Genetic Studies of the Etiology of Asthma

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Asthma is a heterogeneous disease for which a strong genetic basis is firmly established. Although the generally accepted definition includes three domains of symptoms (variable airway obstruction, airway hyper-responsiveness, and airway inflammation), there is general agreement that, rather than being a single disease entity, asthma consists of related, overlapping syndromes. A considerable proportion of asthma is IgE-mediated, but the observation that not all individuals with asthma are atopic adds to the heterogeneity. Although a genetic basis for asthma is undeniable, elucidation of polymorphisms that are "causal" is greatly hampered by variability in the clinical phenotype, which is likely due to the multiple molecular mechanisms underlying the complex pathological processes involved in disease development and progression. One objective of this review is to consider progress that has been made to date in gene discovery in the field of asthma, with a focus on the evolution of molecular genetic methods that have led to the discoveries thus far, and with a particular focus on the major advances owed to the published genome-wide association studies (GWAS) on asthma to date. A second objective is to consider a Darwinian approach toward understanding the genetic underpinnings of asthma, including evidence supporting a modified Hygiene Hypothesis, which suggests that there are co-associations between asthma risk polymorphisms and polymorphisms associated with another IgE-mediated disease, schistosomiasis. The overall conclusion is that the huge research efforts and expense committed to asthma genetics have changed the perception about disease etiology in general and the functional relevance of the asthma genes identified thus far in particular.

Keywords: asthma; genetics; single nucleotide polymorphism; genome-wide association study; pleiotropy

Asthma is a heterogeneous disease characterized by intermittent inflammation of the airways, which may lead to irreversible airway remodeling and intractable airflow limitation. The definition of asthma, according to international guidelines (1), includes the three domains of symptoms: (1) variable airway obstruction, (2) airway hyperresponsiveness (or bronchial hyperactivity), and (3) airway inflammation. The most probable source of the lower airway inflammation characteristic of asthma is an immunoglobulin E (IgE)-mediated reaction initiated by chronic exposure to environmental allergens (i.e., those associated with house dust). There is general agreement that no one domain is essential to the diagnosis, and to this end, rather than being a single disease entity, asthma consists of related, overlapping syndromes (2). Although a genetic basis for asthma is undeniable, elucidation of polymorphisms that are "causal" for disease is greatly hampered by variability in the clinical phenotype, which is likely due to the multiple molecular mechanisms underlying the complex pathological processes involved in disease development and progression.

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ELUCIDATING CANDIDATE GENES FOR ASTHMA BEFORE THE GENOME-WIDE ASSOCIATION STUDY ERA

Genome-Wide Linkage Studies

Asthma and its associated trait "atopy" were some of the first complex diseases for which a strong genetic basis was established (3). In the early 1990s, the genome-wide linkage approach, whereby the inheritance patterns of chromosomal regions using highly polymorphic, genetic ("microsatellite") markers evenly spaced across all chromosomes were genotyped in large samples of families, identified 10 chromosomal regions for which novel genes were subsequently identified by positional cloning (i.e., *DPP10* [4]).

Genetic Association Studies Using Single Nucleotide Polymorphisms

With the publication of initial efforts in sequencing the human genome (http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml), the opportunity to genotype markers directly in genes of interest was greatly expanded as polymorphisms were identified in the approximately 20,000 to 25,000 genes across the 3 billion chemical base pairs that make up human DNA. Relying upon one of the simplest of these polymorphisms, single nucleotide polymorphisms (SNPs), and relatively simple structural variants, such as insertions/deletions and repeats, this advancement allowed researchers to expand genetic studies beyond linkage toward the genetic association study design. Because many more biallelic markers are required compared with microsatellites to detect linkage and association across the genome, a candidate gene approach was adopted whereby investigators could focus on a specific gene or set of genes believed to be causally involved in the underlying pathology of a certain disease. An advantage of the candidate gene approach is that it is not limited to families and can be applied to independent case-control study designs, which possess certain advantages over family-based studies. For example, although association studies based on case-control designs are sensitive to confounding due to population stratification (i.e., ethnic/racial admixture), they are generally considered to be more powerful in detecting true associations once the gene has been identified. Moreover, it is considerably easier (i.e., less effort required, lower costs in recruitment) to amass reasonably powered groups of affected cases and healthy control subjects than it is to collect complete case-parent trios or nuclear families.

Although it is beyond the scope of this review to describe all results from these two approaches, extensive summaries presented elsewhere indicate that a number of candidate genes have been identified for asthma (5, 6). According to the Genetic Association Database (<http://geneticassociationdb.nih.gov/>), a public archive of published gene-based genetic association studies, there have been over 1,825 published studies on asthma and its associated phenotypes as of March 2011, and this list continues to expand. Far fewer genes have been replicated in independent populations, and fewer still have been interrogated for the functional relevance of the implicated mutation(s).

Genome-Wide Linkage Studies of Asthma: Successes and Shortcomings

Although a major advantage of the candidate gene approach is its hypothesis-driven character, its primary constraint is selec-

TABLE 1. PUBLISHED GENOME-WIDE ASSOCIATION STUDIES

Gene (trait)	Population (n)	Reference
<i>ORMDL3</i> (asthma)	British (2,237) German (2,320) British (3,301)	Moffatt <i>et al.</i> (9)
<i>CHI3L1</i> (YKL-40 levels, asthma)	Hutterites (652) European American (206) German (638), Chicago (296)	Ober <i>et al.</i> (10)
<i>IL1RL1</i> (eos and asthma)	Icelanders (9,392), Europeans (12,118), East Asian (5,212)	Gudbjartsson <i>et al.</i> (11)
<i>FCER1A</i> (tIgE)	German (1,530 + 9,769)	Weidinger <i>et al.</i> (12)
<i>PDE4D</i> (asthma)	European Amer. (1,205) + 5 pops. (not in 3 AA)	Himes <i>et al.</i> (13)
<i>TLE4</i> (asthma)	Mexican (492 + 177 families)	Hancock <i>et al.</i> (14)
<i>DENND1B</i> (asthma)	European American (2,781 + 2,463) African American (3,712)	Sleiman <i>et al.</i> (15)
<i>ADRA1B</i> , <i>PRNP</i> , <i>DPP10</i> (asthma)	African American (1,000) African Caribbean (1,000)	Mathias <i>et al.</i> (16)
<i>RAD50-IL13</i> , <i>HLA-DR/DQ</i> (asthma)	European American (2,365)	Li <i>et al.</i> (17)
<i>IL1RL1/IL18R1</i> , <i>HLA-DQ</i> , <i>IL33</i> , <i>SMAD3</i> , <i>IL2RB</i> , <i>ORMDL3</i> (childhood onset asthma)	European (26,475)	Moffatt <i>et al.</i> (8)

Definition of abbreviations: *CHI3L1* = chitinase 3-like 1; *FCER1A* = Fc fragment of IgE, high affinity 1, receptor for, alpha subunit; *HLA-DR/DQ* = major histocompatibility complex, Class II, DQ Alpha-1; *IL1RL1* = interleukin 1 receptor-like 1; *IL2RB* = interleukin-2 receptor beta; *IL33* = interleukin-33; *ORMDL3* = ORM1-like protein 3; *PDE4D* = phosphodiesterase 4D, cAMP-specific; *RAD50* = *RAD50 S. cerevisiae*, Homolog of; *SMAD3* = mothers against decapentaplegic, *Drosophila*, homolog of, 3.

tion of candidates based on limited knowledge in contrast to the comprehensive search of the genome using a genome-wide association study (GWAS) approach. Nearly a dozen reports of associations with asthma and/or its associated phenotypes using GWAS have been published (Table 1). Important observations include validation of several previously identified genes (i.e., *IL13*, *IL1RL1*, *DPP10*, *HLA-DQ*) and the identification of genes that appear to be distinct for different asthma-associated phenotypes (i.e., *ORMDL3* for childhood onset asthma, *FCER1A* for total serum IgE; see Ref. 7 for review). Most GWASs have only observed associations that pass the strict correction for multiple comparisons in one or several genes per GWAS. However, in the largest asthma GWAS reported to date (>26,000 subjects), the European-based GABRIEL Consortium observed evidence for association in six loci, validating their previous observations in the *ORMDL3* locus as well as the association between *IL1RL1* and asthma (8), demonstrating the value of robustly powered samples for GWAS in yielding multiple, significant hits.

A curious observation drawn from Table 1 is the general lack of overlap of genes identified in these GWAS studies, and the unexpected finding that very few of the genes identified using positional cloning and candidate gene association approaches are not supported by GWAS. Population and phenotype heterogeneity is one potential explanation, but adequacy of representation of previously associated candidates on the commercial chips is another potential explanation. For example, Rogers and colleagues combined SNP data from a comprehensive review of genetic associations for asthma with SNP data from their own GWAS of greater than 500,000 SNPs and only identified 10 significantly associated SNPs in six genes that were on the commercial SNP chip and that overlapped with 160 SNPs in 39 genes previously associated with asthma in the literature (28), concluding the unlikely success in replication at the SNP level.

Most of the GWASs reported to date have been performed in European ancestry populations as the primary or "discovery" population, and, for the most part, even replication has been performed in European ancestry groups (Table 1). It is not clear why there should be this bias, but it is clear that the technology available limits the ability to adequately analyze underrepresented minority cohorts. Consider, for example, that the first asthma GWAS, which showed a reproducible association between childhood onset asthma and markers near the *ORMDL3*

gene on chromosome 17q21 ($P < 10^{-12}$) among European populations (9), has been widely replicated in several European ancestry samples (18–22) as well as samples of Asians (23, 24) and Hispanic ancestry (25, 26). However, the SNPs significantly associated in the discovery (European) population and replicated by other groups were not significantly associated in nearly 3,500 African Americans from Philadelphia (27) or in African American and African Caribbean populations from Baltimore/Washington, D.C. and Barbados (16), respectively. Although investigators studying an African American sample from San Francisco/Oakland observed the same lack of replication, significant associations were observed between a different *ORMDL3* SNP (rs9894164) and asthma, but this marker was not on the original marker panel (25), underscoring the challenges in replicating SNP-for-SNP findings among diverse ethnic groups.

Elsewhere, the requirement of a SNP-for-SNP replication as evidence for replication has been questioned rather, a gene-centric approach has been advocated because the SNP-for-SNP approach is predicated on the assumption that allele frequencies and haplotype structure at a specific locus is identical in two (or more) populations, which is unlikely. In one of the few observations of SNP-for-SNP replication of a GWAS association in asthma, Hakonarson's group observed significant associations between variants in the gene encoding *DENND1B*, a protein expressed by dendritic cells and a potential interactor with the TNF- α receptor and by a reduced risk of asthma in a large sample of European Americans ($P = 7.8 \times 10^{-8}$; estimated odds ratio [OR], 0.60), which was replicated in an independent North American sample of European ancestry and a Northern European group (15). Replication was also evident in an African American case-control group, but the association was with a different allele, and the estimate OR was different ($P = 3.6 \times 10^{-5}$; OR, 1.54). Reports of this "flip-flop" phenomenon in different ethnic groups has been attributed to heterogeneous effects of the same variant resulting from differences in genetic background or differences in linkage disequilibrium across populations, leading to inconsistent patterns of association, especially when noncausal variants are tested (29) (as is typically the case in the commercial GWAS chips). These observations underscore the complexities of drawing definitive conclusions from suboptimal panels of markers in distinct ethnic subpopulations and the need to test beyond simple SNP-SNP replication.

In an analysis to determine the adequacy of commercially available SNP chips in detecting genetic association between complex traits such as asthma in non-European ancestry samples, Bhargava and colleagues resequenced 76 genes in unrelated samples from the International HapMap project ($n = 24$ Yoruban-speaking West Africans from Nigeria [YRI]; $n = 23$ Utah residents with Northern and Western European ancestry from the CEPH collection [CEU]) and examined coverage of all genetic variation data using various commercial genotyping arrays and Phase II HapMap SNPs to capture common variants (29). In direct comparisons of coverage performance of the commercial arrays compared with actual sequence data, the highest-density chip only provided an estimated 55% coverage for African-origin populations. These observations underscored several shortcomings. First, it is clear from this study that detection of true genetic associations to complex traits in samples other than those of European ancestry is significantly compromised due to the modest coverage on commercial chips of SNPs that best represent non-European populations, such as African Americans. Second, this study also supports the notion that a reliance upon one African reference population (i.e., YRI) as a proxy for African Americans may not accurately capture the genetic architecture of this admixed group, especially in light of the recent observations by Tishkoff and colleagues (30) demonstrating that African groups besides the YRI account for at least 8% of African ancestry among African Americans.

The “1000 Genomes Project” (<http://www.1000genomes.org>) is an international research consortium that 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 approximately 22 populations are being sequenced ($\sim 4\times$ coverage), with the goal of finding nearly all DNA sequence variants. Studies aiming to relate genetic variation to disease will use these data to localize regions of the genome-containing variants associated with disease and to choose variants that should be studied further as possibly controlling risk of disease.

As a proof of concept of diversity across the representative continental populations, we mined more than 17 million SNPs from 181 samples in the 1,000 Genomes Project, which include 9,307,170 novel discovery SNPs. Fifty-four percent of these SNPs had not previously been reported in dbSNP and would therefore not have been considered on commercial SNP chips. As illustrated in Reference (31), in the continental African (YRI) data, there were 12,068,821 SNPs identified in 50 samples, of which 5,953,505 were novel SNPs. In an assessment of the overlap in variation between the African (YRI) HAPMAP population and the other three continental populations (the Han Chinese/Tokyo Japanese, or CHB/JPT, and European CEU samples), not only did each population have a set of unique SNPs (0.51–5.65 million), but the largest representation of unique variation is in the YRI samples, with 5.65 million SNPs found only in YRI data. Fortunately, with the relatively recent release of next-generation GWAS chips that have integrated data from the 1000 Genome Project (TGP) reference samples (i.e., Illumina’s Omni 2.5M), greatly improved coverage for non-European samples is anticipated.

GENETIC PLEIOTROPY AND A DARWINIAN APPROACH TOWARD UNDERSTANDING THE GENETIC UNDERPINNINGS OF ASTHMA

The expanding field of evolutionary biology has been increasingly relied upon to explain why modern humans are vulnerable to diseases (32, 33) such as asthma. One area of particular relevance is the impact of adaptations through natural selection against

pathogens. Indeed, there is compelling “proof of concept” data to suggest that human genetic variations that may have been originally selected to increase disease resistance in fact carry a “cost” in that the same variants confer risk to diseases in environments where the benefit of the genetic adaptation is diminished due to a decrease (or absence) of exposures that elicited the adaptation. Examples of pleiotropy, where a locus or allele has more than one distinct phenotypic effect, is the positive selection for the *Fy* locus on the Duffy gene—also referred to as the Duffy Antigen/Receptor for Chemokines (DARC)—and resistance to malaria. DARC acts as the receptor for the parasite *Plasmodium vivax*, and in most sub-Saharan and West African populations, nearly 100% of the population possess the Duffy negative C allele (FY*0) (34). This point mutation selectively abolishes expression of the receptor on erythrocytes, conferring a selective advantage in malaria-endemic regions, because DARC-negative erythrocytes are resistant to infection by *P. vivax*. However, DARC is also expressed on subsets of endothelial cells, binding with high affinity to chemokines of the CXC and CC classes, and for this reason DARC has been described as a chemokine “sink.” Some of these chemokines are persistently up-regulated in asthmatic airways, especially after viral infections, suggesting that this genetic variant, associated with protection against malaria in regions endemic for the parasite, may also be associated with a higher allergic diathesis, specifically among patients with asthma of African descent who are more likely to carry the C variant. In fact, in a study of four independent populations of African descent, asthma and high serum total IgE were significantly associated with the DARC variant (35). Not surprisingly, the frequency of the C allele was higher in the cohorts for which there was a larger proportion of African ancestry as measured by “ancestry informative markers” (Colombian, 32%; northeastern Brazilian, 54%; African American, 75%; African Caribbean-Barbadian, 86%).

Another example of pleiotropy in the context of asthma and allergies is the large body of research addressing the potential beneficial role of microbial exposures for development of asthma and allergies. David Strachan coined the term “hygiene hypothesis” to explain his observations of protection from hay fever among individuals with multiple older siblings, and, since then, the “hygiene hypothesis” has evolved with respect to potential underlying immunological mechanisms and the type of infectious/microbial stimuli. The observation that individuals exposed to helminth infections, which promote a Th2 immune response, have a reduced prevalence of allergic disease suggests a modification to the original hypothesis, and there is support for a modified biological model whereby chronic microbial and parasitic exposures early in life provide important regulatory signals that prevent the development of a dysregulated immune response resulting in allergic disease. Helminthic infection and exposure to certain innocuous allergens elicit a Th2-mediated

TABLE 2. COASSOCIATIONS BETWEEN ASTHMA AND SCHISTOSOMIASIS CANDIDATE GENES

Gene (Variant)	Asthma/Atopy Refs.	Schistosomiasis Refs.
Linkage studies		
Chr 5q31-q33	see 5, 6	38–40
Chr 1p22.2	41	38
Association studies		
ECP(G434C)	42, 43	44
IL10	45–48	49
IL13(Arg130Gln)	17, 50, 51	52
IL13 (C-1111T)	5, 59–63	52, 58, 64
IFNG	53, 54	55
IFNGR1	53, 56	57
STAT6	54, 65–72	58

immune response and stimulate production of IgE (reviewed in Ref. 36), and it has been demonstrated that, in regions endemic for extracellular parasitic diseases (e.g., schistosomiasis), there is a lower prevalence of atopy overall and, among patients with asthma, less severe disease, the thought being that individuals with a history of atopy appear to be protected from helminthic parasites because they mount a stronger IgE-mediated response to worm antigen and demonstrate a lower intensity of parasitic infection compared with nonatopic individuals with the same exposure (reviewed in Ref. 37).

Similar to asthma, there is extensive evidence for a genetic basis for schistosomiasis, but what is of particular interest is how many of these loci overlap with asthma susceptibility. For example, the evidence for linkage to schistosomiasis in chromosome 5q31–q33 is the precise locus where some of the most compelling evidence for linkage to asthma and atopy had been reported, and multiple polymorphisms in genes within the Th2, IgE-mediated pathway are coassociated with both traits (Table 2). Of particular interest are those examples for which the precise polymorphism(s) are associated with a lower risk of schistosomiasis and a higher risk of asthma (i.e., IL-13 Arg130Gln), further supporting an evolutionary perspective for the origins of allergic disease.

SUMMARY

The huge research efforts and expense committed to asthma genetics have arguably changed the perception about the etiology of asthma and allergic disease. Despite the complexity of asthma and heterogeneity of the definition from one study to another, progress has been made in terms of elucidating candidate genes that have been replicated using the candidate approach as well as the unbiased, robust GWAS approach. Although there have been notable shortcomings in the GWASs on asthma, including underrepresentation of non-European populations, insufficiently powered samples, and technical concerns regarding adequate coverage on the SNP chips, recent efforts toward larger scale studies in general and international efforts toward metaanalyses of GWAS data specifically are promising. The role of the Hygiene Hypothesis in the interpretation of findings and a Darwinian approach toward understanding the pleiotropic effects of candidates may be critical in advancing our understanding of the functional relevance of the asthma genes identified thus far.

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