

Genetic risks and childhood-onset asthma

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List of Design Committee Members: William Cookson, MD, DPhil, Miriam Moffatt, DPhil, and David P. Strachan, MD

Activity Objectives

1. To understand different aspects of genetic risk, including individual, population, and familial risks.
2. To access factors that may affect genetic risk of asthma.
3. To discuss heritability as it relates to asthma.

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Recent large-scale genome-wide association studies have successfully identified several genetic loci that influence asthma susceptibility. The loci thus far identified confer a high population attributable risk for childhood-onset disease and have provided a better understanding of the primary mechanisms underlying asthma and a clear focus for new therapies to treat the disease. The loci are of limited utility for diagnostic or predictive genetic testing. This review considers different aspects of genetic risk, including individual, population, and familial risks, and explores how these different measures interact and how the next generation of genetic studies might be best designed. (J Allergy Clin Immunol 2011;128:266-70.)

Key words: *Asthma, childhood, genetic, familial, population, risk, genome-wide association*

The primary motivation for genetic investigations of complex disease has been to develop a systematic understanding of otherwise impenetrable disease processes. In this aspect large-

Abbreviations used

GWAS: Genome-wide association study

OR: Odds ratio

PARF: Population attributable risk fraction

SNP: Single nucleotide polymorphism

scale *genome-wide association studies* (GWASs) have been remarkably successful, so that by the end of 2010, 1212 genome-wide associations to 210 complex disease traits had been established with a stringent genome-wide significance ($P < 5 \times 10^{-8}$; www.genome.gov/gwastudies).¹

Although the ability to predict risk from the result of a GWAS has been of secondary importance, the success of these studies and the rapidly decreasing costs of sequencing individual human genomes have led to a high level of public interest in using DNA to provide an individual risk of disease, with the expectation that high-risk subjects might benefit from preventive and therapeutic interventions.² As a consequence, there are more than 650 currently listed articles in PubMed with “personalized” and “medicine” in the title, and several companies have offered direct-to-consumer “personal genome services” that provide genetic information from individual genomes.³

It is not surprising that there has been resistance to simplistic promulgation of individual genetic screening, and issue has been taken with the interpretation of particular statistical risk estimates,² the quality control of genotyping, and the need to establish the clinical validity of predictive tests from appropriately designed trials.³ Despite these concerns, there is no shortage of

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Terms in boldface and italics are defined in the glossary on page 267.

persons who want to uncover the secrets of their genomes, and the need is growing for physicians and their patients to understand the limitations and real utility of genomic information.⁴

The aim of this review is to help in that process by using the exemplar of asthma to consider what genetics actually tell us about disease susceptibility and whether that information is of help in the management of disease. We will try to engender a sense of perspective by looking at current approaches to nongenetic risk factors for asthma and other complex disorders.

GENETIC RISK OF ASTHMA

There have been a number of GWASs for asthma,⁵⁻¹³ the largest of which contained 10,000 cases of asthma and 16,000 control subjects and was reported by the GABRIEL consortium.¹⁴ The GABRIEL subjects came from clinics and epidemiologic surveys in Europe and could be divided by age of onset, severity, and occupational exposure. The GWASs have identified 10 loci with genome-wide significance. Childhood-onset asthma consistently shows the strongest genetic effects,¹⁴ and this review will concentrate on this subtype of disease. The asthma genetics community is currently consolidating all the data from these international GWASs, so that the number of asthmatic subjects included in a single meta-analysis will increase from 10,000 to 25,000. The experience of other diseases¹⁵ is that this exercise might double the number of verified asthma-susceptibility loci. However, it is likely that the strongest and most consistent genetic effects on asthma have already been identified.

The outstanding finding from asthma GWASs has been the *GSDMB-ORMDL3* locus on chromosome 17q21.^{5,14} In our initial GWAS,⁵ the **odds ratio** (OR) for the most strongly associated single nucleotide polymorphism (SNP) in cases of childhood asthma recruited through hospital clinics and compared with control subjects was 1.84 per additional risk allele. (To facilitate comparisons, we will present all ORs as positive effects rather than their reciprocals throughout the review: the original directed estimates can be gathered from the references.) The per-allele OR at this locus for childhood-onset asthma was 1.32 in the GABRIEL consortium GWAS, which contained a high proportion of asthmatic subjects from epidemiologic surveys,¹⁴ and was 2.02 in subjects with severe therapy-resistant asthma attending tertiary referral clinics.¹⁶ There was no effect of the locus in subjects with adult-onset asthma.¹⁴ These substantial differences in ORs

reflect the different ascertainment criteria and types of asthma for each study and show that genetic risk is highest in children with early-onset disease that is resistant to therapy and that environmental factors are important modifiers of this risk.

The size of these effects can be put in perspective by considering well-known epidemiologic effects on asthma. The OR for the protective effect of farming on the prevalence of childhood asthma in the PARSIFAL study was 0.62 (inverse, 1.61), and it was 0.86 (1.16) in the GABRIEL advanced survey.¹⁷ At school age, the typical ORs associated with smoking by either parent were 1.24 for wheezing in the last year and 1.21 for asthma.¹⁸ Although these figures are derived from differing populations, it seems fair to observe that genetic risks are comparable with those conferred by known environmental factors.

GENETIC RISKS IN POPULATIONS

Population attributable risk, which is also known as the population attributable risk fraction (PARF), is widely used to estimate the burden of a risk factor in population surveys. Applied to genetic studies, the PARF estimates the fraction of cases that would not occur if no one in the population carried the risk allele.²

Use of the PARF as a measure of association in GWASs is attractive because typically such studies use a panel of tag SNPs to detect associations of diseases or traits with common haplotypes. The causative variant might not be the same as the measured or imputed tag SNP, but it can be shown that the PARF is constant for all SNPs, regardless of risk allele frequency, that are in complete *linkage disequilibrium* with the causative variant.¹⁹ Furthermore, the PARF at a given locus encapsulates not just the marginal (gene-only) association but also the possibility that this association is concentrated wholly or partially among a subgroup exposed to an environmental factor (gene-environment interaction) or another genetic risk variant (gene-gene interaction).

We used the PARF to model how genetic variants at the 6 GABRIEL GWAS significant loci jointly affected the burden of childhood disease in the GABRIEL populations.¹⁴ This estimate indicated that 38% of childhood-onset asthma was attributable to SNP combinations at these loci, and in an independent population-based replication sample, we found the same SNPs accounted for 49% of asthma risk from birth to middle age.¹⁴ These findings indicated that the genetic variants at these loci

GLOSSARY

CPG: Shorthand for cytosine-phosphate-guanine or a cytosine base located adjacent to a guanine base in a linear sequence (as opposed to cytosine forming chemical bonds to guanine on the opposite DNA strand).

EPIGENETICS: A field of science that studies heritable changes caused by the activation and deactivation of genes without any change in the underlying DNA sequence of the organism.

EXOME: The part of the genome formed by exons (the coding portions of a nucleic acid).

GENOME-WIDE ASSOCIATION STUDY (GWAS): Cohorts of patients with and without a given disease are examined across the entire genome for single nucleotide polymorphisms that are overrepresented in patients with the disease. This identifies regions of the genome that contain a variant gene or genes that confer disease susceptibility.

Candidate genes are then selected based on how closely they are associated with the disease and whether their biologic function correlates with the disease under study.

LINKAGE DISEQUILIBRIUM: When alleles at adjacent loci are often found together on the same chromosomal segment more frequently than predicted by chance (ie, alleles that are inherited in clusters).

LOCUS: The position in a chromosome of a particular gene or allele.

ODDS RATIO (OR): The odds of exposure in the group with disease divided by the odds of exposure in the control group. ORs are generated in case-control studies in which subjects are selected on the basis of disease status. The OR is often used to approximate the relative risk.

PENETRANCE: The proportion of subjects of a particular genotype that express its phenotypic effect in a given environment.

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and their presumed functional outcomes had a significant effect on the burden of asthma in the community.

A large PARF does not imply that the measured genotypes can provide clinically useful risk predictions,² but it does suggest that therapies directed at the functional consequences of the genes identified by the GWASs are likely to be of benefit to many asthmatic subjects.

GENETIC RISK IN INDIVIDUALS

The GABRIEL GWAS represents the largest set of data currently analyzed for the ability of genetic variants to determine the risk of a subject having asthma.¹⁴ We assessed individual risk for the 7 SNPs associated with childhood asthma in a classification analysis based on a logistic regression model (Fig 1). Taking as a cut point the risk score exceeded by 25% of the nonasthmatic population (specificity, 75%), the sensitivity of classifying asthma was just 35% (false-negative rate, 65%). Assuming the prevalence of asthma in the general population is between 5% and 10%, the positive predictive value of a genetic risk score of greater than this arbitrary cut point would be in the range of 6.9% to 13.5%. The clear conclusion from this is that the common genetic polymorphisms measured or imputed by GWASs are unlikely to be of value in predicting which children or infants are at risk of having asthma.

Rare SNPs that are strongly associated with disease will contribute much more to prediction of individual risk and familial aggregation than common SNPs with weaker effects, although both might be comparable in terms of their effect on population risk (PARF). Before considering the implications of this, we need to explore the mechanics of genetic risk in families and populations.

FAMILIAL RISK AND HERITABILITY

A third component of genetic risk relates to families. The increase in risk in the siblings of affected subjects is known as the familial relative risk. A related measurement is that of heritability, which is defined as the variance in a complex trait caused by inherited factors. The heritability of childhood asthma has been estimated to be as high as 60%, but the variance in asthma prevalence accounted for by the loci in our large-scale GWAS was only 4%. This is consistent with other complex human traits, such as height,²⁰ Crohn disease,²¹ and obesity,²² and the search for the “missing heritability” is a major current focus for geneticists who study complex diseases.²³

Applying heritability estimates from carefully crafted family studies to general population samples, where there will be much environmental variation, is inherently unreliable, but there is a more important and underappreciated statistical problem with considering individual, population, and familial risks as interchangeable. GWASs are designed to investigate SNPs with minor allele frequencies of greater than 5% because the power to detect genetic associations is only high when a disease and its underlying susceptibility alleles are both common²⁴ and because parametric statistical tests of association are unreliable when allele counts are sparse.

Asthma has been investigated more intensively than many diseases by linkage studies based on families and pedigrees, and several previously unsuspected genes were identified by these analyses.²⁵ Population-based studies have generally failed to

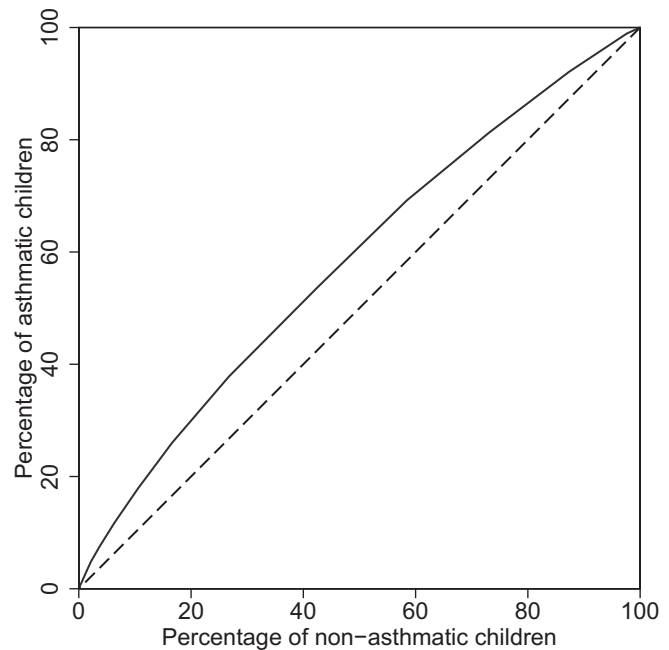


FIG 1. Receiver operating characteristics (ROC) curve for a genetic risk score for childhood asthma based on the genome-wide significant loci identified in the GABRIEL meta-analysis. Data from Moffatt et al.¹⁴

confirm the importance of these positionally cloned loci for determining asthma risk in the general population.^{14,26} This apparent paradox might be reconciled by noting that private mutations, which are rare in the general population, might underlie the clustering of asthma within families. The search for missing heritability and for the ability to predict individual risk of disease should perhaps therefore be concentrated on rare mutations with high *penetrance*.

FINDING RARE MUTATIONS

GWASs are generally directed at SNPs with minor allele frequencies of greater than 5% or 10%, although with the extension of imputation from the HapMap panel to more extensive reference panels derived from the 1000 Genomes project, it has recently become possible to test associations with SNPs with allele frequencies as rare as 1%. The power to detect genetic associations is not only higher when the underlying susceptibility alleles are common²⁴ but also when the per-allele OR is higher.²⁷ For a locus with a given PARF, the power to detect this PARF as being genome-wide significant is greater for rare SNPs with large effects than for more common SNPs with weaker associations with disease. It is possible that elaboration of existing asthma GWASs by means of imputation to 1000 Genomes reference panels might shed important insights on whether asthma risks at established loci are due to rarer or more common variants.

Full genomic sequencing of multiple diseased subjects is the gold standard for identifying rare mutations, but it is still too expensive for general application. However, whole-exome sequencing with enrichment of exonic sequences by means of hybridization before ultra-deep sequencing²⁸ has proved to be extremely effective in the identification of diseases with rare mutations^{29,30} and merits application to complex disorders, such as asthma.

Exome sequencing has been successful with very small subject samples in Mendelian disorders because it is safe to assume that

these diseases are due to mutation rather than to common variants affecting, for example, transcription or subtle aspects of gene function. This assumption allows noncoding mutations to be ignored and common coding mutations to be filtered out of the analysis by means of comparison with published databases, such as HapMap and the 1000 Genomes project. This assumption is unlikely to apply to complex diseases, such as asthma, but it is still possible that patients with severe or strongly familial forms of the disease might be enriched for mutations in particular genes or pathways of relevance to more common forms. It is also possible that detailed resequencing of loci already mapped might find rare functional alleles that are present almost exclusively among asthmatic subjects and thereby confer substantial familial and individual risk.

THE EPIGENOME

Epigenetic effects mediated through mechanisms other than sequence variation are another possible cause of familial clustering. The patterns of gene expression that determine cellular type and function become stably restricted during development, partly through methylation of *CpG* sequences and gene silencing.³¹ Islands of *CpG* sequences are positioned at the 5' untranslated regions of many human genes.³² About one fifth of these islands are variably methylated, and in one third methylation status correlates with transcript abundance.³³ Abnormalities of DNA methylation are well recognized in single-gene disorders and in cancer,³⁴ and it is postulated that *epigenetic* changes in methylation might be of importance to common human diseases,³⁴ such as asthma and chronic obstructive pulmonary disease.

Although it is very likely that genome-wide studies of methylation status at various loci might identify new genes and pathways that mediate airway diseases, it is important to recognize that age,^{33,35,36} sex,^{33,35} genetic polymorphisms,^{37,38} and environmental factors^{36,38} have all been strongly associated with altered methylation at selected loci. The relative contribution of these factors to methylation at loci genome wide is not known, and it is not certain to what extent true epigenetic inheritance with transmission between generations takes place. These factors will have to be taken into account if methylation changes at individual loci are to be used to help in the understanding of complex diseases.

OTHER PHENOTYPES AT RISK

In this review we have discussed the risk of asthma, as defined by a physician's diagnosis in most genetic studies and by age of onset and country of origin in the GABRIEL GWAS.¹⁴ Other phenotypes might also be of interest, including severity; prognosis; different childhood wheezing phenotypes, such as transient and persistent symptoms³⁹; atopy; and response to therapy.

The use of pharmacogenetics to tailor drug treatments to a person's genetic profile has been strongly promoted, but the present limited state of knowledge suggests that it might be another 15 to 20 years before their use is established. Large-scale clinical trials are needed to assess the cost-effectiveness of developing and using pharmacogenetic therapies (<http://royalsociety.org/Personalised-medicines-hopes-and-realities/>). More promisingly, rare high-penetrance genetic variants have been shown to mediate adverse reactions to drugs, with a likely significant effect on clinical care.⁴⁰

CONCLUSIONS

Recent genetic association studies for asthma have discovered genetic loci with high PARF but limited utility for diagnostic or predictive genetic testing. Next-generation sequencing strategies might identify rare variants that are of clinical utility, but this utility will be limited by the presence or absence of effectual courses of action arising from genetic information. It is salutary that the communication of personal risk to patients has had minimal effectiveness in promoting their informed decision making.⁴¹

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