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Asthma Genetics: From Linear to Multifactorial Approaches

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interaction, complex disease, CD14

Abstract

Asthma risk has a clear hereditary component but, unexpectedly, the majority of reported associations between genetic variants and asthma have not been consistently replicated across studies. Methodological flaws have been indicated as a possible explanation for these inconsistencies. However, an alternative explanation is that the effects of genetic variants depend on other factors whose frequency and distribution vary, both across individuals and across populations. Within this framework, we review recent advances in asthma genetics and conclude that a paradigm shift is needed, because a static model in which the DNA sequence is associated with disease risk in a linear fashion fails to consider the interdependence of the diverse components of asthma risk. We propose an integrated approach, linking sequence variation to specific phenotypic manifestations of the disease by taking into account concurrent influences from biological systems and environmental factors that interact within specific developmental windows of opportunity.

Phenotype: the expression of a specific trait, as determined by genetic and environmental influences

GENETICS OF COMPLEX DISEASES: ASTHMA AS A PARADIGM

The identification via positional cloning of the genetic variants that cause known monogenic diseases (e.g., cystic fibrosis, phenylketonuria) was among the major achievements of biomedical science in the twentieth century. Yet, to date, neither linkage nor association studies have proved as successful in dissecting the genetic components of more “complex” diseases, which do not display classical mendelian forms of inheritance but do show strong evidence of genetic involvement. These complex conditions encompass cancer as well as respiratory and cardiovascular diseases (among others) and account for most of the public health burden in many areas of the globe.

The vast majority of genetic associations are not consistently replicated across studies of complex diseases (1, 2). This lack of replication appears even more striking when one considers that, in principle, genetic association studies should be relatively robust to some of the main limitations of traditional epidemiological studies, including the ambiguous temporal sequence between exposure and outcome and the risk of unobserved confounding (3, 4). Methodological flaws—such as inflated risk of type I error, lack of statistical power, and publication bias—have been indicated as a possible explanation for these inconsistencies, and recent meta-analyses suggest that false positives and false negatives do account for a sizable fraction of conflicting genetic associations (1, 5). However, reducing the entire issue to a technical matter would oversimplify the problem. Conflicting findings are not limited to poorly designed studies; they have also emerged from genetic association studies that were well designed, properly powered, based on strong *a priori* hypotheses, and consequently robust to major methodological flaws. We need to explore alternative explanations by critically evaluating the implicit assumptions behind the replication studies.

One of these assumptions is that the main genetic effects on disease risk are homogeneous across different populations. However, this assumption is unreliable if the effects of genetic variants depend on the presence of (and interaction with) other factors whose frequency and distribution vary across individuals and populations. Studies of genetic and environmental factors as determinants of complex phenotypes in animal models support the existence of strong gene-by-environment interactions and suggest that genetic influences cannot be studied separately without considering the concurrent influence of the environment (6, 7). For example, recent studies in the mouse have conclusively demonstrated that gene-by-environment interactions explain a proportion of the variance of complex phenotypes that may be higher than that of either main effect (genetic or environmental) considered separately (7).

Within this framework, we review recent advances in asthma genetics. We do not intend to present a comprehensive review of asthma susceptibility genes, which can be found elsewhere (8–10). Instead we review recent evidence that supports the need for a paradigm shift in studying the genetics of asthma and other complex diseases. We advocate a shift from a static model, in which the DNA sequence is associated with disease risk in a linear fashion (**Figure 1a**), to an integrated approach, in which sequence variation is linked to specific phenotypic manifestations of the disease by taking into account concurrent influences from biological systems and environmental factors that interact within specific developmental windows of opportunity (**Figure 1b**).

ASTHMA GENETICS: MULTIPLE GENES FOR MULTIPLE PHENOTYPES

Even in a simple linear model such as the one depicted in **Figure 1a**, investigators will succeed in detecting an association between a genetic variant and the disease of interest only if

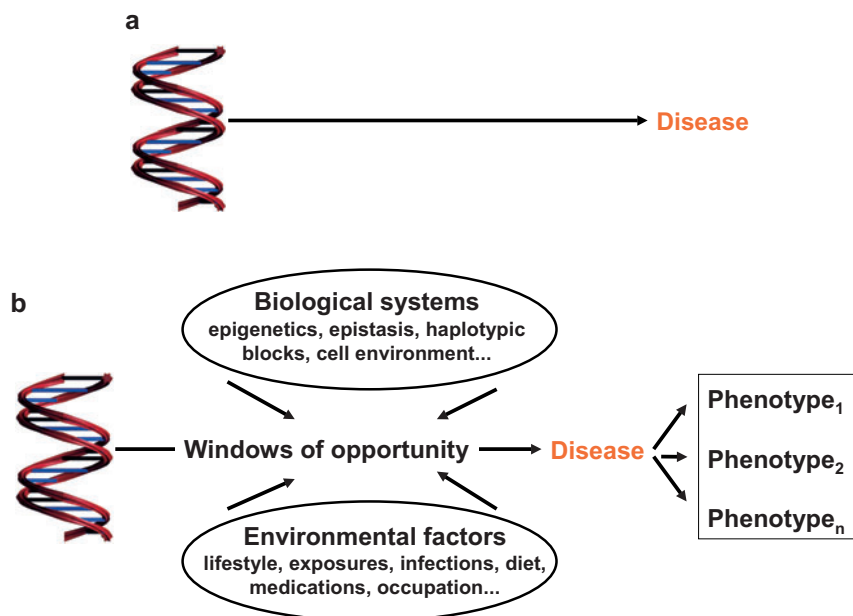


Figure 1

Two different approaches to studying genetic components of human disease. (a) In a static model, DNA sequence is associated with disease risk in a simple linear fashion. (b) An integrated approach links sequence variation to specific phenotypic manifestations of disease by taking into account concurrent influences from biological systems and environmental factors that interact within specific windows of opportunity.

the “right” gene is tested against the “right” phenotype. This is by no means a trivial issue in asthma research.

Finding (All) the Asthma Genes

To date, genetic association studies on asthma have used a candidate gene approach. This means that the genes to be tested were selected a priori on the basis of their location, function, or both. Genome screens have linked asthma-related traits to several chromosomal regions, including 2q, 5q, 6p, 12q, and 13q (11). Among them, chromosome 5q holds particular interest because it is one of the regions most consistently replicated in linkage studies, it corresponds to a region of common evolutionary ancestry that has been linked to airway eosinophil infiltration in mouse models (12), and it contains a cluster of genes—including those encoding the β_2 -

adrenoceptor (ADRB2) (13–20), CD14 (21–30), IL-4 (31, 32), and IL-13 (33, 34)—that are likely to be involved in asthma pathophysiology and, therefore, represent suitable positional and functional candidate genes for this disease. Interestingly, a recent thorough review of the literature found these four genes to be among the top ten asthma candidate genes in terms of number of positive replication studies (9). Other potential candidate genes have been identified in chromosomal regions implicated in asthma predisposition because of significant genetic linkage with asthma. For example, chromosome 6p contains the extensively studied human leukocyte antigen (HLA) genes, including *HLA-DRB1* and *HLA-DQB1* (35), as well as *TNF* and *LTA* (36, 37). Similarly, chromosome 12q harbors, among others, *VDR* (38, 39), *STAT6* (40, 41), and *NOS1* (42, 43). The products encoded by all these genes are involved in

ADRB2: β_2 adrenergic receptor
HLA: human leukocyte antigen
TNF: tumor necrosis factor
LTA: lymphotoxin α
VDR: vitamin D receptor
STAT6: signal transducer and activator of transcription 6
NOS: nitric oxide synthase

Haplotype:

sequence of alleles located on the same chromosome in a specific genomic region

ADAM33: A disintegrin and metalloproteinase domain 33

GPRA: G protein-related receptor for asthma

asthma pathways, and their genetic variation has been repeatedly associated with asthma-related traits.

It is tempting to limit the search to genes that, like these, are both located in asthma-linked chromosomal areas and mechanistically involved in asthma pathways. However, evidence has emerged that questions the specificity (i.e., the ability to capture *only* true asthma genes) and sensitivity (i.e., the ability to capture *all* the true asthma genes) of this approach. First, although many of these positional-functional candidate genes have been replicated in association with asthma in multiple studies, they are by no means free of the inconsistency that plagues most genetic associations. The validity of this argument may not be evident at first because of the large number of replication studies on these genes, which obviously inflates the number of positive replication studies. However, when one considers criteria for consistency that are independent of how frequently a specific gene has been tested—such as the ratio of positive to negative reports, consistency in the direction of the genetic association, and consistency in the specific polymorphisms/haplotypes linked to asthma—striking discrepancies across studies become evident. Second, the positional-functional candidate gene approach can miss asthma genes that are not identified (*a*) as functional, because their function is unknown or cannot be easily associated with asthma pathways, or (*b*) as positional, because their effects on asthma risk are too small to be detected in linkage studies (which have relatively low power compared with association studies) (44). *ADAM33* (45) and *GPRA* (46) are two examples of the former group, as they were identified via pure positional cloning before the function of their encoded proteins was associated with asthma pathways. However, many (if not most) asthma genes are likely to belong to the latter group. Indeed, given our limited knowledge of asthma pathophysiology, it is not unreasonable to believe that a sizable fraction of asthma genes may have

not been tested at all in association studies to date.

Thus, in their search for the asthma genes, scientists face on one side a puzzlingly mounting number of inconsistent genetic associations and on the other the intrinsic limitations of the methodological approaches that have been used so far. The result is that, as of today, not only do we not know how many of the tested genes are truly asthma genes, but we also do not know how exhaustive is the list of candidate genes identified heretofore. Greater methodological rigor and larger sample sizes for replication studies have been advocated to minimize false positives and false negatives. Similarly, the anticipation of large genome-wide association studies (47) is fueled by the expectation that they will overcome some of the limitations of linkage and candidate gene association studies by maximizing statistical power and capturing influences of novel genes and pathways on disease risk in a hypothesis-free fashion. We argue that rigorous methods and new genomic strategies, albeit necessary, will not be sufficient to provide definitive answers on the genetics of asthma and that future studies, no matter how rigorous the methodology or large the sample size, will fall short in identifying the asthma genes (let alone explaining the functional nature of these associations) if they do not embrace a new, integrated approach that recognizes the influence of additional factors on the link between genetic variation and disease. **Figure 1b** shows a model for such an approach. We propose the inclusion of three “modifying” factors:

1. Interactive influences from the environment
2. Interactive influences from biological systems
3. Developmental processes (i.e., windows of opportunity)

In addition to these factors, the importance of a precise characterization of the phenotype (rather than the disease) of interest is highlighted in the proposed model. We discuss

phenotypic characterization below and cover each of the three modifying factors in the second part of this review.

Phenotypic Characterization

All complex diseases are to some extent heterogeneous. Heterogeneity is manifest in many domains—age at onset, severity, response to treatment, intermediate phenotypes, natural history, and so on. Asthma is no exception. Indeed, this disease shows extreme heterogeneity. It can have its onset at any age; it recognizes a large number of specific and unspecific triggers; it can take any form from a mild, transient phenotype to a severe, persistent, life-threatening illness; and it is associated with a large number of intermediate phenotypes, including atopy, bronchial hyperresponsiveness, eosinophilia, and others, which can be assorted in any combination. The implications of this heterogeneity for genetic research are remarkable, as different phenotypes and clinical manifestations are likely to recognize different pathways and different genes.

The case of *ADAM33*, located on chromosome 20p13, is illustrative. The original linkage signal detected between physician-confirmed asthma and 20p13 became substantially stronger when the phenotype was further characterized by the presence of bronchial hyperresponsiveness (BHR), but not when a combined phenotype of asthma plus elevated total IgE was used (45). The preferential link of *ADAM33* with the BHR phenotype was confirmed in association analyses in the same original report (45) and is indirectly supported by previous mapping of BHR to a region of common evolutionary ancestry on mouse chromosome 2 (48). Other preferential genetic associations with specific asthma-related phenotypes have emerged from association studies, some of which are discussed in the following sections, as well as from multipoint linkage analyses of regions linked to asthma. One of them (chromosome 12q), for instance, was found to har-

bor three distinct loci that were separately linked to asthma, BHR, and forced expiratory volume in one second (FEV1) in a subset of families identified through the Childhood Asthma Management Program (49).

Another potential source of phenotypic variability in asthma is that disease inception and disease progression appear to be influenced by different factors. Some environmental exposures may even have opposite effects on asthma initiation and progression. For example, exposure to endotoxin (50) and pets (51) has been suggested to exert protective effects against asthma-related traits early in life, whereas such exposure triggers symptoms and airway inflammation once the disease has developed. The potential implications of these developmental-specific processes on genetic research are discussed later in this review. Here, it will suffice to note that, if the mechanisms involved in the inception of asthma differ from the mechanisms involved in its clinical course, genes may be found that are associated with the severity of the disease even in the absence of any detectable effect on asthma susceptibility (and vice versa). The β_2 -adrenergic receptor gene, *ADRB2*, might be one of these genes. *ADRB2* has been extensively studied in asthma for several reasons. First, it is located in a chromosomal region (5q) repeatedly linked to asthma. Second, its two best-characterized polymorphisms are missense mutations in codons 16 and 27 (Arg16Gly and Gln27Glu), which are both functionally important (17) and representative of the most common haplotypic blocks in the gene (15). Third, its encoded protein is expressed on several lung cells, including bronchial smooth muscle cells, and plays a key role in controlling bronchial tone and response to the most commonly used asthma medication (i.e., albuterol). Yet, a meta-analysis suggested no direct effects of *ADRB2* on susceptibility to asthma or BHR per se, but it did find direct influences of the Gly16 allele on asthma severity (18). A recent large prospective study reported that the same polymorphism, but opposite

BHR: bronchial hyperresponsiveness

IgE: immunoglobulin E

FEV1: forced expiratory volume in 1 sec

Allele: one of a pair or series of genes that occupy a specific position on a specific chromosome

allele (Arg16), was associated with persistence of asthma symptoms from childhood to adult life (19).

This evidence suggests that, although inconsistencies across studies on asthma genetics cannot be entirely explained by their phenotypic heterogeneity, a detailed characterization of the phenotype of interest needs to be an essential part of every association study and is likely to result in increased statistical power, better comparability across replication studies, and new insights into the multiple genetic and molecular pathways involved in this disease.

INTERACTIVE INFLUENCES FROM THE ENVIRONMENT

Before we examine possible interactive influences on asthma susceptibility, the biological and epidemiological meanings of “interactive” should be distinguished. It can be argued that any factor that affects disease susceptibility does so as part of complex biological interactions. For example, in order for genetic variants to be able to influence disease susceptibility, their DNA sequence must be placed in the context of the many other cellular elements that are required for DNA organization, recombination, transcription, translation, etc. Similarly, once a genetic variant has altered the quality or quantity of its encoded protein, that protein will be part of complex biological networks at the cell, organ, and systemic levels. However, although these biological interactions illustrate the complexity of identifying genetic effects, they do not limit in principle our ability to detect an association between a genetic variant and a disease. Indeed, they are part of the scientific rationale behind any genetic association study that seeks to identify the downstream effects of genetic variants on disease susceptibility.

In contrast, from an epidemiological standpoint, interaction is defined on the basis of effect modification and is present when the main effect of factor A on disease risk differs significantly across different levels of ex-

posure to factor B. This interaction is defined as a gene-by-environment interaction if A is a genetic factor and B is an environmental factor (or vice versa). The impacts of these potential gene-by-environment interactions on association studies are crucial because our ability to detect significant genetic effects will depend entirely on the presence or absence of specific environmental factors in the study population. Interactions are usually quantitative. In those cases, the association between factor A and disease risk has different magnitude—but the same direction—across different levels of exposure to factor B. However, interactions can also be qualitative, when the direction of the association between factor A and disease risk depends on factor B. In those cases, the same factor A will be associated with increased or decreased risk of disease according to the level of exposure to factor B. Recent work on two key innate immunity genes (*TLR2* and *CD14*) provides evidence of both quantitative and qualitative interactions in asthma.

Innate immunity genes are involved in the organism's first line of defense against microbes. Since the late 1980s (53), epidemiological observations suggested that exposure to microbial products early in life could confer protection against atopic diseases by triggering precoded maturational mechanisms of the immune system. This idea was later dubbed the hygiene hypothesis. It is thus not surprising that, as innate immunity receptors with specificity for different microbial products were characterized at the molecular level, variation in their genes was also studied in relation to asthma-related traits (54). *TLR2* is one of these receptors and has specificity for a wide variety of components of the cell membrane of gram-positive and gram-negative bacteria. In vitro studies have shown that monocytes from healthy adult volunteers carrying two copies of the T allele at position –16934 in the first intron of *TLR2* express significantly more membrane *TLR2* than monocytes from carriers of the A allele, suggesting a functional role of this polymorphism (55). In rural communities of farmers, children

carrying the TLR2/–16934T allele were found to be protected against asthma, hay fever, and atopy as compared with children carrying the AA genotype (56). However, these associations were not present among children from the same communities who were not living on farms and, therefore, were exposed to lower levels of the microbial products that are abundant in the animal-farming environment. This difference suggests the existence of quantitative interactions that modulate the effects of TLR2/–16934 on asthma and atopy.

In this framework, CD14 may arguably represent the most interesting example of qualitative interaction in asthma genetics. CD14 is a pattern recognition receptor with specificity for endotoxin [lipopolysaccharide (LPS), a component of gram-negative bacteria] and is expressed both anchored on the membrane (mCD14) of several cell types, including mature myeloid cells, and in soluble form (sCD14) in human fluids, including blood and breast milk. In 1999, the first single-nucleotide polymorphism (SNP) of the CD14 gene was identified at position –159 of the 5' flanking region (21). Since then, the functional nature of this SNP has been demonstrated by linking the CD14/–159T allele to increased sCD14 levels in plasma (21) and breast milk (57), increased mCD14 expression on antigen-presenting cells (58), and increased gene expression in functional studies (59). CD14/–159T was originally associated with protection against atopic sensitization (21). However, the relation of CD14/–159 to atopic sensitization and asthma has been inconsistent across subsequent studies. For example, consistent with the original CD14 report (21), in a Dutch urban population adults carrying the T allele were found to have on average a lower number of positive skin tests than subjects homozygous for the C allele (22). However, two large epidemiological studies from Germany failed to find any association between CD14/–159 and IgE levels or the prevalence of atopic diseases (23, 24). Even more strikingly, reports emerged regarding

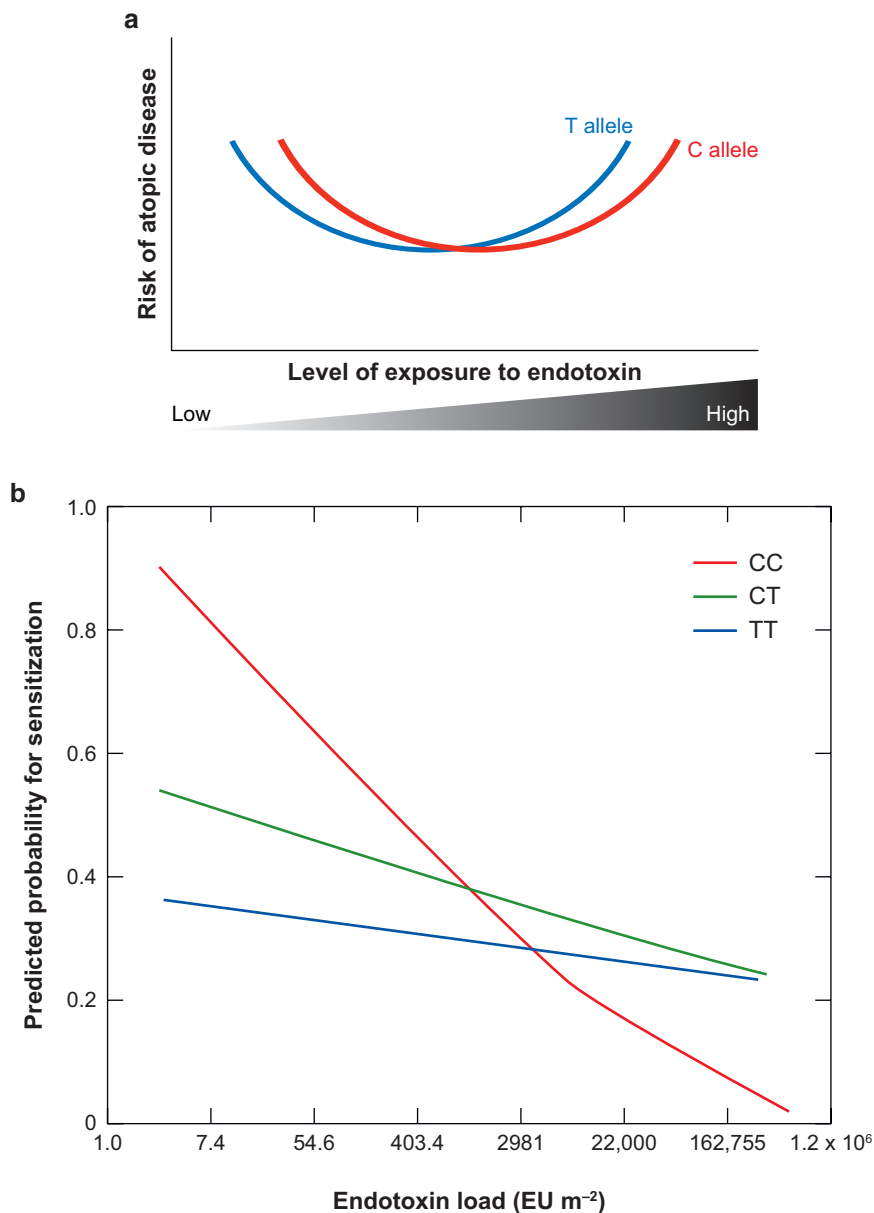
subjects supposedly heavily exposed to endotoxin that questioned the very direction of the association between CD14/–159 and atopy. These reports suggested a protective role for the C rather than the T allele (25, 26). Such conflicting findings support the existence of qualitative gene-by-environment interactions and suggest that the same allele can exert protection or enhance risk for asthma-related traits depending on the study population and its level of endotoxin exposure (**Figure 2a**).

Final proof of the existence of such interactions in asthma genetics will require interdisciplinary approaches and convincing experimental evidence of their biological bases. However, three recent studies that have investigated interactive effects of CD14/–159 and endotoxin load within the same population have provided important support to this scenario (27–29). In two of these studies, CD14/–159T was protective against atopy at low levels of endotoxin load, whereas it was associated with an increased risk for atopy at high levels of endotoxin exposure (27, 28). Similarly, in the third study, endotoxin load was inversely associated with allergic sensitization among five-year-old children carrying the CC genotype, whereas no association was evident among their peers carrying the T allele (29) (**Figure 2b**).

What have we learned from the CD14 case? Probably the most important lesson is that dissecting the complex interplay between genetic variants and environmental factors will not be possible by studying either of these main effects independent of the other. Much as we would not study the effects of a drug-metabolizing enzyme on disease progression independently of whether patients are or are not treated with that medication, genetic association studies will increasingly need to include assessments of environmental exposures that interact with specific genetic variants as an essential part of their design. This will prove a challenging task. In association studies, environmental exposures are not controlled by the investigators, can change dramatically in time and space, and are

Figure 2.

Qualitative interactions between CD14/–159 and endotoxin load in affecting risk of atopic disease. (a) A hypothetical model in which the genetic effects of CD14/–159T will be opposite at low and high levels of exposure to endotoxin. At middle ranges of exposure to endotoxin, CD14/–159 will have no effect on disease risk. (b) Fitted predicted probability curves for allergic sensitization associated with CD14/–159 genotypes in relation to environmental endotoxin load among five-year-old children from the Manchester Asthma and Allergy Study (from Reference 29).



highly sensitive to residual confounding. Yet, it is highly unlikely that we will understand the genetics of asthma until we understand the complexity of these interactions.

In this framework, pharmacogenetics studies can be considered as a special type of gene-by-environment studies, in which medication use represents the environmental exposure. Clinical applications of genetic research on

asthma management are indeed likely to come first from pharmacogenetics studies rather than observational genetic association studies for several reasons. First, in clinical trials, exposure to medication is controlled to a large extent by the investigators within protocols that include drug dose and treatment duration, whereas in association studies the measurement error in assessing environmental

Table 1 Polymorphisms in genes encoding proteins involved in regulation, metabolism, and/or response to asthma medications that have been associated with *in vivo* pharmacogenetic effects (modified from Reference 56a)

Gene	Medication class	Polymorphism	Pharmacogenetic effects
β 2-adrenoceptor (ADRB2)	β 2 agonists	Arg16Gly	Arg16 associated with increased acute response to albuterol independent of asthma;
			Arg16 associated with reduced response to regularly scheduled treatment with β 2-agonists in patients with asthma
		Thr164Ile	Ile164 associated with reduced vasodilator response to agonists
glucocorticoid receptor (GR)	glucocorticoids	Asp363Ser	Ser363 associated with higher sensitivity to exogenously administered glucocorticoids
corticotrophin-releasing hormone receptor 1 (CRHR1)		Haplotype tagging SNPs rs1876828, rs242939, and rs242941	Diplotype GAT/GAT associated with enhanced response to inhaled corticosteroids in multiple asthmatic populations
leukotriene C4 synthase (LTC4S)	leukotriene modifiers	–444/A→C	C allele associated with larger FEV1 improvements in response to zafirlukast and pranlukast
5-lipoxygenase (ALOX5)		Tandem repeats of Sp1-binding motifs	Wild-type repeat number (i.e., five) associated with FEV1 improvements in response to a selective inhibitor of ALOX5 (unknown effects on response to leukotriene receptor antagonists)

exposures is likely to be quite large most of the time. Second, findings from pharmacogenetics studies have strong potential for immediate and straightforward clinical applications, such as designing personalized pharmacological treatments based on patients' genetic background with the ultimate goal of maximizing benefits and minimizing side effects. A complete discussion of pharmacogenetics studies conducted on asthma is beyond the scope of this review and can be found elsewhere (see Reference 56a and **Table 1**). Here, we summarize some findings from studies that have investigated the effects of the ADRB2 Arg16Gly polymorphism on clinical response to β -agonists. This polymorphism has been extensively investigated in *in vitro* functional studies and has shown opposite effects on acute versus chronic response to albuterol in clinical studies. In a population-based cohort of 11-year-old children, subjects homozygous for Arg16 were found to be 5.3

times more likely to respond to albuterol than subjects homozygous for Gly16, and this association appeared to be independent of asthma status (14). In contrast, findings from the NIH-funded Beta-Adrenergic Response by Genotype (BARGE) study, the largest pharmacogenetic clinical trial on asthma, supported deleterious effects of the Arg16 allele on response to regularly scheduled treatment with albuterol, both in terms of lung function and symptoms (16). A potential explanation for these conflicting results has been proposed in the frame of the so-called dynamic model (52). According to this model, alleles (such as Gly16) that are associated with increased downregulation by endogenous catecholamines may be less responsive to acute exposure to exogenous β -agonists but relatively resistant to the tachyphylactic effects of regular β -agonist use. Findings from the BARGE and other future prospective studies on ADRB2 have the potential to impact

Tachyphylactic: the condition of having decreased response to a medicine over time, requiring larger doses to produce the original effective response

significantly the clinical use of β_2 agonists in patients with asthma.

INTERACTIVE INFLUENCES FROM BIOLOGICAL SYSTEMS

We think of the environment as the totality of circumstances that surround us and/or with which our body interfaces. Yet, the soma itself can be considered another type of environment, an internal one that provides the biological systems that are required to translate the information contained in the DNA sequence into meaningful biological events. Why should this internal environment be any less important than the external environment in influencing the relation of our genes to disease?

As discussed in the previous section, a complex sequence of biological interactions is required for a genetic variant to impact downstream disease risk and, therefore, it provides the fundamental assumption of any genetic association study. However, when key elements of these biological systems differ substantially across individuals and populations, they can influence a gene-disease relationship differently in different individuals, impacting the validity and power of replication genetic studies much like we have seen in the case of gene-by-environment interactions. The simplest example is probably represented by the interaction between polymorphisms at different loci. Indeed, studies using haplotypes have shown stronger power in defining molecular (57) and clinical (15) phenotypes as well as disease risk (37), compared with single SNP analyses, although the latter can sometimes offer statistical advantages. Interactions can also occur between variants that are located on different genes (epistasis). This has been conclusively shown in plants. For example, two quantitative trait loci located within a short 210-kb genomic segment in *Arabidopsis thaliana* were recently found to exert antagonistic epistatic interactions—that is, an allele at one locus could have opposite effects on the phenotype under study depending on which al-

lele was present at the other locus (60). Thus, in the case of epistatic interactions, main genetic effects can be completely missed or erroneously interpreted if the effects of one locus are considered separately from the concurrent effects of the other. Examples of interactions between genes involved in similar molecular pathways are also found in asthma research. For instance, IL-4 receptor alpha (IL-4R α) plays a key role in modulating both IL-4 and IL-13 receptor binding (61). Both cytokines are produced by Th2 cells and are involved in the production of IgE, an important asthma-related phenotype. Interestingly, in a Dutch population, the combination of risk genotypes for SNPs in both IL-4R α and IL-13 genes was found to confer a risk for asthma that was significantly higher than that expected under a simple multiplicative model (34). Only carriers of risk genotypes for both genes (not carriers of risk genotypes for only one gene) were at increased risk of asthma compared with carriers of no risk genotype. These findings have important implications for replication studies across different geographic areas or ethnic groups, as the power of these studies in detecting main effects of these variants will be strongly affected by the corresponding allele frequencies in different study populations.

SNP-by-SNP and gene-by-gene interactions are only two of many possible types of interactive effects exerted by biological systems. We still know very little about the potential influence of epigenetics on susceptibility to complex diseases, but processes of gene regulation are also very likely to modulate the functional impact of variation in DNA sequence, and their measurement should be considered in future functional and association studies. The cell environment is another important modulator of the functional impact of genetic variation. For example, CD14/–159 was found to affect significantly gene expression in monocytic Mono Mac 6 cells, but not in liver HepG2 cells (59), possibly because of the different milieux of transcription factors in these two cell types. Similarly, another SNP located 391 bp upstream of CD14/–159 was

found to affect sCD14 levels in opposite directions in plasma and breast milk, suggesting that the same SNP exerted different effects in hepatocytes than in mammary epithelial cells (57). To add a further layer of complexity, it is likely that different clinical manifestations of asthma are mediated to some extent by different biological systems. For example, asthma symptom attacks that are triggered by allergen exposure will involve different pathways than exacerbations that are infectious in origin. The implication is that the influence of biological systems themselves needs to be studied within pathway-specific phenotypes to maximize our ability to detect genetic determinants of disease.

Biological systems are also known to differ between males and females, and the potential modifying effect of sex on genetic associations is receiving increasing attention. Recently, heritability and genome-wide linkages for 17 quantitative traits (including four asthma-related phenotypes) were compared between males and females, and significant sex interactions were detected for the majority of them (62). Whether these interactions are related to biological or social differences (i.e., internal versus external environment) between sexes remains to be determined, but these findings indicate the importance of considering potential sex interactions in genetic association studies. Indeed, the overall impact of multiple biological systems—including haplotype effects, epistatic relationships, and pathway- and cell-specific events—should be increasingly evaluated in future genetic epidemiological research.

WINDOWS OF OPPORTUNITY

Inception of many complex diseases occurs preferentially within windows of opportunity, that is, within limited developmental and/or temporal intervals during which genetic and environmental exposures exert their maximum effect on disease risk. For instance, many children who develop asthma show early markers of allergy and Th2 polarization that

appear to be related to alterations of developmental processes of the immune system in the first years of life. Because these alterations are likely related to the presence or absence of specific environmental exposures, we conclude that, in order to understand the impact of genetic variants on asthma susceptibility, we must take into account not only the level but also the timing of exposure to environmental stimuli. Once again, this concept can be best illustrated by studies on CD14.

Consistent with the hygiene hypothesis, microbial exposure early in life has been suggested to reduce risk of atopic sensitization and asthma, and studies in animals have shown that exposure to endotoxin prior to (but not after) sensitization abolishes Th2-type responses linked to asthma phenotypes (63). In addition, plasma sCD14 levels at birth correlate directly with early production of IFN- γ and inversely with the risk for recurrent wheezing by the age of 1 year (64), indicating the importance of adequate CD14-mediated responses to endotoxin early in life for immune maturation and protection against subsequent asthma phenotypes. However, once asthma has occurred, endotoxin exposure is a well-established trigger of disease symptoms and an aggravating factor of disease severity (50). These results suggest that activation of the endotoxin receptor system needs to occur at the right time during the sensitization process to exert its protective effects on asthma risk. Consistent with this scenario, in a longitudinal cohort from Belmont, New South Wales, CD14/-159T (the allele associated with increased gene expression) was found to be protective against atopy during the school years, but this protection disappeared as the children entered adulthood (30). Indeed, among adult farmers occupationally exposed to high levels of endotoxin, the same allele was associated with an increased risk of wheezing and reduced lung function (65).

To date, the potential modifying effects of developmental processes on asthma genetics have been rarely studied. Yet, emerging evidence strongly suggests that, as in the case of

gene-by-environment and gene-by-gene interactions, genetic effects on asthma risk may vary in magnitude, and even in direction, with age and disease stage.

CONCLUSIONS

In a dispassionate analysis, the most appropriate word to describe the literature on asthma genetics is “inconsistent.” This inconsistency applies to the presence, the magnitude, and even the direction of genetic effects across studies on asthma-related traits. In this review, we have discussed three possible reasons for these inconsistencies: methodological flaws, phenotypic heterogeneity, and the existence of modifying factors. Although at present we cannot determine what proportion of inconsistent findings is accounted for by each of these issues, future genetic association studies will need to take all of them into account if final answers on the genetic components of

asthma and other complex diseases are to be found.

Association studies using rigorous statistical approaches and applying genome-wide searches on large populations have been advocated. These studies will certainly be necessary, but they will not be sufficient to resolve the discrepancies that have characterized genetic studies thus far, unless they are coupled with a detailed characterization of the phenotype and with the dissection of interactive effects from environmental factors, biological systems, and developmental processes. In this framework, a different word describes the evidence in asthma genetics: “multifactorial.” To capture this multifactorial nature, we need to decrease the emphasis on analytical approaches based on simple linear gene-disease relationships (**Figure 1a**) and move toward an integrated approach (**Figure 1b**) that reflects the heterogeneity of this complex disease.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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