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Genomewide association studies in allergy and the influence of ethnicity

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Abstract

Purpose of review—Asthma and allergic diseases are common and disproportionately affect racial and ethnic minorities. Large-scale research efforts and the expense committed to multiple genome-wide association studies (GWAS) have led to the identification of numerous susceptibility loci for the allergic diseases, but few successes have been reported in populations that are not of European ancestry.

Recent findings—Of the more than two dozen GWAS's for asthma and allergic disease performed to date, very few have included racial/ethnic minorities. Lessons learned from the studies conducted so far suggest that the GWAS approach must include considerations unique to the ancestral populations represented in the sample, population stratification due to admixture, and recognition that the current coverage of common variants both in the public database and on commercially available SNP chips is inadequate to detect true genetic associations among ethnic/racial groups.

Summary—Advancements in the GWAS technology for identifying genes relevant to asthma and allergic disease among underrepresented ethnic and racial who suffer most will facilitate the identification and confirmation of validated genetic risk factors that are both unique to minority groups as well as confirm risk factors that are generic to the population at large.

Keywords

genomewide association studies (GWAS); ethnicity; population stratification; admixture

Introduction

Allergic diseases are complex phenotypes wherein the interplay between genetic factors and environmental exposures has significant influence on susceptibility and disease prognosis. Over a decade of genetic mapping and positional cloning have revealed evidence suggesting linkage to over two dozen loci for asthma alone, and association studies have identified a multitude of variants associated with traits associated with allergic disease (asthma, allergic rhinitis, atopic dermatitis). With the emergence of genome-wide association studies (GWAS), the candidate gene approach is being replaced by a more unbiased approach to search for genes controlling risk to complex diseases, including atopy. In GWAS, multiple haplotype tagging SNPs (htSNP) from continental reference populations (*i.e.*, the HapMap

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project¹) theoretically allow for detection of associations to potentially causal variants, and have successfully broadened the scope of gene discovery for many complex traits, including allergic disease. For asthma alone, nearly a dozen reports of associations using GWAS have been published, but nearly all of these have been for European ancestry populations. Of the few that have been performed in minority populations, results are inconsistent with results from GWAS's performed in European-ancestry cohorts, suggesting that populations of different ethnicities, such as those of African descent, may carry unique susceptibility loci. Of concern, however, is that the efficiency of htSNPs chosen from one population (*e.g.*, European), and originally believed to be sufficient for detection of true associations, is questionable in their representation of other continental races (*e.g.*, African) and thereby especially important in GWAS of admixed populations (*e.g.*, African American). This review explores the findings to date from GWAS's performed in ethnically diverse populations to identify polymorphisms that confer risk to asthma and its associated, 'atopy-related' traits in ethnically diverse populations. It further interrogates the pitfalls with the current GWAS technology, and considers the impact of anticipated advancements in the field, making it possible to broaden the focus of genetic studies of complex traits from populations of European-ancestry to better represent minority populations.

Does Genetic Variation Account for Ethnic Disparities in Allergic Disease?

It is well-accepted that allergic diseases, especially asthma, are disproportionately high and continue to increase among many ethnic minorities². For example, the death rate due to asthma among African Americans is 4-6 times higher than among whites³, and certain Hispanic populations (which also share African ancestry), especially Puerto Ricans, have an even higher asthma prevalence and greater morbidity and mortality than African Americans⁴⁻⁶. For other underrepresented minority groups, fewer studies have been conducted to accurately compare prevalence and morbidity, but disparities are likely. Pacific Islanders (*i.e.*, native Hawaiians), have historically suffered from more widespread and severe asthma than other ethnic groups living in Hawaii⁷, and among children 0-14 years old, asthma prevalence is 22% in Hawaiians/part-Hawaiians, compared to 14% in Filipino, 12% in Chinese, and 8% in Japanese and whites (http://hawaii.gov/health/statistics/hhs/hhs_07/index.html). The National Survey of Children's Health, a telephone survey in 2003–2004 of a national random sample of parents and guardians which included over 100,000 children ages 0-17 years, is one of the few large epidemiological studies to include Native Americans and collect data on prevalence of allergic disease. In this survey, disparities in prevalence of asthma and allergies were observed when comparing white, African American, Latino, Asian/Pacific Islander, Native American, and multiracial children⁸. Specifically, Native American children were more likely to be asthmatic (14.2% vs. 11.5%, $P=0.0001$) and have skin-related (*i.e.*, eczema) allergies (12.0% vs. 9.2%, $P=0.0001$) compared to whites.

Outside of the U.S., asthma prevalence is high and continues to increase in African countries and countries with African admixture (*i.e.*, Latin America). In the most recent ISAAC report, prevalence continues to increase in the Caribbean country of Barbados specifically⁹. In the *Asthma Insights and Reality in Latin America* (AIRLA) survey, over 2,000 adults or children in 11 Latin American countries, including Colombia and Brazil, were evaluated, and this report concludes asthma is both underdiagnosed and poorly managed¹⁰. What has become increasingly clear, at least among U.S. populations, is that the striking racial and ethnic disparities in disease prevalence for common disorders, including asthma and allergies, cannot be explained entirely by environmental, social, cultural, or economic factors, and genetic factors are likely at play¹¹.

A Dearth of Validated Genetic Determinants for Asthma and Allergic Disease among Ethnic Minorities

Asthma and its associated trait 'atopy' were perhaps some of the first complex diseases for which a strong genetic basis was established¹²⁻¹⁴. Nearly a dozen genome-wide linkage screens have been performed on asthma and its associated phenotypes¹⁵⁻²⁵, which have identified 10 chromosomal regions giving multiple reports of linkage. From several of these family-based genomewide linkage screens, six novel genes were identified by further positional cloning²⁵⁻³⁰.

The huge research efforts and expense committed to asthma genetics from candidate gene and linkage studies have changed the perception about the etiology of asthma and allergic disease, including a new appreciation for the role of innate as well as adaptive immune-response genes, and the potential importance of the epithelial barrier and its defense mechanisms in asthma^{14, 31}. However, genomewide approaches have the advantage of being completely unbiased from a genetic perspective, and have emerged as a powerful approach for detecting novel genetic variants with plausible effect sizes for a plethora of complex traits, including asthma. To date, nearly 600 GWAS's have been published on a plethora of complex traits³².

Notably, however, the GWAS's performed so far have focused primarily on European ancestry populations as the discovery population, with very limited replication in non-European groups. In a review of 570 references from "A Catalog of Published Genome-Wide Association Studies" (<http://www.genome.gov/gwastudies/>), as of March, 2010, there have only been 72 studies that included cohorts from non-European populations (Figure 1, panel A). Within the asthma community, 13 groups have published results from their GWAS on asthma; however, of the asthma GWAS's completed to date, only one focused on African Americans³³ and two focused on Hispanics^{34, 35} (Figure 1, panel B). To date, one GWAS has been published on AD in a German sample³⁶, replicated in an independent German group, and a second, European-based GWAS is in the replication phase. A GWAS focusing on total IgE as the primary outcome³⁷ leveraged the GABRIEL consortium (<http://www.gabriel-fp6.org/public/index.htm>), the European sample selected for asthma (currently 10,000 asthma cases and 16,000 non-asthmatic controls) from which the first results from a GWAS for asthma were generated.

The initial GABRIEL report demonstrated strong association between asthma and markers near the *ORMDL3* gene on chromosome 17q21 ($P < 10^{-12}$)³⁸, and has been widely replicated in several European ancestry³⁹⁻⁴³, Asian^{44, 45} and Hispanic^{46, 47} populations. However, the SNPs significantly associated in the discovery population were *not* associated in nearly 3,500 African Americans from Philadelphia⁴⁸, an African American sample from San Francisco/Oakland area⁴⁶, or African American and African Caribbean populations from Baltimore/Washington, D.C. and Barbados, respectively³³. The San Francisco group did report significant association between a different *ORMDL3* SNP (rs9894164) and asthma among African Americans, but this marker was not on the original commercial SNP chip, underscoring the challenges in replicating SNP-for-SNP findings from GWAS performed in European populations. In the first asthma GWAS focusing exclusively on populations of African descent, replication at a SNP-for-SNP level was not observed across multiple independent populations; however, three genes (*CTNNA3*, *DPP10* and *KCNMA1*) that showed evidence for association in the African-ancestry discovery samples were replicated in a European population using a gene-based approach³³. Conclusions drawn from this study include the possibility that subtle differences in ancestry between populations can lead to a failure to replicate associations for individual SNPs, even when gene-based evidence does exist.

In one of the few observations of SNP-for-SNP replication of a GWAS for asthma, Hakonarson and colleagues observed significant associations between variants in the gene encoding *DENND1B* in a large sample of European Americans ($P=7.8 \times 10^{-8}$), which replicated in independent European-ancestry samples⁴⁹. Replication was also found in a combined African American case-control group, but the association was with a different allele and the estimate OR was in the opposite direction. This ‘flip-flop’ phenomenon across different ethnic groups may reflect differences in genetic background and/or differences in linkage disequilibrium (LD) across populations, especially when non-causal ‘tag’ markers are tested⁵⁰ (as is typical in GWAS using commercial chips). These observations similarly underscore the complexities of drawing firm conclusions from marker panels in distinct sub-populations, and the need to test beyond simple SNP-SNP replication.

The Complexities of Ethnically Admixed Populations in Genetic Association Studies

Genetic structure and substructure occurs when a population is comprised of more than one or more ethnic/racial groups representing different ancestries. Ethnic or racial admixture is particularly relevant for genetic studies in the U.S. and elsewhere in the Americas, where the unique population history has been characterized by a mixture of European ancestry with West Africans, indigenous groups (northern and southern Native Americans, as in the case of Mestizos in Central and South America), and a multitude of peoples from other distinct geographical regions. A serious problem arises when the disease of interest (*e.g.*, asthma, atopy) is more prevalent in a particular group (or groups) within the population, because any alleles that are more common among the minority group(s) of interest will tend to be associated with the disease, even if completely unlinked to the disease-causing locus. This phenomenon is referred to as ‘population stratification’, and the consequent confounding due to population stratification can lead to spurious associations between genetic markers, especially in population-based (*i.e.*, case-control) studies, and can limit generalizability of results to the broader population⁵¹⁻⁵³.

While matching the self-reported ethnic backgrounds of cases and controls in study design is one possibility, it is still possible to have hidden, or >cryptic= stratification⁵⁴. GWAS has accelerated the development of statistical approaches toward detecting stratification and controlling for ancestral differences in association testing. Currently there are a variety of algorithms that employ non-hierarchical cluster analysis or principal component analysis (PCA) for these statistical approaches^{55, 56}. Correction for population stratification is typically performed using information on either: (1) markers for which allele frequencies differ substantially between ancestral populations referred to as ‘ancestry informative markers’ (AIMs); or (2) leveraging the large number of markers available in GWAS studies where it is easy to argue most are in fact unlinked to the true susceptibility loci for the disease under consideration^{55, 57-61}.

African Americans represent a racial/ethnic group that is both disproportionately affected by asthma and allergic disease (as described above) and a classic admixed population. To this end, ancestry estimation in this group has been extensively pursued. Relatively early studies demonstrated that African Americans are comprised of ~18%-20% European ancestry, but within the U.S. this proportion can vary regionally⁶². African Caribbean populations are believed to be similar in terms of genetic ancestry, but using a relatively modest panel of AIMs, Torres and colleagues estimated the West African component to be higher (up to 90%) among certain West Indian groups⁶³.

In the first GWAS focused on populations of African descent, 417 AIMs were used combined with similar genotypic data from 210 unrelated individuals drawn from the

HAPMAP data resource (60 YRI, 60 CEU, and 120 CHB/JPT) to define “ancestral populations” while estimating admixture in the ~2,000 study samples. Cases and controls were shown to be highly admixed with a balanced estimated proportion of African genes of 72.2 and 72.4%, respectively³³. It was further demonstrated that, after expanding this analysis to a large subset (~14,000 SNPs) of the full panel of autosomal SNPs from the GWAS chip (>620K) and also including two East African (Maasai and Luhya) populations from the newest version of HapMap, Phase III, the AIMs were just as good for detecting substructure larger numbers of random markers⁶⁴. These approaches for a variety of populations expected to have prominent African admixture component, as illustrated in Figure 2, show the highest proportion of YRI ancestry in the African Americans, Barbadians and Jamaicans (73-88%), and lower proportions in the Brazilian and Colombian data (38-50%).

Admixture and the impact of population stratification are not limited to overtly admixed populations, as demonstrated by Tian and colleagues, who demonstrated a spurious correlation between SNPs in strong linkage disequilibrium with a candidate gene for rheumatoid arthritis in a set of rheumatoid arthritis cases recruited from multiple U.S. sites compared to a group of self-identified European Americans recruited in New York city⁶⁵. In this study, the investigators used a set of putative European substructure AIMs (ESAIMs), and demonstrated that the large differences in allele frequency for a gene initially showing a strong association with RA was actually due to the difference in allele frequency among different European subpopulation groups, whereby the largest allele frequency difference was between predominantly Irish controls from New York city and cases of Northern, Central, and Eastern European descent. These studies together underscore the importance of considering population stratification in any population-based genetic association study, perhaps most importantly in populations with a known strong admixture component.

Technological Limitations: Coverage on Commercially-Available GWAS SNP Chips and Power for GWAS

The GWAS approach is predicated on the notion that data cataloged in the International HapMap Project, combined with more accurate approaches in selecting tagging markers, will be sufficiently dense to capture most of the common variation in the human genome. As described elsewhere, HapMap was initially generated from 269 DNA samples representing four biogeographical groups. This has recently been expanded to 1,115 individuals from 11 populations⁶⁶. However, the commercial enterprises that create the chips have, for the most part, relied upon the European ancestry reference panel as a proxy for all continental groups. Not surprisingly, the GWAS approach is most successful in situations where the genetic variants evaluated are selected from populations most representative of the study population at hand (*i.e.*, European Americans genotyped on markers largely selected from the CEU HapMap sample). Next generation sequencing technologies are rapidly driving the costs of assessing variation at every site in the genome down at a somewhat constant rate, and these may eventually replace commercially available genomewide platforms and at the very least compete with the price of capturing just a fraction of variable sites (*e.g.*, via high density SNP arrays). Nevertheless, currently, the cost of a whole genome sequence is still prohibitive, so using SNP arrays will, for the immediate future, continue to be the most cost-effective strategy for GWAS.

A recently published perspective on the scientific value of GWAS⁶⁷ suggested currently available commercial chips do capture the bulk of common genetic variation, and SNPs with large effects may have already been discovered, implying there is no need for additional genomewide scans, and focus should therefore shift towards a detailed search for rare variants. However, it is difficult to argue most important SNPs have already been

discovered, if what constitutes one of the largest and most genetically heterogeneous continental populations – Africans – is so severely underrepresented in the GWAS literature. Second, while the collective findings of GWAS do support the notion that most identified genetic risk factors only account for a small fraction of observed heritability of most complex diseases and typically have an effect size <1.5 , it is probable that multiple rare causal variants are more likely to be found in African ancestry populations and further argues for greater focus on this ethnic group.

In a recent analysis by co-investigator Bhangale and colleagues⁶⁶, 76 genes were resequenced in unrelated HapMap samples (24 YRI, 23 CEU) and they examined coverage of all genetic variation data using various commercial genotyping arrays and Phase II HapMap SNPs to capture common variants in these 76 genes. In direct comparisons of coverage performance of the commercial arrays compared to the SeattleSNP-based coverage at $r^2 \geq 0.8$, the commercial chip with the highest proportion of YRI SNPs only provided an estimated 55% coverage.

The limitations of the current technology are especially apparent in data that are emerging from the “1000 Genomes” project (<http://www.1000genomes.org>), an international research consortium which aims to create the most detailed catalog to date of human genetic variation and provide a resource that will be useful for human disease studies. Samples from about 2,000 individuals from ~22 populations are being sequenced with the goal of finding nearly all DNA sequence variants. Relying on Phase 1 data, which includes the greatly expanded whole genome sequencing of the original four continental populations represented in HapMap, >17 million SNPs from 181 samples have been identified, which includes >9M novel discovery SNPs. In other words, ~54% SNPs were not previously reported in the public database (Mathias, Qin, Abecasis, *unpubl data*). As illustrated in Figure 3, in the YRI data specifically, there are 12,068,821 SNPs identified in 50 samples, of which 5,953,505 were novel SNPs. Moreover, in an assessment of the overlap in variation between the four HAPMAP populations, each population has a set of unique SNPs (0.51 – 5.65 million), with the largest representation of unique variation in the YRI samples, with 5.65 million unique SNPs.

Conclusion

Despite the major advancements in the identification of novel genes associated with asthma and allergic disease through GWAS, few studies have been conducted in non-European populations. GWAS in ethnically and racially diverse populations demands consideration of characteristics unique to underrepresented cohorts, including population heterogeneity due to admixture. A critical limitation in conducting GWAS on ethnically and racially diverse samples arises because commercially available chips were constructed using SNPs identified in European derived samples and the density of these SNP panels does not provide adequate coverage. New technologies are on the horizon that promise to more accurately capture the genetic diversity of all continental populations, and advance the field.

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Figure 1.

Summary of GWAS by ethnicity of the discovery population (as of March, 2010). **Panel A:** All GWAS's published in the "Catalog of Published Genome-Wide Association Studies" (<http://www.genome.gov/gwastudies/>) according to ethnicity/race; **Panel B:** Published asthma GWAS's to date according to ethnicity/race.

Figure 2.

Heterogeneity of African admixture in five populations. African Americans (N= 906), GRAAD Barbadians (N = 294), Jamaicans (N = 174), Brazilians (N= 119) and Colombians (N=668), relying on autosomal SNPs that qualify as ancestry informative SNP markers (AIMs; 416, 416, 79, 79 and 52 AIMs, respectively) with absolute differences >0.4 in allele frequencies between pairs of putative parental populations (Utah residents with ancestry from northern and western Europe, or 'CEU', indicated in blue; Asians collectively represented by Japanese in Tokyo, Japan, or 'JPT'/Han Chinese in Beijing, China, or 'CHB', indicated in red; and Yoruba in Ibadan, Nigeria, or 'YRI', indicated in green). Color combinations (blue, red, green) for the five test populations reflect the degree of admixture of the three parental populations. The semi-parametric Bayesian algorithm implemented in STRUCTURE (v2.2; <http://pritch.bsd.uchicago.edu/software>) was used to infer both mean and individual West African, Northern European and Asian ancestry proportions in these five populations along with the Phase II HapMap groups.

Figure 3.

A graphical representation of the overlap in 17million SNPs between four HAPMAP populations according to Phase 1 data from the 1000 Genomes Project (August, 2009). Purple SNPs are unique to YRI, red SNPs are unique to CEU, blue SNPs are unique to JPT, and green SNPs are unique to CHB. Adapted from HapMap (<http://www.hapmap.org/>).