A genome-wide association study meta-analysis of clinical fracture in 10,012 African American women

Kira C. Taylor [a,b,*], Daniel S. Evans [c], Digna R. Velez Edwards [d], Todd L. Edwards [e], Tamar Sofer [f], Guo Li [g], Youfang Liu [h], Nora Franceschini [i], Rebecca D. Jackson [j], Ayush Giri [e], Macarius Donneyong [a,k], Bruce Psaty [l], Jerome I. Rotter [m], Andrea Z. LaCroix [n], Joanne M. Jordan [o], John A. Robbins [h], Beth Lewis [p], Marcia L. Stefanick [q], Yongmei Liu [r], Melissa Garcia [s], Tamara Harris [t], Jane A. Cauley [u], Kari E. North [v,b]

[a] School of Public Health and Information Sciences, University of Louisville, 485 E Gray St., Louisville, KY 40202, USA
[b] Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 137 E. Franklin St., Chapel Hill, NC 27514, USA
[c] California Pacific Medical Center Research Institute, 550 16th Street, Box 0560, San Francisco, CA 94158-2549, USA
[d] Vanderbilt Epidemiology Center, Department of Obstetrics and Gynecology, Vanderbilt Genetics Institute, Vanderbilt University, 2525 West End Avenue, Nashville, TN 37203, USA
[e] Vanderbilt Genetics Institute, Division of Epidemiology, Department of Medicine, Vanderbilt University, 2525 West End Avenue, Nashville, TN 37203, USA
[f] Department of Biostatistics, University of Washington, UW Tower 15th floor, 4333 Brooklyn Ave NE, Seattle 98105, USA
[g] Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Metropolitan Park East Tower, 1730 Minor Ave, Suite 1360, Seattle, WA 98101, USA
[h] Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, 3300 Thurston Bldg., CB# 7280, Chapel Hill NC 27599-7280, NC, USA
[i] University of North Carolina at Chapel Hill, 137 E. Franklin St., Chapel Hill, NC 27514, USA
[j] The Ohio State University, 376 W 10th Avenue, Suite 260, Columbus, OH 43210, USA
[k] Division of Pharmacoepidemiology and Pharmacoeconomics, Department of Medicine, Brigham and Women's Hospital, 1620 Tremont St, St 3030, Boston, MA 02120, USA
[l] Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology and Health Services, University of Washington; Group Health Research Institute, Group Health Cooperative, Metropolitan Park East Tower, 1730 Minor Ave, Suite 1360, Seattle, WA 98101, USA
[m] Institute of Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute and Department of Pediatrics, Harbor-UCLA Medical Center, 1124 W. Carson Street, Bldg., E-5, Torrance, CA 90502, USA
[n] Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, WA 98109, USA
[o] Department of Medicine, University of California at Davis Medical Center, PSSB Building, 4150 V St., Sacramento, CA 95817, USA
[p] University of Alabama, Medical Towers 614, 1717 11th Avenue South, Birmingham, AL 35205, USA
[q] Stanford Prevention Research Center, Stanford University School of Medicine, Medical School Office Building, 1265 Welch Road, Mail Code 5411, Stanford, CA 94305, USA
[r] Wake Forest Baptist Medical Center, Medical Center Boulevard, Winston-Salem, NC 27157, USA
[s] Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, 7201 Wisconsin Ave, Suite 3C309, Bethesda, MD 20892, USA
[t] Laboratory of Epidemiology and Population Science, Intramural Research Program, National Institute on Aging, National Institutes of Health, 31 Center Drive, Bethesda, MD 20892, USA
[u] University of Pittsburgh Graduate School of Public Health, Department of Epidemiology, A510 Crabtree Hall, Pittsburgh, PA 15261, USA
[v] Carolina Center for Genome Sciences, 250 Bell Tower Dr., Chapel Hill, NC 27514, USA

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ABSTRACT

Background: Osteoporosis is a major public health problem associated with excess disability and mortality. It is estimated that 50–70% of the variation in osteoporotic fracture risk is attributable to genetic factors. The purpose of this hypothesis-generating study was to identify possible genetic determinants of fracture among African American (AA) women in a GWAS meta-analysis.

Methods: Data on clinical fractures (all fractures except fingers, toes, face, skull or sternum) were analyzed among AA female participants in the Women's Health Initiative (WHI) (N = 8155), Cardiovascular Health Study (CHS) (N = 504), BioVU (N = 704), Health ABC (N = 651), and the Johnston County Osteoarthritis Project (JoCoOA) (N = 291). Affymetrix (WHI) and Illumina (Health ABC, JoCoOA, BioVU, CHS) GWAS panels were used for genotyping, and a 1:1 ratio of YRI:CEU HapMap haplotypes was used as an imputation reference panel. We used Cox proportional hazard models or logistic regression to evaluate the association of ~2.5 million SNPs

African American
Genetic association study

with fracture risk, adjusting for ancestry, age, and geographic region where applicable. We conducted a fixed-effects, inverse variance-weighted meta-analysis. Genome-wide significance was set at $P < 5 \times 10^{-8}$.

Results: One SNP, rs12775980 in an intron of *SVIL* on chromosome 10p11.2, reached genome-wide significance ($P = 4.0 \times 10^{-8}$). Although this SNP has a low minor allele frequency (0.03), there was no evidence for heterogeneity of effects across the studies ($I^2 = 0$). This locus was not reported in any previous osteoporosis-related GWA studies. We also interrogated previously reported GWA-significant loci associated with fracture or bone mineral density in our data. One locus (*SMOC1*) generalized, but overall there was not substantial evidence of generalization. Possible reasons for the lack of generalization are discussed.

Conclusion: This GWAS meta-analysis of fractures in African American women identified a potentially novel locus in the supervillin gene, which encodes a platelet-associated factor and was previously associated with platelet thrombus formation in African Americans. If validated in other populations of African descent, these findings suggest potential new mechanisms involved in fracture that may be particularly important among African Americans.

1. Introduction

Osteoporosis is a major public health problem that over a lifetime results in fractures in 40% of aging women (Melton et al., 1992). Although the lifetime risk of fractures is slightly lower in nonwhite women, the absolute risk is still substantial and may be rising (Farmer et al., 1984; Baron et al., 1994; Baron et al., 1996). By 2025, 21% of all fractures in the United States will occur in nonwhite women (Burge et al., 2007). Importantly, the consequences of fractures may be greater among nonwhite women. For example, African-American women who suffer a hip fracture have longer hospitalization stays and are more likely to be non-ambulatory compared with European-American women (Furstenberg and Mezey, 1987). Furthermore, mortality after a hip fracture is higher among African-American than European-American women, pointing to disparities in access to health care or differences in comorbidities (Jacobsen et al., 1992). The Surgeon General's Report on Bone Health and Osteoporosis noted the lack of information on ethnic and racial minorities as a priority (United States Public Health Service Office of the Surgeon General, 2004).

Osteoporosis has a strong genetic component, with 25–85% of the variation in osteoporosis-related traits (such as bone mineral density) being attributable to genetic factors (Ralston, 2002; Ferrari, 2008). The heritability of osteoporotic fracture (OF) has been estimated to be 0.5–0.7 (Deng et al., 2002; Michaelsson et al., 2005). Genetic interactions with both ethnicity and sex may be an important source of heterogeneity (Johnson et al., 2009). To date, genome-wide association scans have focused on bone mineral density (BMD) and osteoporosis in populations of primarily European or Asian descent. The largest relevant genome-wide association study (GWAS) to date is a meta-analysis conducted by the Genetic Factors of Osteoporosis (GEFOS) consortium, which included individuals of European and East Asian descent, and identified 56 BMD-associated loci (Estrada et al., 2012). Fourteen of these loci were also associated with fracture (any type) at a Bonferroni-corrected level of significance. Richards et al. identified an additional gene that increased risk of fracture independent of bone mineral density in a European sample (Richards et al., 2008). Two other GWAS (Xiong et al., 2009; Guo et al., 2010a) and two genome-wide copy number variation studies (Oei et al., 2014; Yang et al., 2008) in have identified a few other putative fracture loci in Chinese and European samples. In one published GWAS that used radiographic vertebral fractures as the primary outcome of interest, one genome-wide significant locus was identified in the discovery sample, but did not replicate (Oei et al., 2013).

Although some progress has been made, genes identified to this point only explain a small portion of the total heritability of osteoporosis-related traits, and it remains to be determined how these genes and others affect risk of fracture in populations of African ancestry. Moreover, it has been argued that more genome-wide association studies should focus on fracture (the most severe osteoporosis-related-

phenotype) rather than BMD or other intermediate endpoints (Liu et al., 2014).

Here, we meta-analyze GWAS data from 5 studies examining clinical fracture in African-American women in an effort to identify potentially novel genome-wide significant loci. Because there is not a replication sample within this study, the results are viewed as hypothesis-generating. We also examine the generalizability of previously discovered osteoporosis-related loci to this population of African-American women.

2. Methods

2.1. Subjects

Subjects for this study were African-American women from five studies with information on clinical fracture: the Women's Health Initiative (WHI) (N = 8155) (Design of the Women's Health Initiative clinical trial and observational study, 1998); the Cardiovascular Health Study (CHS) (N = 504) (Fried et al., 1991); BioVU (N = 704) (Roden et al., 2008); the Health ABC study (N = 651) (Newman et al., 2003); and the Johnston County Osteoarthritis Project (JoCoOA, N = 291) (Jordan et al., 2007). Women included in this study were over 50 years of age (for WHI, CHS, BioVU, and Health ABC), or over 45 (for JoCoOA). Women from the WHI were part of the WHI-SHARe (SNP Health Association Resource) project, which included randomly selected African-American and Hispanic-American women who consented to genetic research from both the observational study and the clinical trial arms of the WHI. See Supplementary materials (Cohort Descriptions) for additional descriptive information regarding each study. All studies were cohort studies except BioVU, which defined cases and controls for fracture by linking a DNA repository to electronic medical records.

2.2. Fracture phenotype

All studies measured fractures, excluding fingers, toes, face, skull, or sternum. Radiographic vertebral fractures were not included. Fractures were incident after age 45 (for JoCoOA) or age 50 (WHI, CHS, BioVU, and Health ABC). Fractures included in this analysis were adjudicated in WHI, BioVU, and Health ABC, and self-reported in CHS and JoCoOA. For WHI, hip fractures were centrally adjudicated. All other fractures were centrally adjudicated for women in the clinical trial and for women in the observational study enrolled at three BMD sites (Pittsburgh, Birmingham, Tucson/Phoenix). For Health ABC, Fractures were adjudicated at the clinical centers based on radiograph confirmation. For BioVU, fracture phenotype was determined from medical records in the BioVU database. For the other two studies (JoCoOA and CHS), self-reported fractures were used. However, good reliability has been found between self-reported fracture and adjudicated fracture (Chen et al., 2004). More information about the ascertainment of fracture in

each of the studies can be found in the Supplementary materials (Cohort Descriptions).

2.3. Genotyping and quality control

Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 (WHI), the Illumina 1M-Duo platform (Health ABC, JoCoOA, BioVU), or the Illumina Human Omni1 Quad_v1 (CHS). Hardy-Weinberg P-value inclusion criteria were set at $>10^{-6}$ (WHI, Health ABC, and BioVU), 10^{-5} (CHS), or 10^{-4} (JoCoOA). Call rates required for inclusion ranged from $>90\%$ (WHI) to $>98\%$ (BioVU, JoCoOA). See Supplementary Table 1 for further quality control and genotyping information for each cohort. A sensitivity analysis was conducted excluding SNPs with minor allele frequency (MAF) <0.15 from BioVU, because an association was detected between MAF and P-values in the BioVU GWAS.

2.4. Imputation

A 1:1 combination of the YRI and CEU HapMap (version 2) samples provided the reference panel for imputation (see (Marchini and Howie, 2010) for a review of the advantages of using mixed reference panels for imputation). Imputation was done using MACH (WHI, JoCoOA, Health ABC), BEAGLE v3.2.1 (CHS), or IMPUTE v2.1.2 (BioVU).

2.5. Association analyses

The tests for single-SNP associations across the genome was accomplished through the application of survival analysis methods to the observed data where possible (WHI, Health ABC), or logistic regression (BioVU, CHS, JoCoOA). Survival analysis methods used time to first fracture (any fracture excluding fingers, toes, face, skull, or sternum) as the survival time. Fracture was treated as dichotomous (yes/no) for logistic regression analyses. The genotypes for each SNP were coded as the number of copies of the minor allele that each individual carries (additive genetic model), which has been shown to have reasonable power under all genetic modes of inheritance (Ziegler et al., 2008).

This ordinal genotype variable was entered into a logistic regression or survival analysis model, and a P-value representing the significance of the association between the ordinal genotype variable and the outcome was obtained. The trend test (for increasing minor allele count: 0, 1, or 2) provides a t-distributed statistic that does not rely on the Hardy-Weinberg equilibrium (HWE) of the SNP (contrary to a simple *t*-test based on allele counts), and assumes an additive genetic model for associated alleles. The assessments of association between the imputed SNPs and fracture were performed in R, using ProbABEL or survival packages.

We used a minimum model including adjustment for ancestry (*frappe* ancestry estimates for WHI (Tang et al., 2005) or top 10 principal components for the other studies), age and geographic region (clinical center), where applicable. In a secondary analysis in WHI, we also ran a model additionally adjusting for body mass index (BMI), since obesity is protective for fracture risk. In WHI, we also ran a fully adjusted model which included age, geographic region, ancestry and a covariate for the number of risk factors for fracture. The risk factors were identified in a paper by Cauley et al. (Cauley et al., 2007) and for African-Americans include: height > 161.9 cm; higher than high school education; broken bone at/after age 55 years (but before study enrollment); diabetes; myocardial infarction; have any arthritis; use corticosteroids >2 years; depressed (CES-D or medication use); use sedatives/anxiolytic; parental history of fracture; and greater than two falls during the last 12 months of follow-up or year before the fractures.

2.6. Meta-analysis

METAL software was used for fixed-effects inverse variance-weighted meta-analysis (Willer et al., 2010). We applied genomic control on the study-level results and at the meta-analysis stage (Devlin and Roeder, 1999). We applied additional filters of MAF > 0.02 and minor allele count (MAC) > 10. Regional plots were created using LocusZoom software (Pruim et al., 2010). Presence of heterogeneity across studies was evaluated using the I^2 statistic (Thorlund et al., 2012).

2.7. Multiple comparisons

The standard genome-wide significance level is a P-value $< 5 \times 10^{-8}$. Suggestive significance was reported if the P-value was below the threshold of $<1 \times 10^{-5}$.

2.8. Functional analysis

We performed an association analysis of genetically predicted gene expression (GPGE) with fracture risk using the method MetaXcan (http://dx.doi.org/10.1101/045260), with reference data from the GTEx (Genotype-Tissue Expression) Project (Genotype-Tissue Expression Consortium, 2013). GTEx is a large-scale effort where DNA and RNA from multiple tissues were sequenced from almost 1000 deceased individuals of European, African, and Asian ancestries. MetaXcan is a gene-level approach that uses regression weights for the effects of SNPs on gene expression derived from GTEx Project data and association summary statistics from GWAS to test the association between GPGE and GWAS phenotypes. MetaXcan is a meta-analysis extension of PrediXcan (Gamazon et al., 2015), which performs the same gene-based tests in individual-level data. Among the benefits of this approach are that it a) directly tests a biological mechanism, b) provides gene level results, c) provides estimates of effect (which can suggest treatments since GPGE that is positively correlated with risk may reduce risk when knocked down), d) multiple testing for gene-based tests are reduced, e) provides tissue-specific results, and f) can study GPGE-disease relationships in consortia with large sample sizes where individual-level data are not available to the coordinating group.

We scanned over 40 tissues from the GTEx Project using the entire set of summary statistics from the GWAS. Sample sizes for the GTEx reference data are presented in Supplementary Table 2, and only tissues with at least 100 observations were used here. This software and all prediction models necessary to apply to new datasets are publicly available on https://github.com/hakyimlab/PrediXcan.

2.9. Generalization analysis

To test for generalization of previously reported SNP associations with bone density-related traits, we applied the framework of Heller et al. (Heller et al., 2015). The generalization null hypothesis states that a SNP is not associated with the outcome in the discovery study, the generalization study, or both, and is rejected if there is enough evidence that the SNP is associated with the traits in both studies. Under directional control, associations that have consistent directions of effects in both studies are also of interest. For each reported analysis (each published manuscript and trait), a false discovery rate (FDR)-controlling multiple testing adjustment procedure was applied to control multiple testing of the generalization null hypothesis at the 0.05 level. When effect alleles and directions of associations were available, we calculated directional FDR *r*-values to decide whether an association generalized. The *r*-value is similar in interpretation to an adjusted P-value, and represents the probability that generalizations with *r*-values smaller than the given *r*-value are false. When the trait reported in the "discovery study" (the study initially reporting the association) was bone mineral density (BMD), an association was considered as having a consistent direction of association if it had different signs in the discovery study and

in our study. Otherwise, we required the same effect direction. For two analyses effect alleles were not available (results reported by Zhang et al. from an investigation of SNP-trait associations known to them at the time (Zhang et al., 2014a)), and a single association reported by Kou et al. (Kou et al., 2011), and hence we calculated non-directional FDR r-values.

3. Results

3.1. Study sample

The sample size for each cohort ranged from 291 (in JoCoOA) to 8155 (in WHI), and the number of fractures ranged from 35 (JoCoOA) to 313 (WHI) (Table 1). In all, there were 10,305 women included in the meta-analysis and a total of 540 fractures. The cumulative incidence of adjudicated fractures during follow-up varied from 0.038 (WHI) to 0.077 (BioVU) to a maximum of 0.135 (Health ABC); the cumulative incidence of self-reported fractures was 0.10 in CHS and 0.12 in JoCoOA. The incidence rate for adjudicated fractures in WHI was 4.0 fractures/1000 person-years; in Health ABC the rate was 12.4 per 1000 person-years.

3.2. Results of meta-analysis

The meta-analysis revealed one genome-wide significant SNP (Table 1; Fig. 1). There was no evidence of residual confounding by population stratification according to the quantile-quantile plot of observed vs. expected P-values ($\lambda = 0.983$, Fig. 2). The genome-wide-significant SNP, rs12775980 (minor allele frequency = 0.03, $P = 4.0 \times 10^{-8}$), is located in an intron of the gene supervillin (SVIL) on chromosome 10. It was genotyped on the Affymetrix 6.0 chip but not Illumina 1M or the OmniQuad. Therefore it was genotyped in the largest cohort (WHI) but imputed for the other cohorts (imputation quality scores: 0.98 in BioVU; 0.97 in Health ABC; 0.96 in JoCoOA; and 0.77 in CHS). In HapMap the minor allele frequency for this SNP is 0.066 in CEU (CEPH-Utah), 0.022 in YRI (Yoruban), and 0.052 in ASW (African ancestry individuals from Southwest USA). After conducting a sensitivity analysis excluding BioVU SNPs with MAF < 0.15 (to reduce the chance of type I errors; see Section 2.3), the P-value for this SNP was still GWA-significant ($P = 4.6 \times 10^{-8}$). The regional plot shows that rs12775980 is in linkage disequilibrium (LD) with several other nearby SNPs analyzed in the meta-analysis (Fig. 3). The other notable SNPs in this regional plot (based on small P-values) include rs7893141 ($P = 1.36 \times 10^{-7}$), rs17768169 ($P = 1.58 \times 10^{-7}$) and rs12767247 ($P = 1.36 \times 10^{-5}$); rs12771815 ($P = 3.53 \times 10^{-4}$) and rs11819549 ($P = 7.81 \times 10^{-4}$), all of which are intronic.

We examined whether this SNP (rs12775980) was significantly associated with either femoral spine BMD or lumbar spine BMD among women in the GEFOS study, using their publicly available GWAS results (http://www.gefos.org/?q=content/data-release) (Estrada et al., 2012). The SNP was not significantly associated with either phenotype in this meta-analysis of cohort studies (with individuals primarily of European and east Asian ancestry).

There were also several loci with suggestive significance ($P < 1.0 \times 10^{-5}$) (Table 1; Fig. 1). Regional plots for these are provided in the Supplementary materials (Supplementary Figs. 1–8). For three of these SNPs, there was evidence of moderate (rs1228431, rs7547923) or substantial (rs2275731) heterogeneity of effects among the 5 studies, according to the I^2 statistic.

3.3. Additional secondary analyses in WHI

In WHI, we were also able to run a fully adjusted model which included a term for the number of fracture risk factors (see list in Section 2.5). A disadvantage of this approach is that it assigns equal weight to each of the risk factors. However, the WHI results using the minimally adjusted model and the fully adjusted model were not meaningfully different for the top results, and the results from the minimally adjusted model are presented in the meta-analysis to maintain consistency with the other cohorts.

After running the meta-analysis, we observed that among the top results with suggestive significance, three of the loci are related to BMI. PTER has been reported as a genomewide-significant finding for morbid obesity (Meyre et al., 2009); MOGAT2 is a gene involved in dietary fat absorption and was associated with HDL cholesterol levels in a GWAS (Willer et al., 2013); and LUZP2 was associated with the ratio of visceral adipose tissue to subcutaneous adipose tissue (rs16912285) (Fox et al., 2012). For PTER and MOGAT2, the SNPs identified in our study were not in LD with the SNPs discovered in previous GWAS. But for LUZP2, both analyses may be tagging the same signal (R^2 between rs7113850 and rs16912285 = 0.42 in CEU). The risk allele for increased adiposity in the prior study (rs16912285) was the minor allele (MAF = 0.10), and in the present study, the allele associated with decreased risk of fracture for rs7113850 was also the minor allele (MAF = 0.22).

In WHI, we examined whether adjusting for BMI might attenuate the results for these three loci. Adjusting for BMI had very little effect on the results (P-values and effect estimates) for all three loci; therefore, we did not pursue this analysis with the remaining cohorts, since WHI represents nearly 80% of the sample.

3.4. MetaXcan analysis

For this analysis, which examined the association between genetically predicted gene expression (GPGE) in specific tissues with fracture risk, the most highly associated genes and tissues ($P < 1.0 \times 10^{-5}$) are presented in Supplementary Table 4 ($P < 1.0 \times 10^{-5}$). We also specifically examined the results for any associations between GPGE and fracture risk for the 9 loci shown in Table 2. Of these, genetically predicted expressions of RAB31, SLC40A1, PTER, DOK6, and COL7A1 were nominally associated ($P < 0.05$) with fracture risk in at least one tissue type. Notably, genetically predicted expression of PTER in skeletal muscle was inversely associated with fracture risk (Z-score = −2.02; $P = 0.043$), and genetically predicted expression of COL7A1 in the tibial nerve was positively associated with fracture risk (Z-score = 2.30; $P = 0.022$). For the other genes, nominally significant associations were identified in brain tissue (RAB31 and DOK6) and ovary (SLC40A1).

Table 1
Sample size and number of clinical fractures for the five studies included in a meta-analysis of clinical fractures among 10,305 unrelated African-American women.

Study	N	Number of fractures	Mean age (SD[a])	Mean follow-up time[b] (years) (SD)
BioVU	704	54	65.1 (10.1)	n/a
Cardiovascular Health Study	504	50	73.1 (5.7)	n/a
Health ABC	651	88	73.4 (2.9)	8.5 (3.3)
Johnston County Osteoarthritis Project	291	35	63.8 (9.6)	n/a
Women's Health Initiative	8155	313	61.6 (7.0)	10.8 (3.0)
Total	10,305	540		

[a] SD = standard deviation.
[b] Mean follow-up time is provided for studies that conducted survival analysis of clinical fractures.
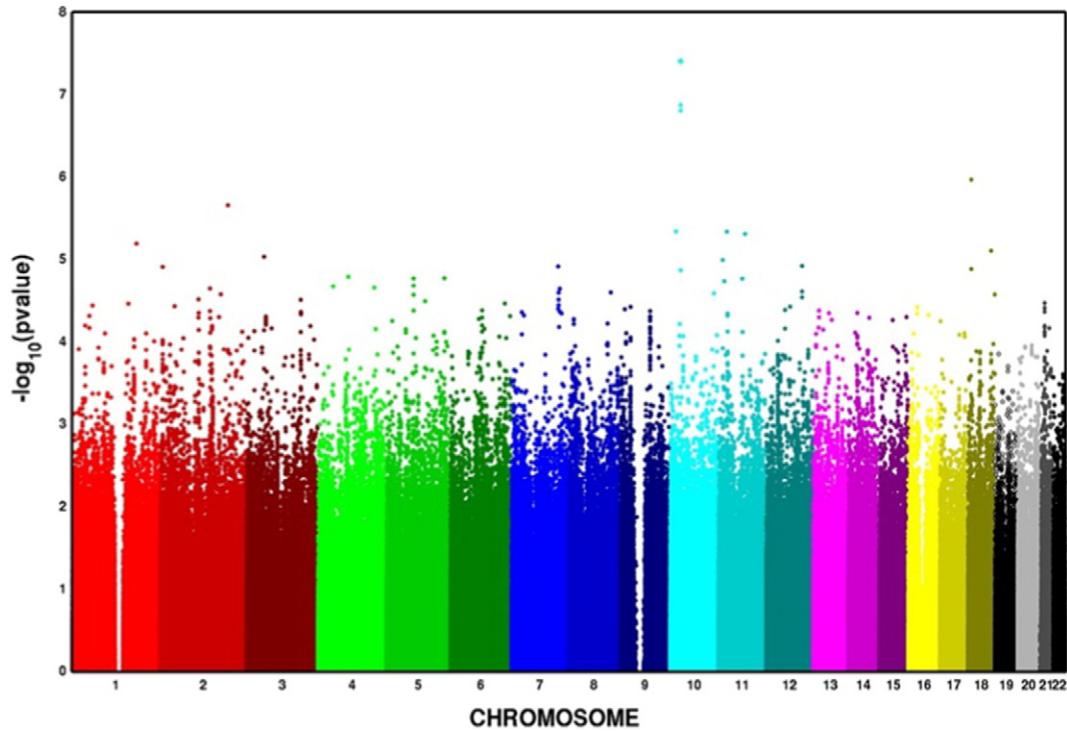
Fig. 1. Manhattan plot of a GWAS meta-analysis of clinical fracture in up to 10,302 African-American women.

3.5. Generalization of loci previously reported in osteoporosis-related GWAS

We examined the generalization of SNPs for loci reported in previous GWA studies or meta-analyses for bone mineral density or other osteoporosis-related traits in populations of primarily European or Asian descent (Estrada et al., 2012; Richards et al., 2008; Xiong et al., 2009; Zhang et al., 2014a; Kou et al., 2011; Styrkarsdottir et al., 2008; Rivadeneira et al., 2009; Paternoster et al., 2010; Duncan et al., 2011; Kiel et al., 2007; Zheng et al., 2013; Medina-Gomez et al., 2012; Guo et al., 2010b; Koller et al., 2010; Hsu et al., 2010; Hwang et al., 2013). Three of the studies included some individuals (<1000) of African descent, either in their discovery or replication sample (Xiong et al., 2009; Zhang et al., 2014a; Koller et al., 2010). There were 62 independent regions (at least 1 MB apart) identified in the collective list of all GWA-significant loci reported for osteoporosis-related traits in the GWAS catalog (as of 7/16/2015). Out of 140 associations in 62 loci, one association generalized: rs227425 in *SMOC1* (r-value = 0.02, AA P-value = 0.01), which was initially reported as a BMD locus by Zhang et al. (Zhang et al., 2014b). A few other associations were borderline significant: rs7117858 (r-value = 0.054, AA P-value = 0.006) and rs7108738 (r-value = 0.059, AA P-value = 0.004), both near *LUZP2*; and rs12272917 (r-value = 0.092, AA P-value = 0.01) in *PPP6R3*. While it is surprising that rs227425 (*SMOC1*) generalized while rs7117858 and rs7108738 did not (the latter two had lower P-values than rs227426 in the AA cohort), this is because the number of generalization tests was higher in the generalization analyses that included rs7117858 and rs7108738. The full results for all previously published SNPs across the 62 loci are given in the Supplementary materials (Supplementary Table 3).
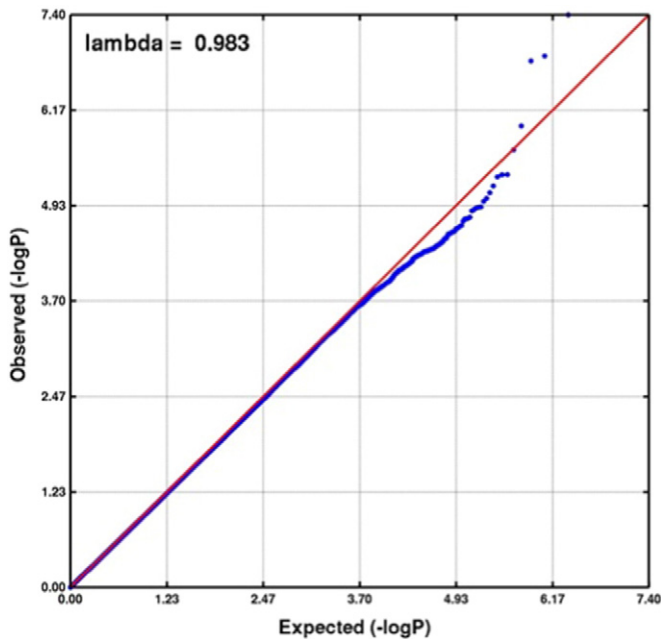
4. Discussion

4.1. Summary of findings

In this GWAS meta-analysis of fracture in African American women, we identified one potentially novel locus, *SVIL*, and eight other suggestive loci. We also investigated generalization for 62 genome-wide significant loci previously identified for bone mineral density or other osteoporosis-related phenotypes in populations of primarily European or Asian descent. One locus (*SMOC1*) generalized, and two others (*LUZP2* and *PPP6R3*) were suggestive.
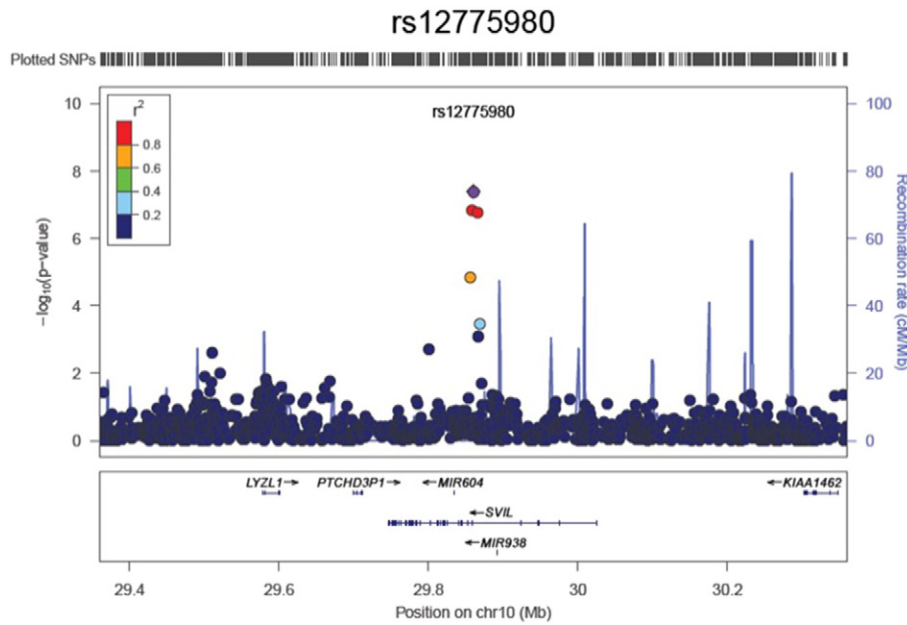

Fig. 2. Quantile-quantile plot comparing the distribution of observed vs. expected – log P-values for a GWAS meta-analysis of clinical fracture in up to 10,302 African-American women.

rs12775980

Fig. 3. Regional plot (LocusZoom) for the *SVIL* locus. The diamond (shown in purple) is the most statistically significant SNP in the region, according to P-value. All other SNPs are shown as circles. The colors represent the degree of linkage disequilibrium with the index SNP (see R^2 values on the left of the figure.) For additional documentation, please see http://locuszoom.sph. umich.edu/locuszoom/.

4.2. Significance of potentially novel findings

The *SVIL* locus is a promising candidate gene for fracture and other osteoporosis-related phenotypes based on its biological function. The tissue expression pattern of *SVIL* revealed that it is most highly expressed in skeletal muscle (the Genotype-Tissue Expression Project; gtexportal.org) (Supplementary Fig. 9) (Genotype-Tissue Expression Consortium, 2013). The supervillin protein is a platelet-associated factor that plays a role in thrombus formation, and human *SVIL* variants have already been shown to be associated at a genomewide-significant level with platelet thrombus formation in African Americans, but not European Americans (Edelstein et al., 2012). This could potentially explain why this association was not detected in previous GWAS, which mostly had European-American or European participants. The same study also showed that several *SVIL* SNPs are significantly associated with *SVIL* mRNA expression, and that *SVIL* mRNA expression is inversely associated with platelet thrombus formation, suggesting an inhibitory effect. Higher *SVIL* mRNA levels would thus be expected to result in lower platelet function. As injection of platelets at a fracture site has

been reported to aid in fracture healing (Sampson et al., 2008), the *SVIL* SNP might indicate a new pathway involved in fracture. Since the minor allele of *SVIL* variant rs12775980 was associated with increased risk of fracture (OR = 2.12), it could be reasoned that the variant may be associated with increased *SVIL* mRNA levels, resulting in decreased platelet thrombus formation and higher vulnerability to fracture. Although this association is therefore biologically plausible, replication in larger studies is especially warranted considering the low minor allele frequency of the SNP (0.03 in AAs). This SNP may contribute to the lower fracture rate and higher BMD observed in AA, since the fracture-increasing allele is less frequent in individuals of African descent (MAF = 0.022 in YRI vs. 0.066 in CEU).

Some research suggests that obesity may be a protective factor for fracture; likewise, adiposity was recently shown to be causally related to increased bone mineral density in a Mendelian randomization study of BMI loci and BMD in children (Kemp et al., 2016). Three of our top loci (*PTER, MOGAT2,* and *LUZP2*) have been previously associated with obesity or obesity-related traits, though only for *LUZP2* does the signal appear to be the same for both traits (in this case, visceral adipose

Table 2
Top loci ($P < 1.0 \times 10^{-5}$) associated with clinical fracture in African-American women.

Top SNP in region[a]	Chr	Position[b]	Effect allele	EAF[c]	P	Effect ratio[d] (95% CI[e])	Directions of effect[f]	N	$I^{2(g)}$	Nearest gene
rs12775980	10	29,899,862	A	0.03	4.0×10^{-8}	2.12 (1.61, 2.79)	++++−	10,302	0	*SVIL (intron)*
rs11872467	18	9,838,002	A	0.11	1.1×10^{-6}	2.29 (1.64, 3.20)	?++++	2147	0	*RAB31 (intron)*
rs10931422	2	190,084,680	T	0.07	2.5×10^{-6}	1.62 (1.33, 1.97)	++++−	10,302	0	*SLC40A1(nearby)*
rs2275731	10	16,566,846	A	0.37	3.5×10^{-6}	1.36 (1.21, 1.53)	+−+++	10,302	67.9	*PTER (intron)*
rs7113850	11	24,151,165	G	0.78	4.7×10^{-6}	1.54 (1.29, 1.83)	+++++	10,302	0	*LUZP2 (nearby)*
rs12284314	11	75,116,572	C	0.21	5.0×10^{-6}	1.39 (1.21, 1.60)	++−+−	10,302	43.7	*MOGAT2 (intron)*
rs7547923	1	180,976,245	A	0.03	6.5×10^{-6}	3.22 (1.94, 5.39)	?++++	2147	35.8	*NPL (nearby)*
rs12606715	18	65,062,093	C	0.42	8.0×10^{-6}	1.32 (1.18, 1.49)	+++++	10,302	0	*DOK6 (nearby)*
rs6792156	3	48,629,942	C	0.46	9.3×10^{-6}	1.34 (1.17, 1.53)	+++++	10,302	0	*COL7A1 (nearby)*

[a] SNP with lowest P-value within a 1 megabase region. See corresponding LocusZoom plots.
[b] HapMap phase 2, release 22, build 36.
[c] EAF = effect allele frequency.
[d] Effect ratio refers to the meta-analyzed hazard ratios (from WHI and Health ABC) and odds ratios (from BioVu, CHS and JoCoOa).
[e] CI = confidence interval.
[f] WHI, HealthABC, CHS, BioVU, JoCoOA.
[g] I^2 = heterogeneity statistic.

tissue and fracture). In WHI, the associations of these loci with fracture risk were virtually unchanged after adjusting for BMI, suggesting an independent effect on fracture. For example, these genes may affect bone morphology, density or quality.

4.3. Generalization of loci identified in prior studies

Overall, only a small fraction of previously reported SNP associations with bone-density related traits generalized in this population of AA women. *SMOC1*, the only locus which generalized at a statistically significant level, is a bone morphogenetic protein (BMP) antagonist (Thomas et al., 2009) and plays an important role in limb development (Okada et al., 2011; Rainger et al., 2011) and osteoblast differentiation (Choi et al., 2010).

Lack of generalization could be due to several reasons. First, different traits were used in the previously reported analyses ("discovery studies") and our analysis. Other studies have demonstrated that a minority of GWA-significant BMD loci were also associated with fracture (Estrada et al., 2012; Guo et al., 2012). In the large GEFOS study, fourteen of the 56 BMD loci (25%) were also associated with fracture (any type) at a Bonferroni-corrected level of significance in their meta-analysis; and this was within the same dataset (Estrada et al., 2012). In a study of 700 Chinese Han subjects, 350 hip fracture cases and 350 controls, 4 of 23 previously identified BMD-related loci were associated with fracture, though only one remained significant after correction for multiple testing (Guo et al., 2012). In our case, we attempted to replicate BMD-associated loci for risk of fracture in individuals of African ancestry; given the results discussed above, it is unclear how much replication should be expected. Second, there are different linkage disequilibrium (LD) patterns between the populations in the discovery studies and this study of AA women, and therefore the best tag SNP for the causal SNP is different between populations. Third, it is possible that some associations are false positive in the discovery study; or that the true effect size is smaller than reported in the discovery study due to the winner's curse, and our study did not have enough power to detect the true effect size. Finally, lack of power could be also due to lower MAF in the AAs compared to the discovery study population, and low power may also result from genetic heterogeneity within the AA population.

4.4. Challenges and limitations

There are several sources of heterogeneity both within and across the 5 studies used in this analysis which may have limited our power to discover novel associations with fracture. In addition to the inherent genetic heterogeneity of African-American populations, especially considering that European admixture among AA ranges widely and the different geographic regions represented among the studies, there were different methods of ascertaining fracture (adjudicated vs. self-report) and different modeling strategies (survival analysis vs. logistic regression) resulting from differences in how the data were collected. Effects of two loci (*MOGAT2* and *PTER*) were meaningfully heterogeneous across the 5 studies according to the I^2 statistic and inconsistent directions of effect (Table 2). Heterogeneity may indicate different patterns of LD between typed markers and causal variants, deviation of HWE, and gene-environment interactions that vary among studies (Ioannidis et al., 2007; Salanti et al., 2005), in addition to the reasons given above.

Low minor allele frequencies for some of the top SNPs may have led to spurious results, particularly in the smaller cohorts. However, rare variants and "goldilocks" (MAF between 1% and 5%) variants may be important contributors to the total genetic risk for fracture. A recent whole-genome sequencing study of BMD identified novel noncoding conserved variants with low MAF but with large effect size (Zheng et al., 2015), similar to the *SVIL* locus. In addition, a genome-wide study of copy number variants identified a rare deletion associated with

fracture in a European sample, again highlighting the potential importance of rare variants to fracture risk (Oei et al., 2014).

The causes of fractures in this meta-analysis were not identified. It may be rationalized that genetic susceptibility to fracture may play a more important role in some fractures (e.g., minor falls) than others caused by more severe trauma (e.g., fractures caused by car accidents). However, low BMD has been associated with risk of trauma-related fractures, demonstrating that genetic susceptibility may also play an important role in these fractures (Mackey et al., 2007). Finally, vertebral fractures were not included in this analysis because they were not always available; this may have influenced the results.

4.5. Conclusion

In the only genome-wide association scan of clinical fracture among exclusively African Americans to date, we have also identified one potentially novel locus and several suggestive loci that may contribute to the lower fracture rate among AA. Because this was a hypothesis-generating study, replication is needed in other populations of African descent to confirm these findings.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bonr.2016.08.005.

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Conflicts of interest

None.

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