Journal of Human Genetics (2009) 54, 624–628

www.nature.com/jhg

REVIEW

The era of genome-wide association studies: opportunities and challenges for asthma genetics

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In the era of genome-wide association (GWA) studies, delineating pathogenic asthma genetic pathways has provided both challenges and opportunities. Initial GWA studies on asthma and asthma-like phenotypes provided some successes in terms of ascertaining new potential asthma candidate genes. However, due to asthma having a heterogeneous etiology, replications of these genotype–phenotype association studies are generally lacking. Furthermore, genes by environment interactions are generally not considered when GWA studies are conducted. Therefore, there is a need for extensive collaborations in multi-disciplinary research fields, including different environments and populations, to investigate the functional importance of variations in the human genome in relation to asthma pathogenesis.
Journal of Human Genetics (2009) **54,** 624–628; doi:10.1038/jhg.2009.97; published online 9 October 2009

Keywords: asthma; atopy; genetics; genome-wide

INTRODUCTION

Asthma is a multifactorial, and complex, trait with a fundamental genetic predisposition in disease etiology. Genetic factors have an important role in the development of asthma as evidenced by its familial aggregation (heritability of liability).[1] A wide spectrum of epidemiological and genetic studies on asthma have been conducted worldwide in the past decade, with asthma genetics research showing many promising results. In this regard, more than 100 genes have been reported to be associated with asthma-related phenotypes by candidate gene, genome-wide linkage and recently evolved genome-wide association (GWA) studies.[2–4] However, the exact pathogenetic roles of the many distinct genetic variants reportedly associated with the complex asthma phenotypes remain obscure. Many reported associations of specific genetic variants with asthma have not been reproduced in replicate studies. In view of these inconsistencies in asthma candidate gene association studies, as well as in genome-wide linkage studies,[5] there is an evolving negative perception that candidate gene association studies are fruitless, as most findings are not reproduced, even in well-designed, sufficiently powered, epidemiological genetic studies.[6,7] It is therefore postulated that 'the long and winding road to gene discovery' in asthma[3] is still ahead.

Recently, with the advent of high through-put methods of genome-wide genotyping of hundreds of thousands of single-nucleotide polymorphisms (SNPs), and the successes of the HapMap projects,[8] GWA studies have extensively been used for complex trait genetic research[9,10] including asthma.[11] Initial results of GWA studies in complex diseases spawned optimistic views that this approach would significantly improve understanding of the etiology of asthma, would eventually identify all asthma-susceptibility genes and thus lead to better classification of asthma phenotypes and the development of novel therapies. A recent comprehensive review on asthma GWA studies,[12] described the applications of GWA data to asthma research, addressed the potential of GWA studies to identify novel asthma candidate genes and explained the factors likely to influence the success of this approach. Our present review is to highlight the challenges and opportunities confronting asthma genetics research in the era of GWA studies.

ASTHMA GENETICS

Asthma and asthma-related traits cluster in families and clearly asthmatic phenotypes have a fundamental hereditary component. There are three approaches commonly used in asthma genetic studies, namely family-based linkage, candidate gene association and GWA studies.[4] Genome-wide linkage studies have found several regions across the whole genome in linkage to asthma-related phenotypes in different ethnic populations, such as on chromosomes 2q, 2p, 3p, 5q, 6p, 11q, 12q and 17p.[13–15] Candidate gene association studies, although not consistently, have reported more than 100 genes with different sequence variants associated with asthmatic phenotypes.[2–4] The identified asthma-susceptibility genes have been implicated in several pathways that are crucial for asthma pathogenesis and that are associated with innate immunity, T-helper 2 cell immune responses, epithelial biology and hallmarks of asthma, lung function and airway responsiveness.[4]

REPLICATION IN ASTHMA GENETICS

Reproducibility in asthma genetic research, essential for establishing the credibility of a scientific finding, is indicated by replication studies

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[1]Supported by NHMRC
Received 9 August 2009; revised 7 September 2009; accepted 10 September 2009; published online 9 October 2009

that show an association of similar magnitude and direction to the same genetic variant for the same phenotype.[16] Positive findings of genotype–phenotype associations generally require cautious interpretation, as many confounders can distort the true associations. Unfortunately, few published reports of significant genetic associations in asthma have been replicated unequivocally based on the abovementioned definition of reproducibility, even for some well-designed studies with large sample sizes.[6,7] Replication is essential for establishing the credibility of a genotype–phenotype association; however, in consideration of the allelic heterogeneity (complex allelic architecture) in asthma,[17] Ober *et al.*[2,3] evaluated published replication studies on asthma genes at a gene level rather than at a specific variant level (SNP or allele level).

A large number of asthma candidate genes have been examined for SNPs in their promoter and coding regions, in which variants of functional significance are more likely located. Most of these genes have been chosen because of their chromosomal location and the potential function of their coded products in allergic and/or asthma disease processes. However, these candidate gene association studies on asthma, similar to those for other complex traits, have shown inconsistent findings at the allele level.[18,19] Martinez[19] has comprehensively discussed aspects of these inconsistent data for complex diseases, using asthma as an example. There are several possible explanations for these discordant findings including insufficient power, linkage disequilibrium, population admixture, gene/environment (G/E) and gene/gene (G/G) interactions.[6,20] However, more importantly, the lack of replication may be a result of the complex pathogenesis of asthma, which involves genetic heterogeneity and polygenic mode of inheritance, with numerous disease-causing alleles of likely incomplete penetrance, individually small effects, possible parent of origin imprinting and co-existent environmental risk factors.

Although many factors contribute to the failure of replication in asthma genetic studies, reproducibility is still the gold standard for genotype–phenotype association studies. Recommended criteria for replicating genotype–phenotype associations were published in the journal *Nature* and stated that 'the results of replication studies should be consistent (that is, finding repeated associations to the same allele) and reported for the same phenotypes to constitute true validation'.[16]

GWA STUDIES

The GWA studies are now commonly used to investigate for genotype–phenotype associations for complex traits, with an ever increasing number cataloged by the NIH website (www.genome.gov/26525384), with, for example, 23 studies added in March 2009. These GWA studies have identified new disease-susceptibility genes and revealed new pathways that contribute to disease development for a range of complex traits.[10,11,21,22]

A typical GWA study has the following key features: high density SNP chips (Affymetrix GeneChips (Affymetrix, Santa Clara, CA, USA) and Illumina BeadChips (Illumina, Cleveland, OH, USA)); a case–control design; a large sample size and stringent significance levels of association with independent replications.[23,24] The initial GWA studies have consistently shown:[9–11,23,25]

(1) that most associations do not involve previously identified candidate genes.
Many new genes or loci have been identified through GWA studies and most of these have not previously been known to be related to the complex trait, or to be associated with functional mechanisms underlying the phenotypes, in question. Some variants were located in genetic desert regions without nearby functional genes.

(2) that the identified variants explained only a fraction of attributable risk of observed phenotypes.
Most of the risk for the investigated trait was not shown to be attributable to the 'candidate' identified by the association findings from the specific GWA study.[24] The effect of individual SNPs on disease risk was small with the odds ratio less than 2 and in most studies was between 1.2 and 1.6.[26, 27] Whether these SNP associations have any clinical relevance has been questionable.[28]

(3) that the most significant SNPs do not survive the subsequent replication tests.[29]

(4) that there remain significant challenges to clarify the causal relationship between the genetic variant and the phenotypic expression using evidence identified by GWA studies. The translation of allelic associations into a better understanding of pathogenesis is particularly challenging, as the effects of variants identified by GWA studies are likely confounded by the multifactorial processes, genetic and environmental, responsible for the phenotype under investigation.

FINDINGS IN SEVERAL GWA STUDIES ON ASTHMA

Several GWA studies have been conducted for asthma and asthma-related phenotypes (Table 1). The first GWA study on asthma was published in 2007 and it was found that *ORMDL3*, located on chromosome 17q21, was a potential asthma candidate gene, by genome-wide genotyping of more than 317 000 SNP markers from 994 patients with childhood onset asthma and 1,243 non-asthmatics.[11] Several markers identified in this region were found to be independently associated with asthma in the Caucasian population and these findings were independently replicated in both a German childhood population and the British 1958 Birth Cohort.[11] A further microarray analysis for gene expression in Epstein-Barr virus-transformed lymphoblastoid cell lines showed that transcript levels of the *ORMDL3* gene on 17q21 were correlated with asthma-associated SNPs in this region.[11] Subsequently, several studies attempted to replicate the association of 17q21 in different ethnic groups and found that various SNPs were associated with asthma.[30–32] These studies also indicated that in addition to *ORMDL3*, other adjacent genes on chromosome 17q21 may also be related to asthma in ethnically diverse populations.[30,32] Moreover, another study found that the markers on chromosome 17q21 were only associated with early-onset asthma and interacted with smoking exposure.[33]

Weidinger *et al.*,[34] in a GWA study, screened SNPs for associations with total serum IgE in an initial 1530 individuals and conducted further replication studies for the most significant SNPs in four independent population-based samples (n=9769). This GWA study identified *FCER1A* as a novel susceptibility locus for total serum IgE with suggested association evidence for SNPs in *STAT6*.[34] Gudbjartsson *et al.*[29] screened SNPs for associations with eosinophil counts, as a quantitative asthma-related trait, in 9392 Icelanders. The 'top' significant SNPs were further assessed in 12 118 Europeans and 5212 East Asians, and five regions showed significant associations. The SNPs that were suggested to be associated with eosinophil counts were further investigated with atopic asthma in a collection of nine European populations and one East Asian population (7996 cases and 44 890 controls). The SNPs at *IL1RL1*, *WDR36*, *IL33* and *MYB* were found to be associated with atopic asthma.[29] A GWA study for atopic dermatitis identified an SNP (rs7927894) on chromosome 11q13.5 with increased risk for developing atopic dermatitis of 1.47 for AA homozygotes.[22] A well-established atopic dermatitis gene *FLG*[35] was not

Table 1 several genome-wide association studies on asthma-related phenotypes

Authors	*Year*	*Population*	*Replication populations*	*Findings*
Moffatt *et al.*[11]	2007	994 patients with childhood onset asthma and 1243 non-asthmatics.	German childhood population and the British 1958 Birth Cohort.	*ORMDL3*, located on chromosome 17q21, was a potential asthma candidate.
Weidinger *et al.*[34]	2008	Screened SNPs for associations with total serum IgE in initial 1530 individuals.	Replication studies for the most significant SNPs in four independent population-based samples (n=9769).	This GWA study identified *FCER1A* as novel susceptibility locus for total serum IgE.
Esparza-Gordillo *et al.*[22]	2009	In 939 individuals with atopic dermatitis and 975 controls as well as 270 complete nuclear families with two affected siblings.	Independent sample of 1363 German atopic dermatitis cases and 2739 German controls.	SNP (rs7927894) on chromosome 11q13.5 with increased risk for developing atopic dermatitis.
Gudbjartsson *et al.*[29]	2009	Investigated eosinophil counts in 9392 Icelanders.	Replicated in 12 118 Europeans and 5212 East Asians. The SNPs that were, or suggested to be, associated with eosinophil counts were further investigated in nine asthma samples (7996 cases, 44 890 controls).	The SNPs at *IL1RL1*, *WDR36*, *IL33* and *MYB* were found to be associated with atopic asthma.
Blanca E Himes *et al.*[36]	2009	359 cases from the Childhood Asthma Management Program (CAMP) and 846 genetically matched controls.	In seven white and Hispanic replication populations, two PDE4D SNPs had significant results with *P*-values less than 0.05, and five had results in the same direction as the original population but had *P*-values greater than 0.05.	The strongest region of association with asthma seen was on chromosome 5q12 in *PDE4D*.

found to be a 'top' significant signal, but was in close linkage with a significantly associated region located 156 kb away.[22] Himes *et al.*[36] conducted a GWA study on 359 cases from the Childhood Asthma Management Program (CAMP) and 846 genetically matched controls, and also sought the replications in 10 independent populations. In seven white and Hispanic replication populations, two PDE4D SNPs had significant results with *P*-values less than 0.05, and five had results in the same direction as the original population but had *P*-values greater than 0.05. This study identified *PDE4D* as an asthma candidate gene.[36]

A recent study by Rogers *et al.*[37] attempted to assess the reproducibility of 39 of these candidate genes previously reported to be associated with asthma using a genome-wide approach in 422 nuclear families (1169 members) participating in the CAMP. They investigated the replications at both allele (SNP) and gene levels. At the allele level, even with loose criteria for significance ($P<0.05$ with genome-wide correction) only 10 SNPs in 6 of 39 genes were replicated to be associated with asthma in the population, whereas at the gene level an additional 15 genes, each with at least 1 SNP, showed replications.[37] This consistent gene level replication suggested possible allelic heterogeneity in asthma genetics. Another Japanese genome-wide asthma study used multiplex PCR-Invader assay methods at 82 935 SNPs in 288 atopic asthmatics and 1032 controls (Stage 1).[38] The associations of SNPs with asthma were further investigated in samples from asthmatic families (216 families, 762 members, Stage 2) and in 541 independent patients and 744 controls (Stage 3).[38] This study failed to find any associations that attained genome-wide significance. Only one SNP in the 5′ region of *PEX19* (rs2820421) was significantly associated with asthma across all three stages of the analyses.

Collectively, the early evidence from these GWA studies on asthma suggested that replication at allele level was still inconsistent. This is not unexpected considering allelic heterogeneity[17] and gene-by-environment interactions.[20]

SEVERAL POINTS TO CONSIDER WITH GWA STUDIES IN ASTHMA GENETICS

Assumptions in GWA studies

There are two major assumptions in GWA studies. First, the hypothesis of 'common disease and common variants' that assumes that common genetic variants contribute to susceptibility to common diseases,[25] should be true for the pathogenesis of these complex traits including asthma. Although this is still a considerable topic of debate,[17] the assumption is more likely to be true for asthma, as humans experience a high prevalence of asthma, particularly in Western countries. The high prevalence is hardly explained by rare allelic variants that accumulated in a range of ethnic groups as independent mutations. Most likely, a full spectrum of rare and common alleles exist to contribute to the susceptibility to complex traits,[25] which may also be true for asthma. Second, the effect of the genetic variant of interest should be consistent in the whole population regardless of unknown environmental and genetic risk factors. The second assumption is questionable, as it is generally agreed that asthma is extremely heterogeneous and influenced by numerous biological pathways that interact with many unknown environmental factors.[18]

Replication of the association from GWA studies

Associations identified from GWA studies should be significantly replicated, particularly in an independent population. However, a review published 2 years ago suggested that non-replication and inconsistency were still a problem for GWA studies based on early empirical evidence.[39] With the ever increasing numbers of GWA studies, the replication issue requires further investigation. With the limited data from GWA studies on asthma, there is limited allelic level (SNP) replication. An example of issues related to the heterogeneity of a given genetic variant on asthma/atopy is illustrated in Figure 1. If the population investigated in an asthma GWA study has multiple environments (E1 to E5), an average 1.5 effect size of the given genetic

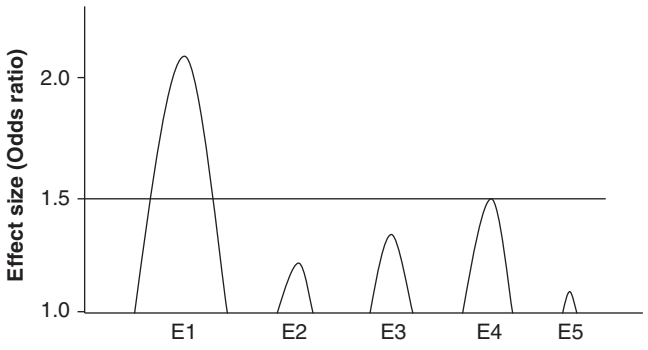

Figure 1 The effect size for a given genetic variant for asthma and atopy in five different environments (E1 to E5).

variant may be observed. The effect size would be hard to replicate in other populations. Indeed, our previous studies showing that the same SNP can produce opposite effects in different environments[7,40] highlighted the importance of investigating environmental exposures in genetic association studies. We investigated associations between allergic diseases and CD14 and CC16 polymorphisms in Finnish versus Russian Karelian women, and found that an Eastern versus Western environment seemed to exert an effect through opposite alleles on risk of allergic diseases.[7] We also investigated genetic and environmental influences and their interactions on allergy and asthma in Inuit living in Greenland and Copenhagen, and found that the magnitude and direction of genetic associations contrasted between the two geographically separate Inuit populations for *ADRB2* and *SCGB1A1*.[40] Further complexity relates to gene-by-gene interactions.[41] If the given genetic variant delivers a different effect size when different co-dominant genes are present, a population consisting of several subpopulations with different co-existent genes will present a mean effect size in that population, which is not expected to be replicated in other populations. These scenarios are more likely to be true for asthma, as many unknown environmental factors and multiple candidate genes interact in contributing to its susceptibility.

GWA studies versus candidate gene studies

If the candidate gene study is compared with a fisherman casting a small net into a pond to catch fish selected on the basis of their earlier fishing experience (hypothesis-driven), then with the GWA study approach the fisherman dries out the pond capturing all the fish. However, in the latter situation only some of the fish are of interest to the fisherman, and although the meaningful fish tend to be larger, they are not always. Similarly, GWA studies investigate million of SNP markers and multi-tests can give many positive findings by chance, providing a challenge for statisticians to ascertain the 'true' positive from many false-positive findings.

Small effects

The GWA studies consistently suggest that common diseases may be attributable to independent multiple genetic variants each of which contribute a small effect and show incomplete penetrance.[42] However, this may be distorted by GWA study design, which usually involves larger sample sizes and has 'internal' replications. To have sufficient statistical power, GWA studies tend to have large sample sizes to compensate for the power loss to obtain stringent genome-wide significance. In these large sample populations, the effect size of a given variant may be diluted by the abovementioned phenomena related to heterogeneity and polygenic etiology. GWA studies also tend to replicate the major findings before being published, and this approach may filter out the variants with a large effect size and finally report a variant with consistent small effect size that survives the replication tests. The clinical relevance of these variants with small effect identified by GWA studies is problematic.[28] However, it is expected that some variants may have large and clinically meaningful effects on asthma in some high-risk populations in high-risk environments.

Finding all asthma-susceptibility genes

It is optimistically expected that the GWA study approach will eventually ascertain most complex trait susceptibility genes. Whether a well-designed GWA asthma study, with sufficient coverage and statistical power, will discover nearly all asthma-susceptibility genes in the given ethnic population is questionable. If the condition shows heterogeneity and a multifactorial etiology, then the answer is definitely 'no', as associations identified by the GWA study will be specific for the investigated population and may not be representative of diverse populations. It will also not be possible to identify all asthma-susceptibility genes until the complex interactions of G/G and G/E on asthma pathogenesis are fully understood, possibly with other research approaches such as microarray expression and functional investigations of the genetic variants of interest. Furthermore, sufficient coverage is hard to achieve and the two commonly used platforms (Affymetrix GeneChips and Illumina BeadChips) for GWA studies have varied coverage for the common alleles (minor allele frequency $>5\%$) and do not sufficiently cover the rare alleles and other structural variants.[43] Another issue is the power to detect all the genes in one study. For asthma, as a complex, multifactorial, polygenic, trait it is expected that more than 100 genes contribute to its susceptibility.[3,4] For example, if a phenotype has two or three true risk variants and the study is designed to detect a risk for a single variant with an 80% power, the statistical power to detect the two and three true causal variants for the given phenotype is 0.64 (0.8^2) and 0.51 (0.8^3), respectively.[44] Therefore, from the mathematical point of view, a GWA study may not have sufficient power to detect all the genes in one study, even with a large sample size.

FUTURE DIRECTIONS

1. Well-designed GWA studies with sufficient power, avoiding biases in recruiting cases and controls and properly addressing population admixture, should be conducted for asthma and its related phenotypes to identify more novel candidate genes. These potentially identifiable genes are 'low-hanging fruits' in GWA studies. They should be more penetrant and less heterogeneous and are relatively uniform in terms of their genetic effects with less variation across individuals or across populations. New genes and pathway are expected to be discovered by GWA studies and these findings will significantly improve the understanding of asthma pathogenesis.

2. There is the need to apply functional genomics approaches such as targeting transcription of specific genes and investigating gene expression by microarray analyses, to better understand how specific genetic variants contribute to the pathogenesis of asthma. Functional studies are important to elucidate the biological mechanisms through which the genetic variants contribute to the asthma susceptibility.

3. Considering the polygenic, multifactorial, mode of inheritance of asthma, it is useful to identify susceptible genetic variants in environment-specific and population-specific contexts. The corresponding statistical method should be developed to investigate the G/E interactions in GWA studies,[45] particularly for asthma.

4. Data on GWA studies should be shared publicly.[46] Meta-analyses can be conducted incorporating possible G/G and G/E interactions to assess the true effects of the previously reported, potentially important, asthma candidate genes.

5. The ultimate goal of asthma genetic studies is to incorporate genetic information into the clinical management of asthma. The findings from genetic studies in asthma will potentially identify treatment options, predict disease outcomes and identify at-risk individuals amenable to preventive strategies.

CONCLUSIONS

Multiple genetic and environmental risk factors are involved in the pathogenesis and pathophysiology of asthma. GWA studies will identify novel asthma candidate genes or confirm the importance of several previously found asthma candidate genes, which are 'low-hanging fruits' for asthma genetic research. Undoubtedly, the era of GWA studies will significantly improve the understanding of asthma pathogenesis. However, due to the multifactorial and polygenic modes of inheritance of asthma, considerable research still needs to be done before integrating genetic findings into clinical management of individuals at risk for asthma.

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