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Genome-Wide Association Meta-Analyses to Identify Common Genetic Variants Associated with Hallux Valgus in Caucasian and African Americans

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version submitted for publication. Drs. Hsu, Liu, Hannan and Jordan had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Hsu, Liu, Hannan and Jordan were responsible for study conception and design.

Supplementary Figure Legends

Supplementary Figure 1. Quantile-Quantile plots (QQ-plots) for gender-specific GWAS results

Supplementary Figure 2. Regional plots on selected men-specific GWAS results

Supplementary Figure 3. Regional plots on selected women-specific GWAS results

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Abstract

Objective—Hallux valgus (HV) affects ~36% of Caucasian adults. Although considered highly heritable, the underlying genetic determinants are unclear. We conducted the first genome-wide association study (GWAS) aimed to identify genetic variants associated with HV.

Methods—HV was assessed in 3 Caucasian cohorts (n=2,263, n=915, and n=1,231 participants, respectively). In each cohort, a GWAS was conducted using 2.5M imputed single nucleotide polymorphisms (SNPs). Mixed-effect regression with the additive genetic model adjusted for age, sex, weight and within-family correlations was used for both sex-specific and combined analyses. To combine GWAS results across cohorts, fixed-effect inverse-variance meta-analyses were used. Following meta-analyses, top-associated findings were also examined in an African American cohort (n=327).

Results—The proportion of HV variance explained by genome-wide genotyped SNPs was 50% in men and 48% in women. A higher proportion of genetic determinants of HV was sex-specific. The most significantly associated SNP in men was rs9675316 located on chr17q23-a24 near the *AXIN2* gene ($p=5.46\times10^{-7}$); the most significantly associated SNP in women was rs7996797 located on chr13q14.1-q14.2 near the *ESD* gene ($p=7.21\times10^{-7}$). Genome-wide significant SNP-by-sex interaction was found for SNP rs1563374 located on chr11p15.1 near the *MRGPRX3* gene (interaction p-value $=4.1\times10^{-9}$). The association signals diminished when combining men and women.

Conclusion—Findings suggest that the potential pathophysiological mechanisms of HV are complex and strongly underlined by sex-specific interactions. The identified genetic variants imply contribution of biological pathways observed in osteoarthritis as well as new pathways, influencing skeletal development and inflammation.

Keywords

hallux valgus; GWAS; genetic variants; candidate genes

INTRODUCTION

Foot disorders affect 20-60% of community-dwelling older adults[1-3] and are associated with disability[1] and falls[1, 4-6]. Hallux valgus (HV), a condition in which the great toe deviates laterally at the 1st metatarso-phalangeal joint, is a common foot disorder in adults that is more prevalent among older than younger individuals[7], women than men[8-10], and African Americans than Caucasians[3, 10]. Body weight and poor shoe wear have reported to be risk factors for HV[8, 11, 12], and genetic predisposition may also contribute to this condition. Pedigree studies have found that 63-90% of individuals with HV report a family history of the condition[13-15]. More recently, HV was found to be highly heritable in the Framingham Heart Study[16] (involving 1,370 European descent Caucasian participants from 429 families) and the Korean Healthy Twin Study (involving 1,265 East Asian participants and 206 twin pairs)[17]. Although these studies suggest that HV may be hereditary, no candidate gene association study or genome-wide association study (GWAS)

has been conducted to examine the genetic predisposition of HV. Identifying genetic factors associated with HV may further our understanding of the biological mechanisms underlying this condition and may help clinicians and researchers target high-risk individuals in need of early intervention approaches to prevent or slow functional decline[18].

The purpose of the present study was to conduct a GWAS meta-analysis of HV in older Caucasian adults from the Framingham Heart Study (FHS), the Johnston County Osteoarthritis Project (JoCo OA), and the Genetics of Generalized Osteoarthritis (GOGO) Study. Due to known differences in the prevalence of HV by sex and by race, sex-specific analyses of FHS, JoCo OA, and GOGO were conducted and the generalizability of the genetic findings was examined in the African American sub-sample of the JoCo OA.

MATERIALS and METHODS

Study Populations

This GWAS meta-analysis was conducted using data from three cohort studies and was approved by the Institutional Review Boards for Human Subjects Research at Boston University and the Hebrew Rehabilitation Center for the Framingham Foot Study and by the University of North Carolina and the Centers for Disease Control and Prevention for JoCo OA. Institutional Review Boards of Duke University Medical Center, University of North Carolina, Rush University, the University of Maryland, Case Western Reserve University, the University of Nottingham, and Sheffield University approved the GOGO study.

Framingham Heart Study (FHS)—The FHS comprised of members from the Framingham Study Original Cohort[19], the Framingham Offspring Cohort[20]. The Framingham Study Original Cohort was formed in 1948 from a two-thirds sample of the town of Framingham, MA in order to study risk factors for heart disease. The Framingham Offspring cohort, formed in 1972, consists of adult offspring who had a parent in the Original Cohort, and the spouses of the offspring. The cohorts have been followed every 2 to 4 years since cohort inception to study familial risk factors for cardiovascular, neurological, metabolic and musculoskeletal diseases. The Framingham Foot Study is an ancillary study of the FHS with the goal to examine the contribution of foot disorders to functional limitation and disability. Members of the FHS who participated at the Exam during 2002-2006 were examined for the Framingham Foot Study (n=2,263).

Johnston County OA Project (JoCo OA)—JoCo OA began in 1990 as a community-based prospective cohort of Caucasians and African-American men and women with and without osteoarthritis (OA)[21]. JoCo OA was designed to estimate the incidence and progression of knee and hip osteoarthritis (OA) among Caucasians and African Americans, and the study has expanded to study the hand, lumbar spine, ankles, and feet during successive follow-up visits (occurring approximately every 5 years). The sampling methods and study protocol have been previously reported[22]. At baseline, 3,187 Caucasian and African American adults aged 45 years or older (35% men; 32% African American; mean age 61 ±10 years) were recruited from 6 townships in Johnston County, North Carolina and completed a clinical examination visit during 1991-97. An additional 1,019 Johnston County residents 45+ years old were enrolled and attended a clinic visit during 2003-2004. The

genotyped sample consists of those participants from baseline who returned for the first follow-up from 1999-2003, and the enrichment cohort of new participants in 2003-04. A total of 915 Caucasians and 327 African Americans with both HV phenotype and GWAS genotypes were in the final analyses.

Genetics of Generalized Osteoarthritis (GOGO) Study—GOGO is an investigator-initiated collaboration involving seven clinical academic sites in the United States and United Kingdom, whose data collection was sponsored by GlaxoSmithKline[23]. Family ascertainment occurred between 1999 and 2002. A qualifying family required self-reported Caucasian ethnicity and at least two affected siblings with clinical hand OA with radiographic evidence involving at least 3 hand joints distributed bilaterally and at least one distal inter-phalangeal joint, with two of the three involved joints within a single joint group. Clinical examination data of the hands, knees, hips, and feet, including HV, were collected. Additional siblings and living parents from qualifying families, both affected and unaffected, were invited to participate. A total of 2,728 participants from 1,145 families met the pre-specified radiographic criteria for affected status. The mean age of all the samples was 66 years and women constituted 80.4% of the sample. Among them, 1,231 participants had information on both HV phenotype and GWAS genotypes for the analyses.

Assessment of Hallux Valgus

HV was assessed in the FHS by podiatrist-trained clinical examiners who conducted a visual and physical examination of a participant's feet during weight-bearing[24]. In JoCo OA and GOGO studies, participants stood on a laminated foot mat with an illustration of two lines drawn at a 15 degree angle. In all three cohorts, HV was considered present if the examiner determined that the lateral deviation of the hallux with respect to the first metatarsal was 15° or more[25]. HV was considered absent if this criterion was not met.

Other Covariates

Possible confounders included age, sex, and body mass index (BMI). Age at exam was recorded. Weight in pounds was measured using a standardized balance beam scale and recorded to the nearest ½ pound. Height (without shoes) was measured in inches using a calibrated stadiometer and recorded to the nearest ¼ inch (to ½ inch in JoCo OA). BMI was calculated as weight in kilograms divided by height in meters squared.

GWAS Genotyping and Quality Controls

Details of genotyping and quality controls in each study are described in **Supplementary Table 1**. In brief, for the FHS, genotyping was conducted by the FHS SHARe (SNP Health Association Resource) Project, for which 549,827 SNPs (Affymetrix 500K mapping array plus Affymetrix 50K supplemental array) were genotyped in over 9,274 FHS subjects from over 900 families[26]. SNPs were excluded due to low call rate (< 95%), significant deviation from Hardy-Weinberg equilibrium ($p < 10^{-6}$ by HWE test), or unknown genomic annotation [26]. A total of 2,593 subjects from the **JoCo OA** cohort were genotyped by Algynomics, Inc. using the Illumina 1M-Duo platform, which interrogates 1,199,187 SNPs. The SNPs chosen for this array were selected to tag 93% of common SNPs in the Caucasian European (CEU) population at $r^2 \geq 0.8$.[27] For the **GOGO Study**, a total of 610,000 SNPs

(Illumina HumanHap 610 Quad v1 array plus 60,000 custom tag SNPs selected from HapMap CEU panel) were genotyped. PEDCHECK[28] and RELPAIR[29] programs were used to check for errors in Mendelian inheritance and inconsistencies within pedigrees at all marker loci.

Population Substructure in each participating cohort

Principal component analysis (PCA) was used to estimate genetic ancestry separately in each of the FHS, JoCo OA and GoGo studies [30]. To account for potential population substructure in the SNP-HV association tests, we adjusted PCs that associated with traits at nominal p-values less than 0.05 (estimated separately in each study), along with other covariates in the regression models. In addition, in the JoCo OA, an identity-by-state matrix was estimated to verify the self-reported race (Caucasian and African Americans). PCA was used to estimate population sub-structure within Caucasian samples and within African American samples, separately.

Imputation for non-genotyped SNPs

For all genotyped individuals, the International HapMap II CEU reference panel was used to impute 2,543,887 SNPs by MACH [31]. To account for the uncertainty of imputation, the additive dosage of the allele from 0 to 2 was used to perform association tests. The ratio of the empirically observed dosage variance (from the imputed genotypes) to the expected (under binomial distribution) dosage variance (computed from the estimated minor allele frequency) was estimated for every SNP as a quality score for imputation. SNPs with the variance ratio < 0.3 were excluded from association analysis. For the African Americans in JoCo OA, IMPUTE v2 [32] with a mixed panel of international HapMap II CEU and YRI references were used to impute un-genotyped SNPs.

Statistical Analyses

Single-SNP GWAS Analysis in Each Study

Gender-specific GWAS analyses were performed for each study. A SNP in additive genetic effect model was the independent variable. The dependent variable was HV dichotomous status (1 as subjects having HV; and 0 as subjects without −HV). HV was analyzed as a dichotomous phenotype. Other covariates included age, sex, cohort-specific sub-studies/ study sites (For FHS and GOGO studies only), BMI and PCs (ancestral genetic background to adjust for population substructure). For **FHS** and **GOGO** studies, to account for the within family correlations of the HV phenotype, the generalized estimating equations (GEE) model with Kinship matrix implemented in R-GEEpack [33] was used.. For **JoCo OA** (Caucasian American only), a logistic regression was applied. To estimate how well the distribution of the null hypothesis was calibrated, for each study, we estimated the genomic inflation factor (λ_{GC}) based on the median chi-squared test of all study participants.[34] Quantile-Quantile (Q-Q) plots were also created to evaluate if there was any systematic bias, such as genotyping errors or population stratification. Gender-combined GWAS analysis was also performed to identify SNPs that were associated with both men and women.

Meta-Analyses—To combine GWAS results from three Caucasian samples, we performed the fixed-effect inverse-variance meta-analyses implemented in METAL package (http://www.sph.umich.edu/csg/abecasis/Metal/). All association results were expressed relative to the forward strand of the reference genome based on the international HapMap project II reference panel. For each study, we excluded ~20% of the imputed SNPs with either imputation quality score "ratio" < 0.3 or minor allele frequency (MAF) < 0.005 . We also applied genomic inflation factor (λ_{GC}) correction to the standard error of the beta coefficients of the SNP-HV associations in each study. After meta-analyses, we further excluded SNPs with association results from only one study, heterogeneity tests p-values less than 5×10^{-6} or heterogeneity $I^2 > 50$. We estimated the genomic inflation factor (λ_{GC}) and plotted Quantile-Quantile plots. Meta-analysis p-value < 5×10^{-8} was considered as the genome-wide significance threshold.

SNP-by-Sex Interactions—To examine the SNP-by-sex interactions for those top associated SNPs found from male or female meta-analysis only, we estimated the SNP-by-sex interaction effects by $\left(\hat{\beta}_{\text{Male}} - \hat{\beta}_{\text{Female}}\right)$ with its corresponding standard error $\sqrt{SE^2\left(\hat{\beta}_{Male}\right) + SE^2\left(\hat{\beta}_{Female}\right)}$, where $\hat{\beta}_{Male}$ and $\hat{\beta}_{Female}$ are sex-specific from meta-analyses. P-values are estimated by Cochran's Q test [35]. This Cochran's Q heterogeneity test in meta-analysis is equivalent to the traditional multivariate interactions model to test the SNP-by-sex interaction term in a general linear regression model.

The Generalizability of the Top Associated SNPs in JoCo OA African American samples

Due to small sample size, we did not perform GWAS on JoCo OA African American samples; instead, to reduce the multiple testing penalties, SNPs with meta-analysis p-values < 10^{-5} from the Caucasian populations were selected to test for associations with HV in the JoCo OA African American samples. In addition, to take into account the potential difference in the LD structure between Caucasians and African Americans, we also selected additional SNPs in high LD ($r^2 \geq 0.8$) with the targeted SNPs (SNPs selected from Caucasian samples) in JoCo OA African American samples and tested their associations with HV. A GEE-based logistic regression model with robust variance was applied. Other covariates included age, sex, BMI and PCs (ancestral genetic background to adjust for population substructure). False discovery rate (FDR),[36] q-value < 0.05 was used to estimate multiple testing corrected p-values.

RESULTS

Prevalence of Hallux Valgus (HV) in study samples

The prevalence of HV, stratified by sex in each study, is shown in **Table 1**. A total of 4,409 Caucasians (2,827 women and 1,582 men) from FHS, JoCo OA, and GOGO studies were included in the GWAS meta-analysis. A sub-sample of 327 African Americans from JoCo OA was analyzed. Key features of the study populations are summarized in **Table 1**. HV was less prevalent in FHS participants than in JoCo OA and GOGO Caucasian samples. The sex-specific prevalence of HV was quite different across the three studies. Among women, HV

was present in 41%, 65% and 44% of FHS, JoCo OA and GOGO, respectively, and among men, HV was present in 19%, 56% and 32%, respectively. The prevalence of HV in both FHS men and women was comparable to the pooled prevalence of older populations (> 65 years old) reported in a recent systematic review of 78 publications (36%)[7].

Sex-specific GWAS meta-analysis

We first estimated the proportion of HV variance that could be explained by the genotyped SNPs in the FHS. The proportion of HV variance explained by genome-wide genotyped SNPs was 50% in men and 48% in women (30% when men and women combined). We found 33% of those genetic determinants were sex-specific, suggesting that a higher proportion of genetic determinants of HV were sex-specific and affected only either men or women. Therefore, we focused primarily on sex-specific GWAS meta-analysis. Q-Q plots of gender-specific GWAS results are shown in **Supplementary Figure 1**. No SNP reached the genome-wide significance level at 5×10^{-8} in either the single cohort GWAS or in the meta-analysis. However, we did observe a set of potentially suggestive SNPs with p-values less than 5×10^{-6} in male-specific and female-specific analyses. The false discovery rate of these suggestive associations ranged from 0.08 to 0.15.

In men, the most significantly associated SNP was rs9675316 located on chr17q23-q24 near the *AXIN2* gene (p-value=5.46×10^{-7}). Regional plots are shown in **Supplementary Figure 2**. SNP rs9675316 was less common in the study populations with a MAF = 1%. Men carrying one copy of the risk allele (G) resulted in a 3-times increased risk of HV comparing to men without “G” alleles. We did not find an association between rs9675316 and risk of HV in women (p=0.1). A significant SNP-by-sex interaction (p=6.8×10^{-5}) of rs9675316 indicated a male-specific genetic effect of rs9675316. The remaining genome-wide suggestive association loci (meta-analysis p-values < 5×10^{-6}) are listed in **Table 2**. All of the suggestive loci showed male-specific effects by testing SNP-by-sex interactions.

Similarly in women, we did not find genome-wide significant (p < 5×10^{-8}) associations with HV. The most significantly associated SNP was rs7996797 (average MAF=39% in study samples) located on chr13q14.1-q14.2 near the *ESD* gene with p-value=7.21×10^{-7}. Regional plots are shown in **Supplementary Figure 3**. Women carrying one copy of the risk allele (A) had a 1.4-times increased risk of HV compared to women without “A” alleles. SNP rs7996797 was not associated with HV in men (p=0.7). A strong SNP-by-sex interaction (p=3.3×10^{-3}) of rs7996797 indicated a female-specific genetic effect of rs7996797. The remaining genome-wide suggestive association loci are listed in **Table 2**. None of the suggestive loci showed nominal association in men (p > 0.05).

Sex-by-SNP interactions

To further explore the potential sexual dimorphism in HV, we performed an autosomal genome-wide meta-analysis of sex-by-SNP interactions to identify genetic variants with different effects by sex. SNP-by-sex interaction with genome-wide significance was found for SNP rs1563374 located on chr11p15.1 near the *MRGPRX3* gene (interaction p-value =4.1×10^{-9}). SNP rs1563374 was less common in our study populations, ~2% in both men and women in the meta-analysis of three studies together. Although the effect size (beta-

coefficient of association between rs1563374 and the risk of HV) was quite similar, the effect direction of SNP rs1563374 was opposite between men and women (**Table 3**). Men carrying the minor allele A had a higher risk of HV (beta coefficient=1.73, meta-analysis p-value=2.6×10^{-5}), but women carrying the same minor allele A had a protective effect (beta coefficient=-1.24, meta-analysis p-value=2.3×10^{-5}). Due to the opposite direction of the effects, the sex-combined association between rs1563374 and HV was not significant (Men + Women p-value=0.31). The genome-wide suggestive interaction loci (p-values < 5×10^{-6}) are listed in **Table 3**. All of the SNPs in the listed loci had similar MAFs, but opposite effects between men and women. We also performed a sex-combined GWAS meta-analysis without sex-by-SNP interaction terms to identify common genetic determinants of HV in both men and women. We did not find genome-wide significant associations. Overall, the association signals diminished compared to the association signals estimated from sex-specific analyses (**Supplementary Table 2**).

African Americans

None of the suggestive loci was associated with HV in the small subset of JoCo African Americans (**Supplementary tables 3 to 6**).

DISCUSSION

HV is one of the most common foot disorders in older Caucasian and African Americans [3, 10] and has been confirmed to be heritable in our recent publication[16]. In this first ever GWAS meta-analysis of HV, we report a set of potentially suggestive SNPs with p-values less than 5×10^{-6} in male-specific and female-specific analyses. We identified SNPs with genome-wide significant interactions with sex. A strong sex difference in the prevalence of HV has been reported in epidemiological studies[7, 37]. One potential explanation for such sex-specific predisposition to HV risk is the possibility that the observed differences between men and women are driven by genetic effects determining skeletal structure (such as an increased intermetatarsal angle, longer first metatarsal and round first metatarsal head[38]). Based on our sex-specific heritability estimation, we also found 33% of those genetic determinants were sex-specific. Several recent studies suggest that sex-specific genetic architecture influences many human diseases and phenotypes, such as blood pressure, hypertension, schizophrenia, bone mineral density, osteoporotic fractures and reproductive relevant diseases[39]. Some of the underlying mechanisms might be attributed to differential gene regulation in males and females, particularly in sex hormonal responsive genes; the potential contribution of sex chromosomes; as well as the interaction between genetic determinants and sex-specific environmental risk factors. Several strong gene-by-sex interactions were observed. However, the identified genes are neither those obviously involved in sex hormones, nor well-studied regarding their differential regulation in men and women. We identified one genome-wide significantly associated sex-interacted SNP rs1563374 located in the 5’ flanking of the*MRGPRX3* gene. The *MRGPRX3* gene encodes a member of the mas-related/sensory neuron specific subfamily of G protein-coupled receptors. The encoded protein may be involved in sensory neuron regulation and in the modulation of pain and/or itch[40]. In men, minor alleles of rs1563374 were associated with a higher HV risk. A recent study[41] has shown increased methylation with age in the

MRGPRX3 gene in males only; and, in addition, that study also showed differential methylation in the *MRGPRX3* gene in male infants associated with peri-conceptional environment, but not in female infants, suggesting a potential sexual dimorphism of *MRGPRX3* gene regulation. The underlying function of these identified variants/genes will need to be further elucidated. Due to the limitation of the quality of the X chromosomal imputation, we did not perform gene-by-sex interactions in the sex chromosomes, which did not allow us to examine the contribution of sex chromosomes.

In men, the most significant finding was the SNP rs9675316 which is located near the gene *AXIN2*. The *AXIN2* gene encodes the Axin-related protein, which plays an important role in the regulation of the stability of beta-catenin in the Wnt signaling pathway[42]. *AXIN2* variants have been reported to be associated with risks of oral clefts[43]. Another top associated SNP, rs10224956, is located near gene *BBS9*. *BBS9* is involved in parathyroid hormone action in bone and is down-regulated by parathyroid hormone in osteoblasts. The SNP-by-sex interaction analysis on rs10224956 also confirmed its male-specific genetic effect. A recent GWAS identified a susceptibility locus for non-syndromic sagittal craniosynostosis within *BBS9*, which indicates that *BBS9* may play an important role in skeletal development[44]. The association between *BBS9* and HV is consistent with the concept that HV may result from a long-term complex process involving interaction between bones and soft tissues of the foot. In addition, the association between *FAT4* and HV identified in our study might be additional evidence to support its regulatory function in bone formation and the importance of bone formation in HV, since *FAT4* gene is critical regulators of osteoblast development in *Fat4* mouse mutant (unpublished data: Fat-Hippo regulation of osteoblast differentiation).

In women, the most significant finding was the SNP rs7996797 which is located in gene *ESD*. The *ESD* gene encodes a serine hydrolase that is involved in the recycling of sialic acids[45]. Interestingly, increased serum sialic acid level is associated with decreased antioxidant levels in people with primary OA [46]. The association between HV and the *ESD* gene suggests that HV, which is associated with OA of the first metatarsophalangeal joint [47, 48], might share similar mechanisms with other OA phenotypes. *ANXA1* (rs4744678), also known as Annexin I, belongs to a family of Ca^{2+} dependent phospholipid binding proteins and is required for biosynthesis of potent mediators of inflammation, prostaglandins and leukotrienes, *ANXA1* may have potential anti-inflammatory activity. Inflammation is well-known to be associated with OA [49], and this association with HV in women suggests that inflammation may be important in HV as well.

Similar to many GWAS analyses, with limited sex-specific sample size, lack of statistical power may be a potential explanation of no genome-wide significant findings ($p < 5\times10^{-8}$). To achieve power ≥ 70% for detecting associations for common SNPs (MAF ≥ 5%) under additive genetic effect model with effect size 1.1 to 2.0 odds ratios (ORs), we will need to have at least 8,240 samples in each sex. To our knowledge, we have gathered all of the available Caucasian studies with HV phenotypes and genome-wide genotyping in the world thus far. Our findings suggest that HV is heterogeneous, with multiple genetic risk factors involved in different biologic pathways and with potential interactions with sex. It is likely that there are multiple causal SNPs, each of which has a small effect; therefore, more

cohorts to be genotyped or phenotyped are needed to have adequate power to detect moderate SNP effects. Although, we estimated > 80% genomic coverage (Linkage disequilibrium, LD, r^2>0.8) of the HapMap Phase I+II common SNPs with MAF ≥ 0.05 for the Caucasian population.[50]; better/more dense genotyping coverage for both uncommon and common variants (such as imputation using the recent available 1000Genome Project Phase III reference panel with 2,050 whole genome sequences) may also improve our effort to discovery genetic variants associated with HV. It is reasonable to consider the hidden heritability in uncommon and rare variants as well. However, based on our sample size and effect size (ORs=1.1 to 2.0) we observed so far, we do not have statistical power (power < 30%) to detect association signals for uncommon variants (MAF < 5%).

The gender-specific loci that we discovered are considered robust due to the same effect direction and similar effect magnitude observed across three Caucasian studies. However, we failed to observe significant associations in the JoCo African Americans for these suggestive loci. It may be due to (1) difference in allele frequencies and LD among Caucasians and African Americans as shown in **Supplementary Tables 3** to **6**; (2) very small sample size (327 African Americans) with limited statistical power; (3) heterogeneity in non-genetic exposure (gene-by-environment interactions); and/or (4) polygenetic etiology.

Study heterogeneity across three participating cohorts may also explain our lack of genome-wide association findings. We found several strong associated SNPs in each cohort (**Supplementary Tables 3 to 6**), but only one SNP (rs9626245 in **supplementary Table 4**), located in an intron of the *PRR5-ARHGAP8* read-through mRNA, was replicated in another cohort (JoCo OA African American, p=0.009) and this did not pass our FDR q-value < 0.05 threshold. This SNP is adjacent to the *ARHGP8* gene for the Rho GTPase activating protein 8 that encodes a member of the RHOGAP family of proteins that participate in signaling pathways that regulate cell processes involved in cytoskeletal changes. The remaining associations in individual cohorts failed to replicate in the other two cohorts. Some of these associations from each cohort may be false-positives, suggesting the possibility of study heterogeneity across three participating cohorts. The heterogeneity can be due to difference in HV assessment, ancestral genetic background and environmental exposures. To identify the source of study heterogeneity, we compared ancestral genetic backgrounds across the three studies by PCA of genome-wide genotypes. We did not find any deviation from the ancestral genetic background of the CEU (in HapMap Project) samples. In addition, the allele frequencies were similar among three studies. Therefore, we ruled out major differences in ancestral genetic background. The prevalence of HV was quite different across the three cohorts, suggesting a potential phenotypic heterogeneity due to assessment of HV, non-genetic host effects (such as age, sex and BMI distribution) and/or environmental exposures. The assessment of HV in FHS was slightly different, but not substantially different, than that used in JoCo OA and GOGO. This potential phenotypic misclassification may also reduce the statistical power to identify SNP-phenotype associations. Due to lack of information, we cannot evaluate whether environmental exposure (such as shoe-wear or physical activities) to risk factors associated with HV were different across the three studies, and we did not have adequate statistical power to test all potential gene-by-environment interactions.

A further caveat is that HV was considered as a dichotomous variable with a cut-off of 15 degrees, with no data available on severity. Statistical power may be further improved by performing association analysis with ordinal[51, 52] or continuous[53] measurement of HV. However, in the current study, not all of the participating studies had such measurements.

In summary, this is the first GWAS meta-analysis to examine SNPs associated with HV. Although we did not identify genome-wide significant SNPs using the stringent p-value cutoff, our identified sex-specific genome-wide suggestive loci and genome-wide significant sex-by-SNP interactions implied that the potential pathophysiological mechanisms of HV may be related to skeletal development and inflammation as well as biological pathways previously observed for other OA phenotypes, suggesting the complexity of the etiology underlying HV. In addition, similar to findings in GWAS of OA phenotypes, the variation in results from sex-specific analysis suggested that the genetic mechanisms behind the development of HV might be quite different between men and women as well as the potential environment-by-sex interactions. Our study provides several important and promising findings that need further replication in additional independent samples of Caucasians and other race/ethnicities, as well as validation for their potential functional involvement in the pathophysiological and biological mechanisms of HV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Principal characteristics of participants by study cohort

	Framingham (N=2,263)		JoCo Caucasian (N=915)		JoCo African Americans (N=327)		GoGo Caucasian (N=1,231)	
	Hallux Valgus		Hallux Valgus		Hallux Valgus		Hallux Valgus	
	Yes	No	Yes	No	Yes	No	Yes	No
N	709 (31.3%)	1554	565 (61.7%)	350	225 (68.8%)	102	512 (41.6%)	719
Female	516 (40.7%)	751	368 (65.1%)	197	152 (69.1%)	68	437 (43.9%)	558
Men	193 (19.4%)	803	197 (56.3%)	153	73 (68.2%)	34	75 (31.8%)	161
Age (yrs)	70.1 (11.54)	66.6 (10.55)	63.2 (9.21)	60.5 (8.34)	61.2 (9.31)	58.8 (8.80)	60.5 (8.34)	67.3 (8.52)
BMI (kg/m^2)	27.5 (4.96)	28.8 (5.53)	29.9 (5.96)	29.8 (5.58)	32.3 (7.83)	33.5 (7.77)	29.8 (5.58)	28.2 (5.19)
Weight (lbs)	162.4 (36.86)	178.9 (39.94)	179.3 (44.06)	185.8 (44.13)	192.1 (50.76)	201.3 (51.01)	164.7 (35.79)	167.8 (38.08)

Number of participants in each study cohort was for persons with both HV phenotype and GWAS genotypes.

Age is age at time of foot examination.

Table 2

Sex-specific GWAS meta-analyses in Caucasians (2,827 women and 1,582 men)

SNP	CHR	Genes	Alleles	MAF	Beta	SE	P-values	Results from opposite sex			SNP-by-Sex
								Beta	SE	P-values	Interaction-P
Top Associated SNPs in Men ($p < 5\times10^{6}$)											
rs9854162	3	NLGN1*	C -> T	0.18	0.63	0.135	2.51E-06	0.05	0.081	5.10E-01	2.20E-04
rs4476613	4	FAT4	G -> C	0.24	0.48	0.105	4.63E-06	0.13	0.072	6.42E-02	6.38E-03
rs10224956	7	BBS9*	G -> A	0.16	0.53	0.118	4.96E-06	−0.08	0.077	2.84E-01	1.29E-05
rs9675316	17	AXIN2	T -> G	0.01	1.14	0.227	5.46E-07	−0.50	0.342	1.45E-01	6.80E-05
Top Associated SNPs in Women ($p < 5\times10^{-6}$)											
rs2242411	5	MRPL36	C -> T	0.46	−0.29	0.061	2.24E-06	−0.15	0.095	1.26E-01	2.07E-01
rs12214759	6	C6orf70	G -> A	0.33	0.33	0.071	2.40E-06	−0.19	0.114	9.99E-02	1.01E-04
rs4744678	9	ANXA1	G -> A	0.49	−0.29	0.062	4.35E-06	−0.11	0.094	2.42E-01	1.19E-01
rs7996797	13	ESD	G -> A	0.39	0.31	0.062	7.21E-07	−0.03	0.095	7.87E-01	3.34E-03
rs17783396	14	C14orf179	G -> A	0.05	−1.05	0.220	2.10E-06	0.52	0.331	1.13E-01	7.97E-05
rs9889124	16	FAM92B	C -> T	0.13	−0.46	0.097	1.72E-06	−0.27	0.153	8.05E-02	2.79E-01

In each locus, only the most significantly associated SNP is listed.

*SNPs are located inside genes

Table 3

Sex-by-SNP Interaction GWAS Meta-analysis in Caucasians (2,827 women and 1,582 men)

SNP	Chr	Gene	Alleles	SNP-by-Sex	Men			Women		
				Interaction-P	MAF	Beta	P-values	MAF	Beta	P-values
rs6953348	7	BBS9*	G -> A	1.05E-06	0.22	0.52	1.23E-05	0.18	−0.10	1.74E-01
rs10283141	8	RUNX1T1; SLC26A7	G -> A	5.86E-07	0.04	0.86	4.43E-05	0.04	−0.49	3.89E-03
rs1519847	8	MLZE	C -> T	4.34E-06	0.37	0.29	2.67E-03	0.39	−0.23	1.32E-04
rs1563374	**11**	**MRGPRX3**	**C -> A**	**4.07E-09**	**0.02**	**1.73**	**2.61E-05**	**0.02**	**−1.24**	**2.30E-05**
rs4550343	13	SLITRK6	G -> A	8.86E-06	0.29	0.39	1.16E-04	0.32	−0.15	2.62E-02
rs6135498	20	MACROD2*	T -> C	2.04E-06	0.41	−0.31	1.65E-03	0.40	0.25	1.17E-04

In each locus, only the most significantly associated SNP is listed.

Bold: SNPs with genome-wide significance (interaction p-values <5E-08)

*SNPs are located inside genes