) and 7q31 (WNT16, $p = 2.96 \times 10^{-5}$), were associated with femoral neck BMD. Effects were in the same direction as previously reported in European ancestry studies and met a Bonferroni-adjusted p value threshold, the criteria for transferability to African ancestry populations. We also found associations that met locus-specific Bonferroni-adjusted p value thresholds in 11q13 (LRP5, $p < 2.23 \times 10^{-4}$), 11q14 (*DCDC5*, $p < 5.35 \times 10^{-5}$), and 17p13 (*SMG6*, $p < 6.78 \times 10^{-5}$) that were not tagged by European ancestry index SNPs. Rare single-nucleotide variants in *AKAP11* ($p = 2.32 \times 10^{-2}$), *MBL2* ($p = 4.09 \times 10^{-2}$), MEPE ($p = 3.15 \times 10^{-2}$), *SLC25A13* ($p = 3.03 \times 10^{-2}$), STARD3NL ($p = 3.35 \times 10^{-2}$), and *TNFRSF11A* ($p = 3.18 \times 10^{-3}$) were also associated with BMD. The majority of known BMD loci were not transferable. Larger genetic studies of BMD in African ancestry populations will be needed to overcome limitations in statistical power and to identify both other loci that are transferable across populations and novel population-specific variants.

Keywords

GENETICS RESEARCH; HUMAN ASSOCIATION STUDIES; GENERAL POPULATION STUDIES; OSTEOPOROSIS; BMD; GENETICS; AFRICAN ANCESTRY POPULATION; DXA; META-ANALYSIS

Introduction

Osteoporosis is a major public health burden in older adults, affecting approximately 54% of the US adult population 50 years and older.[1] It is a skeletal condition characterized by low bone mass and quality that leads to bone fragility and increased susceptibility to fracture.[2] Assessment of bone mineral density (BMD) utilizing dual-energy X-ray absorptiometry (DXA) is key to clinical diagnosis of osteoporosis[2] and remains the single best predictor of fracture.[3] Among individuals of European ancestry, women experience about twice as many fractures as men, but sex differences in fracture rates among African Americans are negligible.[4] African American men and women have higher bone mineral density and lower rates of fracture than similar aged individuals of European ancestry.[5] In 2005, 12% of all fractures occurred in non-whites; this is expected to increase to 21% by 2025.[6] There are ethnic and racial disparities in osteoporosis diagnosis and treatment that need to be better understood to address the rising rates of osteoporotic fractures in older individuals.[7]

Genetic factors may contribute to BMD variation within and between different ethnicities.[8] BMD has a strong genetic component as demonstrated by heritability estimates between 50% to 85%,[9–13] where estimates tend to be higher in twin and other family-based studies.[14–16] Greater African genetic admixture has been associated with higher BMD and biomechanically more favorable hip geometry but larger decreases in bone strength with aging.[17,18] Genomewide association studies (GWAS) to date have been instrumental in identifying genetic determinants of BMD. More than 500 loci have been identified[19–21] in European ancestry populations, providing insights into possible mechanisms underlying osteoporosis. Genetic factors may influence bone accrual even at an early age.[8] A study of BMD in pediatric cohorts found that the number of BMD-increasing alleles is elevated in African ancestry populations compared with Europeans and East Asians, suggesting that BMD variants may play a role in accrual of peak bone mass.[22]

The majority of genetic studies have been conducted in European ancestry populations and may not reflect the genetic architecture of BMD in African Americans.[23,24] Only a small fraction of BMD loci identified from genetic studies of European populations were “transferable” (ie, replicated in non-European populations).[25,26] For example, a GWAS of fracture in African American women found that few BMD loci were transferable to African American populations but identified a novel variant in SVIL that had not been previously identified in European ancestry populations.[27] High transferability of GWAS findings across populations has been reported for other complex traits.[24] Assuming that most underlying causal variants are common and shared across ancestral groups, there still may be differences in genetic architecture and ancestral effects that limit transferability of GWAS findings between human ancestry groups.[28] To date, few genetics studies of BMD have been conducted in African ancestry populations.[29] Genetic studies in African ancestry

populations are needed to assess whether previously identified BMD risk loci confer the same disease risk in African ancestry populations and to identify new genetic associations that may have been missed in studies based on European populations.

We therefore conducted the largest meta-analysis of selected BMD loci in six independent African ancestry cohorts to determine whether BMD loci identified in European ancestry populations are transferable to African ancestry populations. This may provide potential insights into applicability of targeted disease therapies and genetic risk prediction across ethnic populations.

Materials and Methods

Subjects

We included a total of 4967 recent African ancestry individuals from all available cohorts at the time of study initiation with genetic data from either de novo genotyping or existing genomewide genotyping and DXA measures of BMD at the femoral neck and/or lumbar spine: the Tobago Bone Health Study (Tobago) (n = 1414), the Health, Aging, and Body Composition Study (Health ABC) (n = 1093), Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) (n = 908), Women's Health Initiative (WHI) (n = 797), Johnston County Osteoarthritis Project (JoCoOA) (n = 415), and the Bone Mineral Density in Childhood Study (BMDCS) (n = 340).

Tobago—The Tobago Bone Health study is a population-based study of men from the Caribbean island of Tobago.[30] Men aged 40 years and older were recruited without regard to health status except that participants had to be ambulatory, non-institutionalized, and not terminally ill. Men for this analysis were selected to be of African ancestry, as determined by self-report of 1+ African grandparent; non-African ancestry in Tobago has been previously estimated to be <6%.[31] Lumbar spine and femoral neck BMD were measured using a Hologic QDR4500 (Hologic, Inc., Marlborough, MA, USA). The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study, and all participants provided written informed consent before data collection.

Health ABC—The Health, Aging, and Body Composition study is a longitudinal cohort of men and women aged 70 to 79 years at the initial visit focused on identifying risk factors that contribute to functional decline in older persons in relation to changes in body composition with age. Participants were recruited from a random sample of white and black Medicare-eligible residents in Pittsburgh, PA, and Memphis, TN, and were selected at baseline to be free of disabilities related to mobility and activities of daily living. Lumbar spine and femoral neck BMD were measured using a Hologic QDR4500. Baseline assessments were used in the analysis. All respondents provided written informed consent, and all protocols were approved by the institutional review boards at each study site.

HANDLS—The Healthy Aging in Neighborhoods of Diversity across the Life Span study is a community-based, longitudinal study of men and women aged 30 to 64 years at baseline designed to examine the influences of race and socioeconomic status on the development

of age-related health disparities among socioeconomically diverse African Americans and whites in Baltimore.[32] Participants were recruited from 13 contiguous neighborhoods in Baltimore by area probability sampling and randomly selected within strata based on age, race, sex, and socioeconomic status. BMD measurements from the first examination were used in the analysis. Total body, hip, and lumbar spine BMD were measured by Lunar DPX-IQ (GE Healthcare Lunar, Madison, WI, USA) and Hologic QDR Discovery-A. Machine type was included as a study-specific covariate in the analysis. All participants provided written informed consent. The protocol was approved by the institutional review board at the National Institute of Environmental Health Sciences.

WHI—The Women's Health Initiative is a long-term national health study of ethnically and geographically diverse women aged 50 to 79 years at the time of study enrollment. The WHI was designed to address risk factors for diseases that commonly affect postmenopausal women, including cardiovascular disease, cancer, and osteoporosis.[33] At study initiation, there were two major components to WHI, an observational study and four clinical trials. The observational study included women aged 50 to 79 years in a prospective cohort study. The clinical trial enrolled and randomized women aged 50 to 79 years into one of four placebo-controlled trials (one of two postmenopausal hormone therapies, dietary intervention, or calcium and vitamin D supplementation). Bone mineral density was measured in participants recruited at three of 40 clinical centers (Pittsburgh, PA; Birmingham, AL; and Tucson/Phoenix, AZ), chosen to provide maximum racial diversity. Participants of this WHI BMD Cohort underwent DXA measurement of lumbar spine and hip BMD using Hologic machines (QDR2000, 2000+, or 4500).[34,35] Quality assurance methods included cross-clinic calibration phantoms[36] and review of a random sample of scans.[37] When the Hologic QDR 2000 machines were upgraded to QDR 4500 machines, in vivo cross-calibration procedures were performed, and results were adjusted for these correction factors and for longitudinal changes in scanner performance. Values from the baseline assessment were used. All participants provided written informed consent. Institutional review board approvals were obtained at all participating institutions.

JoCoOA—The Johnston County Osteoarthritis Project is a community-based, longitudinal study of white and African American men and women aged 45 years or older from a rural county in North Carolina.[38] The study was designed to determine racial differences in the prevalence and incidence of risk factors associated with the occurrence and progression of hip and knee osteoarthritis. Participants were recruited by probability sampling, with oversampling of African Americans. Hip and lumbar spine BMD were measured using the Hologic QDR Delphi A. All participants provided written informed consent. The study was approved by the institutional review boards at the University of North Carolina Schools of Medicine and Public Health and the Centers for Disease and Control Prevention.

BMDCS—The Bone Mineral Density in Childhood Study is a longitudinal study of BMD in normally developing boys and girls aged 5 to 20 years old who were recruited between 2002 and 2007 from five clinical centers (Los Angeles, CA; Cincinnati, OH; Omaha, NE; Philadelphia, PA; and New York, NY).[39,40] Hip and lumbar spine BMD were measured annually for up to seven measurements with either Hologic QDR4500A, QDR4500W,

Delphi A, or Apex bone densitometers. Values from the baseline assessment were used. One densitometer was used at each clinical center. All participants older than 18 years provided written informed consent. For participants aged 18 years and younger, consent was obtained from the parent or guardian and assent was obtained from the participants. The protocol was approved by institutional review boards at each clinical center.

Genotyping and imputation

We genotyped variants in the Tobago Bone Health Study using a custom Illumina (San Diego, CA, USA) iSelect BeadChip designed to target 3602 single-nucleotide polymorphisms (SNPs) selected to cover 56 genomewide significant loci identified for either lumbar spine or femoral neck BMD.[19] We selected the most significant SNP in each locus (ie, index SNP) and other haplotype tag SNPs to cover 95% of the variation in each locus based on the 1000 genomes reference panel (phase 1). We removed SNPs with low call rates (<0.9) and deviation from Hardy–Weinberg equilibrium ($p < 10^{-4}$). We then phased haplotypes and imputed SNPs to the 1000 genomes reference panel (phase 3) using SHAPEIT[41] and IMPUTE2,[42] respectively. All other cohorts were genotyped with genomewide arrays and similarly imputed to the same 1000 genomes reference panel. Health ABC and JoCoOA were genotyped with the Illumina 1 M-Duo platform, WHI was genotyped with the Affymetrix (Santa Clara, CA, USA) Genome-Wide Human SNP Array 6.0 chip, HANDLS was genotyped with the Illumina Infinium II platform, and BMDCS was genotyped with the Illumina HumanOmniExpressExome-8v1 chip. SNPs with low call rates (<97% for Health ABC, <95% for HANDLS and BMDCS, <90% for WHI, <98% for JoCoOA) and deviation from Hardy–Weinberg equilibrium (significant at $<10^{-6}$ for Health ABC, $<10^{-5}$ for HANDLS, BMDCS, JoCoOA, $<10^{-4}$ for WHI and JoCoOA) were removed before imputation.

Association analyses and meta-analyses

We calculated differences in effect allele frequencies between previously reported European and our observed African ancestry populations and compared the size of the linkage disequilibrium (LD) blocks between European and African ancestry populations by dividing the African ancestry LD block size by the European ancestry LD block size (proportions closer to zero indicate that the African ancestry LD block is much smaller than the European ancestry LD block, and proportions closer to one indicate that the African ancestry LD block is similar in size to the European ancestry block size, while proportions above one indicate that the African ancestry LD block is larger than the European ancestry block size). We additionally calculated power in the African ancestry meta-analyses to detect SNP effect sizes reported in GEFOSII with $p = .05$.

Single-SNP associations in 56 loci originally identified in European ancestry populations that were part of the Genetic Factors for Osteoporosis Consortium (GEFOSII) were tested for association with lumbar spine and femoral neck BMD in each cohort using linear regression. Models assumed an additive genetic model and were adjusted for sex, age, age^2, and weight. In addition, models were adjusted for study-specific genetic principal components to adjust for population stratification[43] and other study-specific covariates such as DXA machine type if two different machines were used. Boundaries of the loci

were defined by high LD SNPs (r^2 European ancestry (EA) ≥ 0.8) furthest up and downstream of the index SNP based on the 1000 genomes European ancestry reference panel.[44] Each cohort performed single-SNP associations on the autosomal chromosomes and X chromosome using the prepScores and prepScoresX functions in the seqMeta R package. We then conducted an inverse-variance weighted fixed effects meta-analysis of lumbar spine and femoral neck BMD associations with common SNPs using summary statistics from each cohort. Only SNPs with good imputation quality (INFO ≥0.4) and common SNPs with minor allele frequencies ≥0.01 were included. Associations that met a $p < .05$ were considered nominally statistically significant. To account for multiple testing, we used a Bonferroni corrected p value threshold to account for testing 56 independent loci ($p = .05/56 = 8.93 \times 10^{-4}$) if SNPs were in high LD with the index SNP (r^2 African ancestry (AA) ≥ 0.8) based on the 1000 genomes African ancestry reference panel. If SNPs were not in high LD ($r^2_{AA} < 0.8$) with the index SNP, we used a locus-specific p value threshold that corrected for the number of SNPs tested in each locus (p = .05/number of tested SNPs in a single locus; Supplemental Table S1). We assessed study heterogeneity with the I^2 index. For associations where the I^2 index exceeded 60, we repeated analyses with a random effects model. We considered a SNP to be transferable between European and African ancestry populations if the SNP was in high LD ($r^2_{AA} \geq 0.8$) with the index SNP, reached statistical significance at the Bonferroni corrected p value threshold, and had an effect in the same direction as reported in European populations. In sensitivity analyses, we repeated meta-analyses leaving out the only pediatric cohort.

To comprehensively assess the entire allele frequency spectrum, in secondary analyses, we also conducted locus-based analyses of less common SNPs using SKAT-O, a method that optimally combines the burden and SKAT tests used in rare variant analyses.[45] We only included SNPs with good imputation quality (INFO ≥0.7) and less common SNPs with minor allele frequencies <0.01. To account for multiple testing, we used a Bonferroni corrected p value threshold to account for testing 56 independent loci ($p = .05/56 = 8.93 \times 10^{-4}$).

Fixed and random effects meta-analyses were implemented with GWAMA (Genome-Wide Association Meta-Analysis) software.[46] SKAT-O analyses were implemented using the seqMeta R package (https://cran.r-project.org/web/packages/seqMeta/seqMeta.pdf).

Results

We included a total of 4967 individuals from six African ancestry cohorts with measures of lumbar spine and/or femoral neck BMD and genotyping data (Table 1). One cohort, Tobago, included only men and another cohort, WHI, included only women. The proportion of women in the other four cohorts ranged from 52% to 63%. Mean age ranged from 49 to 73 years and mean weight ranged from 78 to 86 kg in the adult cohorts. The mean age was 12 years and the mean weight was 44 kg in BMDCS, a pediatric cohort.

We tested 56 loci that were originally identified to be genomewide significant for lumbar spine and/or femoral neck BMD in European ancestry populations from the Genetic Factors for Osteoporosis Consortium (GEFOSII) (Supplemental Tables S2 and S3). Differences in

effect allele frequencies between European and African ancestry populations ranged from 0.01 to 0.53, where about a quarter had differences less than 0.1. Only nine loci (*AKAP11, C6orf97, LEKR1, MEPE, SLC25A13, STARD3NL, WLS, WNT16, ZBTB40*) had at least 80% power to detect nominally significant associations with lumbar spine BMD and only seven loci (*CTNNB1, MEF2C, MEPE, SLC25A13, SOX6, WNT16, ZBTB40*) had 80% power to detect nominally significant associations with femoral neck BMD. Loci were defined by regions of high LD ($r^2_{AA} \geq 0.8$) around the index SNP. African ancestry LD block sizes ranged from 0 kb to 526 kb (median = 16 kb; interquartile range = 3 kb to 55 kb) and proportions of European ancestry LD block sizes ranged from 0% to 775% (median = 50%; interquartile range = 4% to 80%) (Supplemental Table S4). About a third of the loci tested had African ancestry LD blocks that were 70% smaller than the LD block size in European ancestry populations (Fig. 1).

At least one SNP in high LD ($r^2_{AA} \geq 0.8$) with the index SNP or the index SNP itself met nominal statistical significance ($p < .05$) in fixed effects models for associations with lumbar spine BMD in *C6orf97, GPATCH1, JAG1, KLHDC5/PTHLH, LEKR1, LIN7C, MARK3, MAPT, RSPO3, SOX9, STARD3NL, WLS, WNT16,* and *ZBTB40* and for associations with femoral neck BMD in *ARHGAP1, C6orf97, ERC/WNT5B, GPATCH1, IDUA, NTAN1, SLC25A13, SP7, WLS, WNT16,* and *ZBTB40* (Table 2). All of these loci except *LIN7C* and *ERC1/WNT5B* had shorter LD blocks in African ancestry populations than European ancestry populations (Fig. 2, Supplemental Fig. S1, Supplemental Table S5). Study heterogeneity was low to moderate ($I^2 < 60$) for the majority of nominally significant loci, except the *WNT16* association with lumbar spine BMD and *ARHGAP1* and *GPATCH1* associations with femoral neck BMD (Table 2). In random effect models, only the association between *WNT16* and lumbar spine BMD met nominal significance ($p < .05$) but did not meet the p value threshold after correction for multiple testing ($p = 8.93 \times 10^{-4}$) (Supplemental Table S6). Few loci met the more stringent significance threshold. Only *C6orf97* ($\beta = -0.01$, $p = 8.87 \times 10^{-4}$) with lumbar spine BMD and *SLC25A13* ($\beta = 0.01$, $p = 2.84 \times 10^{-4}$) and *WNT16* ($\beta = -0.01$, $p = 2.96 \times 10^{-5}$) with femoral neck BMD met the multiple testing p value threshold; all effects were in the same direction as the reported effect in the GEFOS European ancestry analyses, thus meeting criteria for transferability in African ancestry populations (Table 2). Only the African ancestry lead SNP in *SLC25A13*, but not *C6orf97* and *WNT16*, was previously assessed in GEFOSII (Supplemental Table S7). In sensitivity analyses, after removal of BMDCS from the meta-analysis, *C6orf97* remained significant for lumbar spine BMD and *WNT16* remained significant for femoral neck BMD (Supplemental Table S8), meeting the multiple testing p value threshold in both fixed and random effects models (Supplemental Table S8).

Other SNPs in the locus that were not in LD with the index BMD SNP from the GEFOS Consortium ($r^2_{EA} < 0.8$) were tested and were required to meet locus-specific Bonferroni-corrected p value thresholds (Supplemental Table S1). SNPs in *DCDC5* and *SMG6* met locuswide significance for lumbar spine BMD and SNPs in *C16orf38, DCDC5, FAM9B*, and *LRP5* met locuswide significance for femoral neck BMD (Table 3). Study heterogeneity was low to moderate ($I^2 < 60$) for the majority of loci except *C16orf38* and *FAM9B* associations with femoral neck BMD. In random effects models, these associations did not remain significant at the locuswide threshold (Supplemental Table S9). Only two

SNPs, rs978751 and rs7950105, both in *DCDC5* were previously assessed in GEFOSII (Supplemental Table S10). In sensitivity analyses, after removal of BMDCS from the meta-analysis, SNPs in *DCDC5* remained locuswide significant for lumbar spine BMD and SNPs in *DCDC5* and *LRP5* remained locuswide significant for femoral neck BMD (Supplemental Table S11).

In secondary gene-based analyses of rare variants (minor allele frequencies <0.01), associations with lumbar spine BMD in *AKAP11, SLC25A13, STARD3NL,* and *TNFRSF11A* and associations with femoral neck BMD in *MBL2, MEPE,* and *TNFRSF11A* met nominal statistical significance (Table 4), but none remained significant after adjustment for multiple testing.

Discussion

The majority of genetic studies of BMD have been conducted in European ancestry populations. The largest study for lumbar spine and femoral neck BMD was conducted in the GEFOS Consortium.[19] Few loci have been replicated in non-European populations and there have been no large-scale genetic studies of BMD in African ancestry populations. We therefore tested 56 BMD loci originally identified in the largest GEFOS Consortium meta-analysis and found that only three loci, *C6orf97* (also known as *CCDC170*), *SLC25A13*, and *WNT16*, were transferable to African ancestry populations. We also found significant associations in *DCDC5*, *SMG6*, and *LRP5* that were not tagged by European ancestry index SNPs, suggesting that there is between-population heterogeneity in tag SNPs for BMD. Furthermore, we found evidence that rare genetic variants in *AKAP11, MBL2, MEPE, SLC25A13, STARD3NL,* and *TNFRSF11A* were associated with BMD, although these loci did not meet a more stringent threshold for significance after correction for multiple testing. Our results are consistent with other studies that have shown low transferability of BMD loci between different genetic backgrounds and underscore the need to consider differences in genetic architecture between populations when assessing targeted interventions and genetic risk prediction in osteoporosis. Larger genetic studies of BMD in African ancestry populations comparable to genetic studies in European ancestry populations will be needed to overcome power limitations of the current study and to identify other loci that are transferable between populations and to identify novel population-specific variants.

While only three loci, *C6orf97, SLC25A13*, and *WNT16*, reached stringent criteria for transferability, we found 17 other loci that reached a less stringent threshold of significance, of which all except two loci had effects in the same direction as that reported in European ancestry populations. In fact, the majority of all 56 loci tested, 38 loci for lumbar spine BMD and 48 for femoral neck BMD, had allelic effects consistent with the direction of effects reported in the GEFOS Consortium. BMD genetic risk scores that combine the effects of SNPs discovered in the GEFOS Consortium have been shown to be associated with lumbar spine and femoral neck BMD in African American women[47] and children of Sub-Saharan African ancestry,[22] although the variance explained by genetic risk scores is lower than European ancestry populations and it is unclear whether a select few loci are driving associations. In previous reports, genetic risk scores explained more variation in hip BMD compared with lumbar spine BMD,[47] consistent with our finding that femoral

neck BMD had more associations than lumbar spine BMD in the same direction of effect as European ancestry populations. Our study was limited in power to detect associations at genomewide significance thresholds for all previously identified BMD loci, but suggests that with a larger sample size, more loci transferable between European and African ancestry may be identified.

Our findings are consistent with other genetic studies of non-European descent. In East Asian populations, at least 16 known loci discovered in European ancestry genetic studies were associated with BMD, including *AKAP11, C6orf97, C17orf53, CTNNB1, FOXL1, LRP5, MEF2C, MEPE, SLC25A13, SPTBN1, STARD3NL, SOX6, TNFRSF11A, TNFRSF11B, WLS,* and *ZBTB40*.[25,26,48] We identified 20 loci associated with BMD, of which five loci, including *C6orf97, SLC25A13, STARD3NL, WLS*, and *ZBTB40*, were also associated with BMD in East Asian populations. The three loci that met our criteria for transferability, *C6orf97, SLC25A13,* and *WNT16*, were also found to be associated with BMD at genomewide significance in a multi-ethnic genomewide association study, although this study only included a small sample of African American women.[49] Another study showed that SNPs in *WNT16* and *C6orf97* were associated with BMD in premenopausal women, which were replicated in a multi-ethnic sample that included a small sample of African American women.[29] On the other hand, *SLC25A13* has been associated with fracture risk in European populations[19] but not in African Americans.[27] Taken together, our findings in the largest sample of African ancestry individuals corroborate previous findings from multi-ethnic cohorts and underscore the role of Wnt signaling in bone biology.

In particular, *WNT16* and *C6orf97*, which were strongly associated with both lumbar spine and femoral neck BMD, may point to important aspects of bone biology in African ancestry populations. *WNT16* encodes a non-canonical Wnt ligand that regulates cortical bone homeostasis by inhibiting osteoclast differentiation indirectly by increasing osteoprotegerin and directly by acting on osteoclast progenitors.[50] Loss of *WNT16* in mice results in decreased cortical thickness and increased cortical porosity but does not affect trabecular bone.[50] Similarly, in humans, genetic variants in *WNT16* are associated with cortical bone thickness.[51] Overexpression of *WNT16* in mice increases bone mineral density but may not protect against bone loss.[52–54] The function of *C6orf97* (also referred to as *CCDC170*) is unknown but has been implicated in studies of breast cancer. *C6orf97* is located near the gene that encodes estrogen receptor 1. Estrogen plays an important role in bone homeostasis and prevents bone loss by attenuating bone resorption through estrogen receptor α in osteoclasts.[55] Both *C6orf97* and *WNT16* may play a critical role in acquisition of peak cortical bone mass.[29] Interestingly, even at the early stages of puberty, African American children have greater cortical bone mineral density, mass, and size compared with those of European ancestry.[56,57] Our findings highlight the possibility that factors related to acquisition and maintenance of cortical bone may be particularly relevant to African ancestry populations.

We acknowledge that there are limitations to our study. The study was not originally designed to determine genomewide associations, as the largest contributing cohort only had limited genotyping of 56 loci selected based on their genomewide significant associations with BMD in the previously published, largest GWAS meta-analysis of femoral neck and

lumbar spine BMD to date.[19] Our original intent was not to discover new loci using an agnostic GWAS approach but rather to determine whether already-verified European ancestry loci were transferable to African ancestry populations. We also sought to determine whether there may be genetic associations in these loci that may have been missed in studies based on European populations but could be identified in African ancestry populations due to differences in genetic architecture. There are few genetic studies of BMD in African ancestry populations and no genetic studies in African ancestry populations as large as ours, allowing us to provide unique insights into genetic factors underlying BMD variation in African ancestry populations, which has long been understudied.[58] Our study underscores the possibility that genetic variants associated with BMD based on European populations may not be simply applied to African ancestry populations because these variants may not necessarily confer the same disease risk, which could be attributed to differences in allele frequencies between European and African ancestry populations and allelic heterogeneity.

Additionally, despite assembling the largest meta-analysis of nearly 5000 individuals with African ancestry, we still had limited power to detect all previously identified BMD GWAS loci, let alone an agnostic GWAS. Because of limited sample size, the intent of this analysis was not for new discovery but rather to determine racial differences in variant associations that have already been identified. Large GWAS for new discovery in multiple ancestries is a much-needed direction for the bone field. Interpretation of our findings are also complicated by the use of tagging SNPs that may not necessarily capture all variation in a locus and are unlikely to be the causal variants. However, most variants identified by GWAS are common and likely to be ancient in origin and shared by different populations.[59] Assuming that the causal variant is sufficiently tagged by one or more SNPs in the LD block, we were able to narrow the region of association for several loci because the majority of LD blocks were shorter in African ancestry than European ancestry populations. Most loci had associations in the same direction of effect as previously reported associations and are likely transferable to African ancestry populations, although there may be effect size heterogeneity that impacted our ability to detect significant effects. We cannot exclude the possibility that there may be population-specific SNPs, since we found evidence for associations in *DCDC5, SMG6,* and *LRP5* that were not tagged by European ancestry index SNPs and identified rare variant associations in *AKAP11, MBL2, MEPE, SLC25A13, STARD3NL,* and *TNFRSF11A*. Also, we were not able to take admixture into account because our study was based on summary-level meta-analyses rather than individual-level data. We also were not able to assess gene–environment interactions that may affect BMD because our sample size was limited. Some may consider the inclusion of a pediatric cohort in the meta-analyses as another limitation. However, genetic effects on BMD have been shown to be detectable in pediatric cohorts because the majority of bone mineral mass is gained during adolescence and there is high familial resemblance for most bone traits before puberty.[22,60] Despite these limitations, our study provides the first insights into the genetic architecture underlying BMD in African ancestry populations. Additionally, the limited sample size underscores a well-appreciated limitation of the field of human genetics, namely that the study of populations other than those of European ancestry continue to be limited and there must be greater expansion of the field to include large numbers of other ethnicities.[58]

In summary, we conducted the largest African-ancestry meta-analysis of BMD. In six independent samples, we identified three BMD loci, *C6orf97, SLC25A13,* and *WNT16*, that are transferable to African ancestry populations. Larger genome-wide association studies and even whole-genome sequencing studies in African ancestry populations will be needed to identify other transferable BMD loci and population-specific variants that may impact the applicability of targeted interventions and genetic risk prediction for osteoporosis in diverse populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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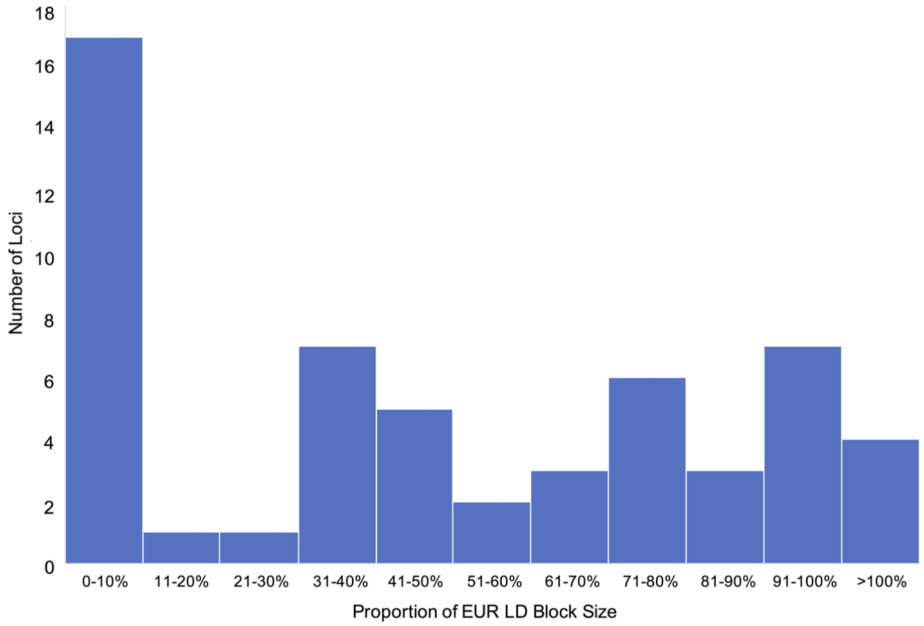

Fig 1.

Distribution of African ancestry linkage disequilibrium (LD) block sizes. We identified LD blocks around index bone mineral density SNPs in African ($r^2_{AA} \geq 0.8$) and European ancestry ($r^2_{EA} \geq 0.8$) populations. A proportion was derived by dividing the African ancestry LD block size by the European ancestry LD block size. A smaller proportion indicates that the African ancestry LD block is much smaller than the LD block in European ancestry populations, whereas a larger proportion indicates that the African ancestry LD block is similar in size to the European ancestry block size.

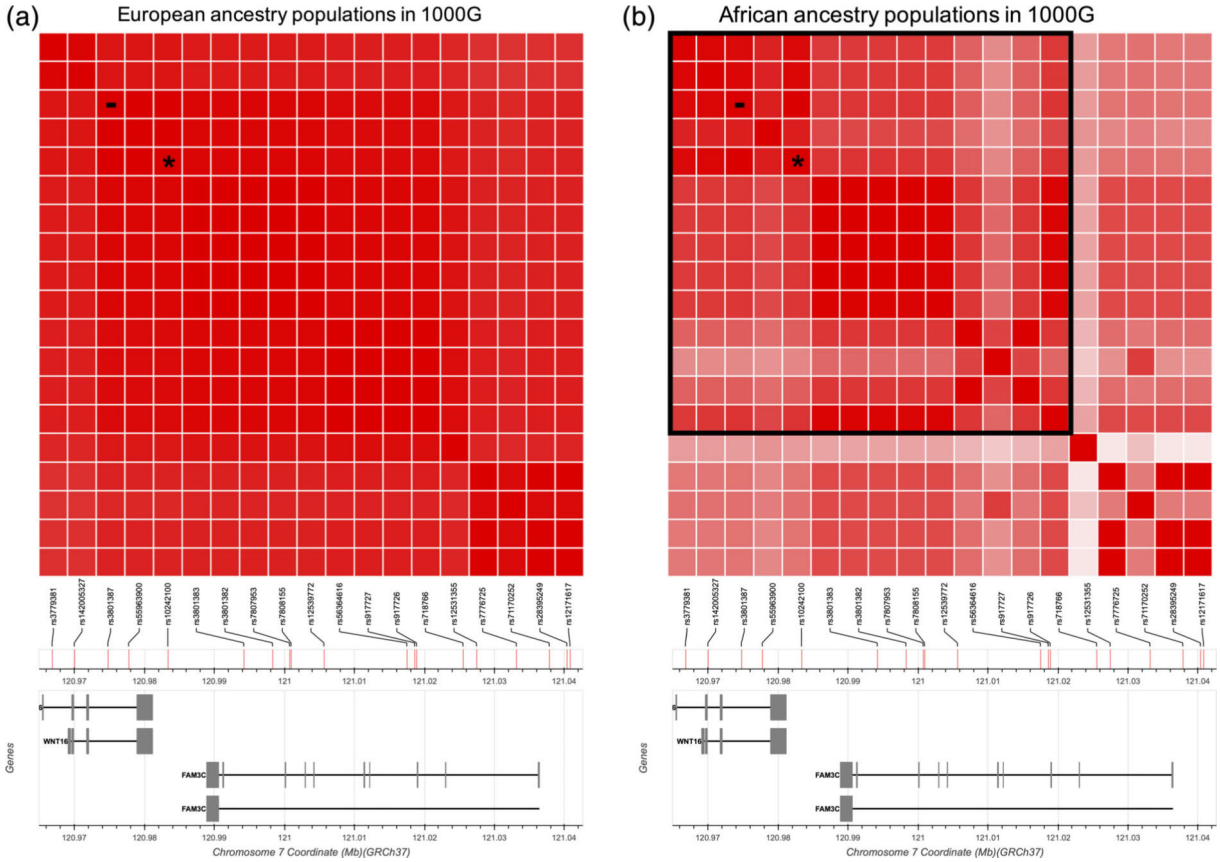

Fig 2.

African ancestry meta-analysis narrows *WNT16* locus from 74 kb to 59 kb. Linkage disequilibrium (LD) blocks represent correlations between SNPs listed along the bottom, where the red intensity represents strength of the r^2 value calculated from 1000 genomes. *(A)* SNPs in high LD ($r^2_{EA} \geq 0.8$) with the *WNT16* index SNP in European populations. *(B)* r^2_{AA} values in African ancestry populations for the same set of SNPs presented in *A*. The asterisks (*) represent the lead SNP identified in the African ancestry meta-analyses; the hyphens (-) represent the index SNP identified by the GEFOS Consortium if it is not the same as the lead SNP; a black box is drawn around SNPs in high LD with the lead SNP.

Table 1.

African-Ancestry Cohort Characteristics

	Tobago	HABC	HANDLS	WHI	JoCoOA	BMDCS
No. of participants	1414	1093	908	797	415	340
% Female	0	57.4	55.2	100	63.1	52.4
Age (years) (SD)	58.8 (10.4)	73.4 (2.9)	48.7 (8.9)	68 (7.1)	61.4 (10.6)	11.5 (4.4)
Weight (kg) (SD)	84.5 (15.9)	77.8 (19.0)	83.6 (19.5)	82.8 (18.4)	86.1 (18.7)	43.8 (18.5)

HABC = Health, Aging, and Body Composition; HANDLS = Healthy Aging in Neighborhoods of Diversity across the Life Span Study; WHI = Women's Health Initiative; JoCoOA = Johnston County Osteoarthritis Project; BMDCS = Bone Mineral Density in Childhood Study.

Table 2.

Lead SNP Associations in BMD Loci (p Value <.05 in Fixed Effects Models)

							Lumbar spine BMD			Femoral neck BMD		
Locus	**Lead SNP**	**Closest gene(s)**	**A1**	**A2**	**Freq 1 in EA**	**Freq 1 in AFR**	**Beta**	***p* Value**	**Het I^2**	**Beta**	***p* Value**	**Het I^2**
1 p31.3	rs36100617	*WLS*	g	-	0.63	0.76	**0.01**	**2.74×10^{-2}**	**0**	**0.01**	**4.94×10^{-2}**	**0**
1 p36.12	rs 10493013	*ZBTB40*	C	t	0.17	0.28	**0.01**	**3.15×10^{-2}**	**0**	**0.01**	**3.50×10^{-2}**	**5**
3q25.31	rs344081	*LEKRl* [a,c]	t	c	0.88	0.47	**0.01**	**1.48×10^{-2}**	**0**	**0.00**	**2.31×10^{-1}**	**0**
4p16.3	rs56079856	*IDUA*	t	g	0.17	0.11	**−0.01**	**2.46×10^{-1}**	**0**	**−0.01**	**4.80×10^{-2}**	**0**
6q22.32	rs 13204965	*RSPO3* [a]	a	C	0.76	0.95	−0.02	7.49×10^{-3}	0	**0.01**	**2.64×10^{-1}**	**30**
6q25.1	rs4869745	*C6orf97* [b]	t	c	0.29	0.69	**−0.01**	**8.87×10^{-4}**	**0**	**−0.01**	**6.21×10^{-3}**	**0**
7p14.1	rs6959212	*STARD3NL°*	t	c	0.36	0.35	**−0.01**	**2.23×10^{-3}**	**5**	**0.00**	**7.51×10^{-1}**	**51**
7q21.3	rs4342521	*SLC25A13*	g	t	0.64	0.81	**0.01**	**1.16×10^{-1}**	**0**	**0.01**	**2.84×10^{-4}**	**54**
7q31.31	rs 10242100	*WNT16* [b]	a	g	0.74	0.63	**−0.02**	**1.88×10^{-6}**	**70**	**−0.01**	**2.96×10^{-5}**	**19**
11p11.2	rs 10769205	*ARHGAP1* [d]	g	a	0.29	0.82	**0.01**	**2.56×10^{-1}**	**63**	**0.01**	**3.95×10^{-2}**	**63**
11p14.1	rs 11030048	*LIN7C*	t	c	0.48	0.86	0.01	2.23×10^{-2}	0	0.01	1.67×10^{-1}	0
12p11.22	rs7953528	*KLHDC5/PTHLH* [a,c]	a	t	0.16	0.05	**−0.02**	**3.39×10^{-2}**	**0**	−0.01	8.92×10^{-2}	0
12p13.33	rs35223785	*ERCl/WNT5B* [b]	a	g	0.73	0.81	**−0.01**	**5.21×10^{-2}**	**21**	**−0.01**	**2.12×10^{-2}**	**0**
12q 13.13	rsl 0783573	*SP7* [b]	a	g	0.67	0.41	**−0.01**	**5.28×10^{-2}**	**13**	**−0.01**	**3.82×10^{-3}**	**9**
14q32.32	rs7145113	*MARK3* [b]	g	a	0.36	0.17	**−0.01**	**2.03×10^{-2}**	**39**	**0.00**	**1.58×10^{-1}**	**0**
16p13.11	rs4985155	*NT AN I* [a]	a	g	0.67	0.66	**−0.00**	**1.76×10^{-1}**	**0**	**−0.01**	**3.75×10^{-2}**	**0**
17q21.31	rsl 2185268	*MAPf* [b,c]	g	a	0.24	0.04	**−0.03**	**4.14×10^{-3}**	**0**	**−0.01**	**2.63×10^{-1}**	**0**
17q24.3	rs7213040	*SOX9*	C	t	0.51	0.72	**0.01**	**4.15×10^{-2}**	**25**	**0.00**	**1.78×10^{-1}**	**61**
19q 13.11	rs2287679	*G PATCH 1* [d]	t	c	0.76	0.25	**−0.01**	**1.55×10^{-2}**	**0**	**−0.01**	**4.22×10^{-2}**	**69**
20p12.2	rs3790161	*JAG1*	a	t	0.51	0.40	**0.01**	**2.13×10^{-2}**	**0**	**0.00**	**1.23×10^{-1}**	**0**

SNP = single-nucleotide polymorphism; BMD = bone mineral density; A1 = effect allele; A2 = non-effect allele; Freq1 = frequency of A1; EA = European ancestry population in 1000 Genomes; AFR = African Ancestry meta-analysis; Het I^2 = heterogeneity I^2 index.

Bolded associations are in the same direction of effect as the associations reported in Genetic Factors for Osteoporosis Consortium (GEFOSII).

[a] Same as the index SNP.

[b] Index SNP met nominal significance for associations with femoral neck and/or lumbar spine BMD.

[c] In GEFOSII, LEKR1 and MAPT were significant for lumbar spine BMD only; *KLHDC5/PTHLH* was significant for femoral neck BMD only.

[d] Associations with femoral neck BMD no longer meet nominal significance (p value $<.05$) in random effects models.

Table 3.

Locuswide Significant SNPs Not in LD With the GEFOS Consortium Index SNP in Fixed Effects Models

									Lumbar spine BMD			Femoral neck BMD		
Locus	**SNP**	**Closest gene(s)**	**r^2 in EA** [a]	**r^2 in AA** [a]	**A1**	**A2**	**Freq 1**	**Beta**	***p*-value**	**Het I^2**	**Beta**	***p*-value**	**Het I^2**
11p14.1	rs7950105	*DCDC5*	0.62	0.14	t	c	0.81	0.02	5.97×10^{-6}	17	0.01	1.31×10^{-3}	0
11p14.1	rs2145795	*DCDC5*	0.60	0.01	a	g	0.67	0.01	8.00×10^{-6}	23	0.01	3.59×10^{-5}	0
11p14.1	rs650489	*DCDC5*	0.61	0.13	a	g	0.18	−0.02	1.88×10^{-5}	7	−0.01	2.69×10^{-3}	0
11p14.1	rs36122686	*DCDC5*	0.63	0.15	a	at	0.82	0.02	1.93×10^{-5}	36	0.01	1.53×10^{-3}	0
11p14.1	rs67243215	*DCDC5*	0.63	0.15	c	t	0.82	0.02	2.04×10^{-5}	39	0.01	1.38×10^{-3}	0
11p14.1	rs978751	*DCDC5*	0.62	0.10	g	a	0.55	0.01	3.22×10^{-5}	0	0.01	1.54×10^{-4}	0
17p13.3	rs79682102	*SMG6*	0.00	0.00	g	a	0.96	−0.04	2.31×10^{-5}	0	−0.02	4.53×10^{-3}	0
16p13.3	rs66725354	*C16orf38* [b]	0.36	0.02	g	a	0.88	0.01	1.14×10^{-2}	53	0.01	2.00×10^{-3}	65
Xp22.31	rs5934498	*FAM9B*	0.13	0.20	t	c	0.37	0.02	7.76×10^{-4}	29	0.02	9.89×10^{-5}	56
Xp22.31	rs12863157	*FAM9B* [b]	0.15	0.19	g	t	0.36	0.02	7.13×10^{-4}	41	0.02	1.06×10^{-4}	61
Xp22.31	rs1919627	*FAM9B* [b]	0.13	0.24	a	g	0.36	0.02	1.58×10^{-3}	52	0.01	2.57×10^{-4}	64
Xp22.31	rs5978281	*FAM9B*	0.13	0.21	a	g	0.39	0.01	3.28×10^{-3}	55	0.01	2.75×10^{-4}	59
11q13.2	rs111507948	*LRP5*	0.03	0.00	c	g	0.88	0.01	5.44×10^{-3}	0	0.02	1.90×10^{-5}	26
11q13.2	rs12793818	*LRP5*	0.03	0.00	c	t	0.88	0.01	5.88×10^{-3}	0	0.02	2.40×10^{-5}	25
11q13.2	rs12793822	*LRP5*	0.03	0.00	c	t	0.88	0.01	5.92×10^{-3}	0	0.02	2.40×10^{-5}	25
11q13.2	rs12362712	*LRP5*	0.03	0.00	c	t	0.88	0.01	5.16×10^{-3}	0	0.02	4.17×10^{-5}	20
11q13.2	rs59553449	*LRP5*	0.03	0.00	t	g	0.88	0.01	6.62×10^{-3}	0	0.02	8.47×10^{-5}	13
11q13.2	rs113307519	*LRP5*	0.03	0.00	c	t	0.88	0.01	6.60×10^{-3}	0	0.02	8.48×10^{-5}	13

SNP = single-nucleotide polymorphism; LD = linkage disequilibrium; GFOS = Genetic Factors for Osteoporosis; BMD = bone mineral density; EA = European ancestry 1000 genomes populations; AA = African ancestry 1000 genomes populations; Freq1 = frequency of A1 in African ancestry meta-analyses; Het I^2 = heterogeneity I^2 index.

[a] r^2 between SNP and GEFOSII index SNP.

[b] Associations with femoral neck BMD no longer meet locuswide significance in random effects models.

Table 4.

SKAT-O Rare Variant Associations in BMD Loci (p Values <.05)

	Gene	p Value	No. of SNPs[a]
Lumbar spine BMD	*AKAP11*	2.32×10^{-2}	2228
	SLC25A13	3.03×10^{-2}	1396
	STARD3NL	3.35×10^{-2}	1684
	TNFRSF11A	4.71×10^{-2}	1018
Femoral neck BMD	*MBL2*	4.09×10^{-2}	506
	MEPE	3.15×10^{-2}	1897
	TNFRSF11A	3.18×10^{-3}	1018

[a]SNPs included have minor allele frequencies <0.01.

BMD = bone mineral density; SNP = single-nucleotide polymorphism.