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Transferability of genome-wide associated loci for asthma in African Americans

Mezbah U. Faruque, MD, PhD^a, Guanjie Chen, MD^b, Ayo P. Doumatey, PhD^b, Jie Zhou, PharmD^b, Hanxia Huang, MD^b, Daniel Shriner, PhD^b, Adebawale A. Adeyemo, MD^b, Charles N. Rotimi, PhD^b, and Georgia M. Dunston, PhD^a

^aNational Human Genome Center, Howard University College of Medicine, Washington, DC, USA

^bCenter for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

Abstract

Objective—Transferability of significantly associated loci or GWAS “hits” adds credibility to genotype-disease associations and provides evidence for generalizability across different ancestral populations. We sought evidence of association of known asthma-associated single nucleotide polymorphisms (SNPs) in an African American population.

Methods—Subjects comprised 661 participants (261 asthma cases and 400 controls) from the Howard University Family Study. Forty-eight SNPs previously reported to be associated with asthma by GWAS were selected for testing. We adopted a combined strategy by first adopting an “exact” approach where we looked-up only the reported index SNP. For those index SNPs missing from our dataset, we used a “local” approach that examined all the regional SNPs in LD with the index SNP.

Results—Out of the 48 SNPs, our cohort had genotype data available for 27, which were examined for exact replication. Of these, two SNPs were found positively associated with asthma. These included: rs10508372 (OR = 1.567 [95%CI, 1.133–2.167], $P = 0.0066$) and rs2378383 (OR = 2.147 [95%CI, 1.149–4.013], $P = 0.0166$), located on chromosomal bands 10p14 and 9q21.31, respectively. Local replication of the remaining 21 loci showed association at two chromosomal loci (9p24.1-rs2381413 and 6p21.32-rs3132947; Bonferroni-corrected P values: 0.0033 and 0.0197, respectively). Of note, multiple SNPs in LD with rs2381413 located upstream of *IL33* were significantly associated with asthma.

Conclusions—This study has successfully transferred four reported asthma-associated loci in an independent African American population. Identification of several asthma-associated SNPs in the upstream of the *IL33*, a gene previously implicated in allergic inflammation of asthmatic airway, supports the generalizability of this finding.

CONTACT Mezbah U. Faruque, mfaruque@howard.edu, Howard University Cancer Center, 2041 Georgia Avenue, NW, Rm. 612, Washington, DC 20060, USA.

Declaration of interest

The authors report no conflicts of interest.

Keywords

Asthma; replication; single nucleotide polymorphism; genetic association; African Americans; *IL33*; ethnicity

Introduction

With an estimated 25.7 million people having asthma in 2010 and an increase of its prevalence from 7.3% in 2001 to 8.4% in 2010 [1], asthma has reached epidemic proportions in the United States. It is one of the diseases that shows disproportionately higher incidence and disease-associated mortality in African Americans. Current asthma prevalence is higher among non-Hispanic Blacks (9.5%) compared to their White counterparts (7.8%) [2]. The asthma death rate per 1,000 persons with asthma was 0.15 for the period 2007–2009, and was more than 75% higher for Black persons compared to Whites [1]. In addition, asthma appears to vary phenotypically according to ancestry, with African Americans showing different patterns of allergic sensitization, IgE levels, and bronchial hyperresponsiveness, when compared with Americans of European ancestry [3].

Asthma is a complex disease in which multiple gene-gene and gene-environment interactions play key roles in its development [4]. Overall estimates for heritability of asthma and related traits suggest that 36–75% of asthma risk is attributable to genetic factors [5]. Genetic role in asthma predisposition is suggested by twin, linkage, and association studies [6,7]. Candidate gene and linkage studies followed by positional cloning have already identified a large number of asthma susceptibility genes [8]. The advent and application of genome-wide association studies (GWAS) has greatly improved our understanding of the genetic factors responsible for asthma. To date, more than 20 broadly defined major susceptibility regions for asthma and related traits have been identified by GWAS [6,7] of which at least 9 loci have been replicated in independent populations.

Findings from meta-analysis of GWASs further substantiated the heterogeneous nature and complex etiology of asthma [9,10]. Although disproportionately afflicted by asthma, very few GWAS and meta-analysis have been conducted in African Americans as the discovery population. The first report of a genome-wide association study for asthma focusing on populations of African descent implicated three SNPs mapping to *ADRA1B*, *PRNP* and *DPP10* genes, all known to have potential biological relevance in asthma and allergic disease [11]. Torgerson *et al.* performed a genome-wide ancestry analysis and reported a single nucleotide polymorphism (rs1361549) on 6q14.1 that was associated with asthma exclusively in African American subjects [12]. Meta-analysis of GWAS in diverse populations identified several asthma-associated loci in three ethnic groups representing European Americans, African Americans, and Latinos [9]. Multiple SNPs in the *PYHIN1* region showed evidence for association only in the African American/African Caribbean sample suggesting that ancestry-specific associations also contribute to the complex genetic architecture of asthma [9].

Although GWAS offers a powerful approach to identify regions of the genome that harbor genetic variants conferring risk for disease without prior knowledge of location or function

by simultaneously testing hundreds of thousands of SNPs in a cost-effective way, a major challenge is to separate true associations from false positive findings. This can be circumvented by replication of initial finding in subsequent independent studies, and thus, is essential for establishing the validity of the observed association [13]. Replication is distinguished from transferability on the basis of that the latter applies if a follow-up sample is drawn from a different ancestral population than the discovery sample [14]. Both replication and transferability studies are now increasingly performed to validate reported associations as well to examine for their generalizability in diverse ancestral populations, and ultimately towards the final objective to explore their potential for translational application.

We hypothesize that asthma-associated GWAS SNPs reported in previous studies that are primarily of European ancestry populations will also show associations with asthma in an African American population sample. In this study, we examined the transferability of asthma-associated genetic loci identified from reported GWAS and meta-analysis in the Howard University Family Study (HUFS), an independent sample of African Americans from the Washington DC metropolitan area. It is expected that index SNPs tag larger regions in European-ancestry populations compared to their African counterparts because of the longer range of linkage disequilibrium (LD) in European-ancestry population, in general. Therefore, functional variants tagged by the index SNP in European-ancestry individuals might not be in LD with the same SNP in African-ancestry individuals. To address this, we explored for “local” replication by examining SNPs in LD on either side of the index SNPs identified by GWAS.

Methods

Study sample

The study cohort comprised 661 non-related individuals who participated in the Howard University Family Study (HUFS), a population-based family study of African Americans in the Washington, DC metropolitan area [15]. The major objectives of the HUFS were to enroll and examine a randomly ascertained cohort of African-American families, along with a set of unrelated individuals, from the Washington DC metropolitan area to study the genetic and environmental basis of common complex diseases including hypertension, obesity and related comorbidities. To maximize the utility of this cohort for the study of multiple common traits, participants were not ascertained based on any phenotype.

Participants were sought through door-to-door canvassing, advertisements in local print media, and at health fairs and other community gatherings. Ethical approval for the study was obtained from the Howard University Institutional Review Board. All individuals provided written informed consent for the collection of samples and subsequent analysis. During a clinical examination, demographic information was collected from each participant by interview. Asthma was defined as self-report of physician’s diagnosis, and supplemented by medication history collected by the interviewer. The control group included those who were never diagnosed with asthma by a physician and never utilized any asthma-treating medication.

Genotyping and quality control

Genome-wide genotyping was performed using the Affymetrix® Genome-Wide Human SNP Array 6.0, and genotypes calls were made using the Birdseed algorithm, version 2 [16]. SNPs were excluded if they had a call rate < 95% ($n = 41,885$), a minor allele frequency (MAF) < 0.01 ($n = 19,154$) or a Hardy–Weinberg equilibrium (HWE) test P value < 1×10^{-3} ($n = 6,317$). This led to a total of 808,465 autosomal SNPs passing the quality control (QC) filters. The average call rate for this set of SNPs was 99.55%, with concordance of blind duplicates of 99.74%. A check for population stratification was conducted using non-parametric clustering of genotypes using the R package AWclust [17].

Statistical analyses

Power calculations were performed using QUANTO [18]. Assuming an additive genetic model and with a MAF of 0.05, this study had a power of 50% to 86% to detect associations with a genetic relative risk of 1.6 to 2.0, respectively. For transfer in the HUFS, 48 candidate SNPs were selected from published GWAS and meta-analyses reports for asthma in populations from diverse ethnic backgrounds (<http://www.ebi.ac.uk/gwas/>). We refer to this set of variants as index SNPs. We tested this set of index SNPs for association with asthma in our dataset using either “exact” or “local” approaches [19]. Genotype and allele frequencies were estimated by gene counting.

For a reported SNP, which was present in the HUFS dataset, we tested that SNP for direct association. Because different GWASs use different genome-wide chips where the SNP panels do not totally overlap, we did not have genotype data on some of the index SNPs reported by previous studies. For those index SNPs where genotypes were not available in the HUFS dataset, we evaluated for “local” replication. For this, we comprehensively evaluated all HapMap (<http://hapmap.ncbi.nlm.nih.gov/>) Phase II+III SNPs with $r^2 \geq 0.3$ and within a search window of 250kb on either side from the index SNP using the appropriate representative reference population (CEU for studies of European ancestry, YRI for studies of African ancestry, and CHB for studies of Asian ancestry) corresponding to the original reported association. P values obtained from local replication were corrected for multiple testing using the Bonferroni method, and an adjusted P value of ≤ 0.05 was considered statistically significant. Asthma was analyzed as a binary trait (cases versus controls) under an additive genetic model with adjustment for age, gender, BMI and the first two principal components (PC) of the genotypes that was computed using EIGENSTRAT [20]. The appropriate number of PCs necessary to adjust for population substructure in the HUFS has been previously determined [15]. All association analyses were performed using the PLINK software package, V1.07 [21].

Results

Table 1 shows the characteristics of the asthmatic ($n = 261$) and non-asthmatic ($n = 400$) individuals. Mean age was higher in the control individuals whereas BMI was higher in the asthmatic individuals. A list of all 48 SNPs examined for association with asthma in our population is presented in Supplementary Table S1 that also shows their comparative allele frequencies in African, European, and Asian ancestry populations. Out of the 48 SNPs, QC-

passed genotypes were available for 27 SNPs that were examined by exact replication. Detailed results of the exact replication analysis are presented in Supplementary Table S2. Two SNPs, rs10508372 and rs2378383, were found significantly associated with asthma at a nominal replication P value of 0.05 (Table 2). Both rs10508372 ($P = 0.0066$) and rs2378383 ($P = 0.0166$) are intergenic SNPs located on chromosomal regions 10p14 and 9q21.31, respectively. Association of rs10508372, originally discovered in a Japanese population [22], was transferred in our population with the same direction of effect. On the other hand, rs2378383, originally reported in a GWAS in a Mexican population [23], was found associated in our African American population with an opposite direction of effect.

For the remaining 21 SNPs that were not examined by exact replication, we adopted an LD-based local replication strategy. Detailed results of local replication are presented in Supplementary Table S3. We identified a total of 721 SNPs that were in LD at our pre-defined threshold from the 21 loci (Supplementary Table S4). Two loci were found positively associated with asthma after adjusting for multiple testing (Table 3). rs3132947 is located in the intronic region of *NOTCH4* (corrected $P = 0.0197$). It is a proxy for index SNP rs204993, originally discovered associated with asthma in a Japanese population [22]. The other SNP, rs2381413 (corrected $P = 0.0066$) is a proxy for rs2381416, originally reported in a meta-analysis of European, African and Hispanic ancestry populations [9]. rs2381413 is located upstream of *IL33*, a gene known to have biological function in asthma pathophysiology. Of particular interest, in addition to rs2381413, 9 other SNPs in LD with rs2381416 were found associated with asthma (Table 4). Figure 1 shows a LocusZoom plot presenting a visual display of the asthma-associated region flanking the *IL33* gene. Thus, we have transferred 4 reported asthma-associated loci in the HUGO.

Discussion

Replication helps ensure that a genotype-phenotype association observed in a GWAS represents a credible association and is not a chance finding or an artifact due to uncontrolled biases [24]. It also helps in identifying a more reliable estimate of the effect size for the association. Replication in independent studies across different designs and populations extends the generalizability of the association in different environmental or genetic backgrounds. In particular, studies in populations of recent African ancestry are likely to increase the yield of rare variants and narrow the large chromosomal regions of association identified in “younger” populations due to reduced linkage disequilibrium, or the tendency for adjacent genetic loci to be inherited together [25,26].

In this study, we examined the association of 48 loci with asthma in an independent African American population. The variants selected for this study are from on-line databases that catalog published data on GWAS. For exact replication, we have used nominal significance ($P < 0.05$), which has been widely applied by some large consortia [27], and is also an acceptable criterion for publications [28]. By this method, two SNPs were successfully transferred in our study population. The C allele of rs10508372 was previously reported to be associated with asthma in a Japanese adult population [22]. The frequency of the associated allele in our population was much higher (0.8102) than that reported in the Japanese (0.433) [22]. Our observed frequency is similar to the HapMap-ASW data (0.786)

and likewise, higher than the HapMap-JPT data (0.419). Large differences in risk-allele frequency between populations of different ancestral background at some SNPs is consistent with studies of other complex-disease-associated SNPs [29,30]. The direction of effect observed in our association for rs10508372 is consistent with that reported by Hirota et al. [22], a criterion for successful replication [13]. Our observed relative risk (1.567) is somewhat larger compared to their report (1.16), which could potentially result from our modest sample size, a factor known to impact accurate estimation of the effect size.

The other SNP, rs2378383, was originally reported to be associated with childhood-onset asthma in Mexicans [23]. Although the G allele was found protective for asthma in Mexicans (RR = 0.61 [95%CI, 0.49–0.76]), we found the same as a susceptibility allele for asthma in our cohort (OR = 2.147 [95%CI, 1.149–4.013]). The frequency of the associated allele in our population was much lower (0.0354) and is consistent with the HapMap-ASW (0.051). Hancock et al. [23] found the frequency much higher (0.22) in their Mexican population, which is consistent with the HapMap-MXL (0.180). This type of association with opposite alleles associated in primary and replication samples has been reported in other studies of asthma [10], where “flip-flop” association has been attributed to a genotype-environment interaction with maternal asthma status [31] or to differences in LD between population groups [32–34]. Genetic effect (odds ratio) point estimates between ancestry groups have been reported to correlate modestly (pairwise comparisons’ correlation coefficients: 0.20–0.33) and point estimates of risks were found opposite in direction or differed more than twofold in 57%, 79%, and 89% of the European versus Asian, European versus African, and Asian versus African comparisons, respectively [35].

By taking advantage of increased genetic diversity and weaker LD in African-descent populations, we also adopted an LD-based approach to examine for local transferability of associated loci. When a region of high inter-marker linkage disequilibrium is tested to replicate an initial finding that is only weakly associated with disease, the local approach is considered to be a good strategy [19]. We performed appropriate correction of statistical significance for multiple testing for all SNPs examined in the LD window by Bonferroni method to reduce false-positive findings. By this approach, two additional loci that failed to transfer in our population by exact testing were found associated with asthma. Although rs2381413 is an intergenic SNP located about 49kb upstream of *IL33* gene, rs3132947 is located in the intron of the *NOTCH4* gene. SNPs in the *NOTCH4* gene have been reported to be associated with different diseases. rs715299, an intronic SNP in *NOTCH4*, has been reported to be associated with sarcoidosis susceptibility in African Americans [36].

Another intronic SNP, rs206015, was found associated with lung function in European-descent populations [37]. Hirota et al. [22] observed the most significant association from their GWAS study for adult asthma at rs404860, located in the *NOTCH4* intron. rs404860 is about 33kb away from rs2070600, a non-synonymous SNP located in the *AGER* gene, which has been previously reported to be associated with FEV₁/FVC in a GWAS for lung function [38]. Although rs404860 was not replicated in our population (Supplementary Table S2), rs3132947, also a *NOTCH4* intronic SNP and located about 7.5 kb away from rs404860, was found positively associated in our population. These findings support the notion that the disease-associated variants and the previously reported associated SNPs are

not always in LD in our African American population although they were in the discovery population. Alternatively, the index SNP and local SNP represent independent signals where each is significant in only one population representing population-specific allelic heterogeneity.

Of particular importance, we observed association of multiple SNPs upstream of the gene *IL33* with asthma. *IL33* (MIM 608678) is a member of the IL1 family that is a ligand for the orphan IL-1 family receptor ST2 [39]. IL-33 potently drives the production of proinflammatory Th2-associated cytokines including IL-4, IL-5, and IL-13 by *in vitro* polarized Th2 cells [39] and mast cells [40]. Atopic asthma is known as a chronic inflammatory pulmonary disease with an underlying Th2 cell-mediated inflammatory response in the airways.

Bronchial epithelium [41] and airway smooth muscle cells [42] from asthmatics were found to express elevated levels of IL-33 compared with that in healthy controls. IL-33-mediated inflammatory responses of lung tissue cells have been proposed to be involved in the chronic allergic inflammation of the asthmatic airway [43]. Furthermore, a recent genome-wide association study showed that a SNP in *IL1RL1* was strongly associated with asthma in a collection of 10 different populations [44]. A SNP in *IL33* that showed a suggestive association with the circulating eosinophil count was also significantly associated with atopic asthma [44]. Our observation of several association signals upstream of the *IL33* gene may represent independent loci, each with its own effect on asthma. Alternatively, they may result from all being in LD with a single causal SNP.

The majority of the index SNPs we tested were not transferred in our cohort, which could be attributed to multiple reasons. Our sample size is modest and thus may not have enough power to detect all associations. An index SNP may be a poor marker for asthma due to ancestry-specific differences in allele frequency and linkage disequilibrium between the original study and our admixed population. Most GWAS-identified variants are likely to be in LD with the causal variant(s) rather than being causal by themselves. The genetic architecture of African Americans is substantially different and generally shows weaker LD compared with other U.S. populations [45], and thus, may limit the transferability of associations from other ancestral populations.

Phenotypic heterogeneity among the cohorts of the discovery studies and ours, especially in case definition and recruitment strategies, may also impact chances of replication. The use of self-reporting cases and controls without a disease confirmation by additional phenotypic characterization (e.g., lung function measurements, skin prick tests, or specific IgE measurement) is a limitation of our study. Etiologic heterogeneity for complex diseases like asthma resulting from gene-gene or gene-environment interactions may also lead to failure of replication.

Neither the discovery nor our replication study captures exposure to all potential environmental risk factors that can lead to asthma development in the context of individual's genetic background. These factors may pose restrictions from observing the genetic association in an independent study. Even though we adjusted for global ancestry,

differences in local ancestry between these samples could have impacted our ability to replicate associations [46]. It is well known that the age-at-onset of asthma is associated with different phenotypic characteristics [47], and it has recently evidenced that age-varying genetic associations can cause non-replication and, consequently, lead to missing important genetic associations [48]. We also conducted a GWAS for asthma in our study population, however no SNP showed an association at genome-wide significance level (data not shown).

Our study has several strengths. The HUFs is a representative population of African Americans from the Washington, DC area. The lack of selection for disease status makes this an optimal study sample for drawing conclusions regarding transferability to a broader population of African Americans [49]. As our study included only self-reported African Americans recruited from a defined geographic site, population stratification is not expected to be an issue. Many candidate gene studies rely on self-reported ancestry to control for population stratification, an approach that has been shown to be reliable in genetic studies [50,51].

We performed additional ancestry analyses using a set of ancestry informative SNPs selected from the genome-wide panel. To maximize the utility of the study, we carefully characterized each study participant, adjusted for covariates in analysis and adjusted for ancestry (admixture), which can produce spurious association in population-based case-control study designs [52]. In addition to examining for exact replication, we employed a local replication strategy to evaluate transferability of the reported loci in recognition of the well-known differences in LD structure across the genome between African and European ancestry individuals. It is now well documented that testing associations in populations of recent African ancestry is likely to improve the detection of new risk variants [25], as they may offer the opportunity to refine the estimates of the position of the causal variant or to identify the causal variants [53,54].

A limitation of our study is the modest sample size. Small sample size can result in insufficient power to detect minor contributions of associated alleles and contributions of those with low MAF. Similarly, small sample sizes can provide imprecise or incorrect estimates of the magnitude of the observed effects. Thus, non-transfer of associations in our cohort should be interpreted with caution, which could result from insufficient sample power.

Conclusions

In conclusion, we sought to transfer 48 published asthma GWAS signals in a cohort of African Americans. Using a combination of exact and local approaches, we successfully transferred four asthma-associated loci in our population, and thus provided independent support for their role as risk factors for asthma in African Americans. Association of multiple SNPs upstream of the *IL33* gene warrants further investigation. Although we acknowledge both the strengths and limitations of our study, further replication studies with larger sample sizes, deep sequencing and functional studies are required to confirm the generalizability of GWAS signals, to pin-point the causal variants, and to decipher their role in the asthma pathophysiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

LocusZoom plot showing the asthma-association results flanking the IL33 gene. P-values of the SNPs on the $-\log_{10}$ scale are shown on the vertical axis against their corresponding chromosomal position presented in the horizontal axis.

Table 1Clinical characteristics of the study population ($n = 661$).

Characteristic	Asthma cases	Control subjects	<i>P</i> value
No. of participants	261	400	
No. of Females (%)	183 (70.11)	177 (44.25)	< 0.0001
Age, years	41.72 (15.00)	49.35 (8.16)	< 0.0001
BMI, kg/m ²	32.57 (9.93)	29.02 (7.45)	< 0.0001

Note. BMI, body mass index. Data are shown as number (percent) or mean (SD).

Table 2
Exact replication of the previously reported GWAS associations with asthma in theHUFS.

SNP	Chr	Position	Type	A1	MAF	OR (95% CI)	P value
rs10508372	10p14	9012024	Intergenic	G	0.1898	1.567 (1.133,2.167)	0.0066
rs2378383	9q21.31	81229182	Intergenic	C	0.0354	2.147 (1.149,4.013)	0.0166

Note. Position based on NCBI genome build 36.3; A1, associated allele; MAF, minor allele frequency.

Table 3
Local replication of the previously reported GWAS associations with asthma in the HUFs.

Index SNP	Chr	No. of SNPs ^I	Best local SNP	r ²	A1	MAF	P value	P value	OR (95% CI) adjusted
rs2381416	9p24.1	11	rs2381413	0.504	G	0.4169	0.0003	0.0033	0.614 (0.472,0.798)
rs204993	6p21.32	11	rs3132947	0.367	T	0.1824	0.0018	0.0197	1.686 (1.215,2.341)

Note.
^IIndicates total number of SNPs examined for the locus in the ±250 kb window with an r² 0.3 with the index SNP; A1, associated allele; MAF, minor allele frequency.

Table 4

Association of multiple SNPs upstream of *IL33* gene in the HUFs.

SNP	Distance (upstream) from <i>IL33</i> (bp)	Risk allele	r^2	MAF	P value	OR (95% CI)
rs2225537	55,206	C	0.533	0.4321	0.0007	0.627 (0.479,0.821)
rs10124250	54,098	C	0.545	0.4246	0.0004	0.616 (0.472,0.804)
rs10119713	51,961	G	0.545	0.4191	0.0005	0.629 (0.484,0.818)
rs2381413	48,767	G	0.504	0.4169	0.0003	0.614 (0.472,0.798)
rs1929996	28,148	G	0.693	0.3315	0.0097	0.698 (0.531,0.917)
rs1412426	27,132	C	0.677	0.3367	0.0172	0.720 (0.549,0.943)
rs1412425	27,044	C	0.687	0.333	0.013	0.709 (0.541,0.930)
rs2095044	22,988	C	0.96	0.379	0.0233	0.729 (0.554,0.958)
rs4742167	20,449	T	0.732	0.3305	0.0111	0.702 (0.534,0.922)
rs7046661	6,585	C	0.65	0.3324	0.0133	0.708 (0.539,0.931)

Note. r^2 with the index SNP rs2381416.