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Genetics and pulmonary medicine: asthma

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It has been known for centuries that asthma is a disease which runs in families. Initial attempts to quantify the contribution of the heritable element of asthma or its intermediate phenotypes were performed in extended pedigrees. These were complemented by twin studies which examined the risk of asthma developing in monozygotic or dizygotic twin siblings of an asthmatic individual. If the development of asthma is genetically determined, one would predict that the risk of a monozygotic twin sibling developing asthma would be greater than that of a dizygotic twin sibling. This is because monozygotic twins are completely identical for all genetic factors and dizygotic twins share on average half their genes: the contribution of environmental factors should be the same for both monozygotic and dizygotic twins. The estimates of heritability of asthma itself from twin studies have agreed reasonably well with those obtained in family studies and have ranged from around 30% to 70%. In one large study of 7000 same sex twin pairs concordance rates were 19% for asthma in monozygotic twin pairs compared with 4.8% for asthma in dizygotic twin pairs.[1]

In addition to studying asthma per se, estimates for heritability can also be derived for intermediate phenotypes. The major intermediate phenotypes that have been studied are total IgE, atopy, and bronchial hyperresponsiveness. In general, the heritability of total IgE appears to be greater than for atopy, asthma or bronchial hyperresponsiveness, with estimates of the genetic component of the variability of IgE around 70% whilst figures of 30–50% are typical for the other intermediate phenotypes. It can be seen that these values agree reasonably well with those obtained from twin studies.

Although it was clear from these early studies that there is a major heritable component in asthma, remarkably little progress was made in attempting to define the genetic factors underlying the risk of developing asthma until the last 10 years. Indeed, until the last two years there has been little consensus on chromosomal regions or genes likely to be important in contributing to the heritability of asthma. However, the publication of a number of recent studies has at last brought a degree of consensus to the field. Asthma genetics is now at an exciting stage where real progress is likely to be made over the next five years. This review will summarise data published to date and will try to address some of the difficulties inherent in studying the genetics of allergic disease.

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Why study asthma genetics?

There has been major investment by both research funding bodies and industry in asthma genetic studies over the last five years. Given that the identity of some of the major genes involved in determining the risk of developing asthma will soon become clearer, it is worth pausing to ask what use can be made of the information being generated.

The 1980s was the era of determining genes underlying the common single gene disorders such as cystic fibrosis.[2] Knowledge of the structure and function of CFTR and the functional mutations present in patients with cystic fibrosis has been of tremendous scientific value but so far, in therapeutic terms, has made little difference to the management of the disease. This begs the question that, if we are unable to obtain marked benefit from knowledge of the molecular basis for a single gene defect disease, is it really likely that knowing about some of the genes responsible for the molecular basis of asthma will be of any value? The usual answers to this question are that knowledge of the genes (and their mutations) will help (1) to define pathophysiological mechanisms underlying the disease, (2) may suggest novel targets for drug treatment, and (3) may allow the definition of "at risk" populations who may benefit from early intervention—for example, by allergen avoidance or early treatment with anti-inflammatory drugs.[3] However, if one takes a critical view of the data available at present, one would have to say that the identified genes for which good data exist are genes that one might have predicted would be important even in the absence of any information from genetic studies—for example, the Th2 cytokine family. As far as "at risk" populations are concerned, we are probably still no better at predicting the risk of an individual developing asthma from genetic data than we would be by simply asking an individual if they have a strong family history of asthma or allergic disease. Finally, with regard to allergen avoidance and early treatment, whilst there are some encouraging data to suggest that early allergen avoidance may be beneficial, the degree of benefit is far from clear[4]; similarly, there are

Table 1 Disease phenotypes in asthma genetic studies

Asthma
Doctor diagnosed
Questionnaire diagnosed
Video questionnaire diagnosed
Period prevalence of wheezing
Intermediate phenotypes
Total IgE
Specific IgE
Bronchial hyperreactivity
Atopy (e.g. skin prick test positivity)
Eosinophil counts

no adequate studies of early treatment with anti-inflammatory drugs in a defined "at risk" population looking at disease progression. Perhaps judging the real value of genetic studies of asthma will only be apparent in a few years' time when these issues have been clarified.

Disease phenotypes

If one believes that useful information will come from genetic studies, it is crucial that those studies are carefully designed to maximise the ability to detect important genes. A critical issue in genetic studies of asthma and/or allergic disease is the question of which phenotype to study (table 1).[5]

There are a number of important points to consider about disease phenotype. Firstly, the genes controlling an individual variable—for example, total IgE levels—will not necessarily be the same as the genes controlling other variables—for example, airway reactivity—and, whilst both sets of genes might be expected to be important in the risk of developing asthma, the overall contribution to asthma is likely to be lower than the contribution of those genes to the intermediate phenotypes. The interdependence of these variables has been examined in a number of studies: in general, as one would predict, it has been found that IgE is a major contributor to atopy whereas bronchial hyperreactivity is more closely associated with asthma rather than atopy per se.[6] It follows that studies must be carefully designed to look at relevant phenotypes: this is critically important in the statistical analysis of genetic studies because increasing the number of phenotypes studied will mean that a concomitant change must be made in the level of statistical significance accepted to prevent false positive results arising. Secondly, it is important to remember that the genes which are responsible for disease initiation may not be the same genes that are responsible for disease progression or the determination of disease severity in an individual.[7] Indeed, the intermediate phenotypes one might consider important for studies looking specifically at disease progression may be very different—for example, one might look at change in lung function with time, hospitalisation rates, or markers of airway remodelling such as the development of irreversible airflow obstruction. It follows that genes important in a variable such as disease progression are unlikely to be identified by genome screens using the intermediate phenotypes given in table 1. Disease severity genes *may* be identified in genome screens using the phenotypes listed above where asthma is considered not as a categorical variable (that is, asthma present or absent), but as a quantitative trait (that is, where an "asthma score" is generated which will reflect markers of severity.[8] Finally, issues of population heterogeneity must not be forgotten. The genes important in causing asthma in one racial group may be different from the genes in another group because the frequency of mutations within important genes often differs markedly between population groups. Environmental exposures will also differ markedly depending on the population studied.[9] Given the dependence on both genetic and environmental factors, unless one studies an "at risk" population from the environmental viewpoint one will have difficulty in identifying genes important in the host response to that environmental factor. In practice this means that genome screens or association studies performed on populations which have not been selected on the basis of environmental exposure will only identify genes for which the relevant environmental exposure is more or less ubiquitous in that population. To take an extreme example, studying a random population selected from a region in the UK would stand a reasonable chance of identifying genes important in determining the response to house dust mite but would be highly unlikely to identify genes important in the development of isocyanate induced asthma.

Asthma genetics

Despite the problems discussed above, progress has been made in identifying potential candidate genes and chromosomal regions showing linkage. These data are summarised below.

GENOME SCREENS

There have been three completed genome screens to date in which asthma was a major phenotype studied, although others are currently in progress and data will shortly be available from these other studies. In addition, there have been a number of limited screens of individual chromosomes which have been useful in reassessing regions of linkage shown by the major genome screens. The first genome screen to be reported was performed by the Oxford group in a population based in Western Australia.[10] This screen identified six chromosomal regions of interest for the phenotypes studied: Monte Carlo simulations (a method of statistical modelling performed to attempt to identify the likelihood of positive findings being true positives) suggested that only one of these six was likely to be a false positive. The second genome screen to be reported was from the CSGA (Collaborative Study on the Genetics of Asthma)[11]; this screen was performed in three racial groups and four different centres in the USA. Separating the racial groups studied (here Hispanic, Caucasian and Afro-American) prevents problems of genetic heterogeneity but reduces the potential power of the study by increasing the number of comparisons whilst reducing the size of the groups. Finally, a genome screen has been completed

Table 2 Summary of linkage studies (as of May 1998)

Chromosome	*Phenotypes*	*Oxford*	*CSGA*	*Other (references)*	*Candidates*
4	IgE, BHR	+			
5	IgE, BHR	+	+	+[12–14]	IL-4,5,9,13, β_2AR
6	Eos	+			MHC, TNFα
7	BHR	+	+		
11	Atopy, asthma	+	+	+[15 16]	FcεR1β, CC16
12	Asthma			+[17]	IFN-γ
13	Atopy	+			
14	Atopy	+		+	TCR-α
16	Atopy, IgE, asthma	+		+	IL-4Rα

Only chromosomal regions where at least one study has reported an LOD of 3 (or equivalent) are shown. Eos = eosinophil count.

on the population of Tristan da Cunha although data are not currently in the public domain from this study. Tristan da Cunha is an ideal inbred population to study. All the islanders live in a single community with very similar environmental exposures. The islanders can trace their ancestry back to seven founder families. One potential problem of the study is that genes important in these families may not be important in the population outside Tristan da Cunha; to avoid this difficulty a replication sample has been collected in Canada. A summary of the data from these genome screens and relevant chromosomal screens is shown in table 2.[12–17]

A number of points are worth considering when interpreting data from genome screens. The two published genome screens used highly polymorphic micro satellite markers at a spacing of 10–20 cM across the whole genome; hence, individuals were genotyped for around 300 markers. The best estimates of linkage disequilibrium within an outbred population are that it is unlikely to extend over more than 1–2 cM, although in inbred populations—for example, the Hutterites in the CSGA study and the Tristan da Cunha population—linkage disequilibrium may exist over 10–20 cM.[18] Hence, using a screen with markers based at 10–20 cM apart has a real risk of missing important sites of linkage in an outbred population due to poor coverage. The development of an SNP (single nucleotide polymorphism) marker set of 2000 or more markers for genotyping will markedly increase the resolution of genome screens in the near future. Secondly, it is important to look at the design of studies involving a genome screen. For example, performing a genome screen in large extended families may give useful information about genes important in those families but may not be relevant to the population at large. On the other hand, using the nuclear family method of collecting either sib pairs or trios (parents and an affected child), large sample sizes (500+ sib pairs) will be required to find genes of moderate effect in a disease of relatively low heritability such as asthma.[19] Notwithstanding these comments, the genome and chromosome screen data obtained to date give some clear areas of consensus. Most people would now agree that there is an important region on chromosome 5q23–31 controlling the production of IgE and the level of bronchial hyperreactivity, and also that an important region on chromosome 11q is involved in determining the risk of developing atopy.[12–14] In addition, there is reasonable consensus that a region associated with the MHC locus on chromosome 6 is important in atopy,[20] and a number of reports have implicated a region on chromosome 12 to be important in asthma.[17]

Having identified chromosomal regions of interest, the next step is to perform fine mapping of the region with the aim of identifying novel genes. However, because the heritability of asthma is such that genetic studies are operating at the limit of resolution for fine mapping (λs, the risk of disease in the sibling of an affected individual compared to the general population risk, is around 2 for asthma),[19] being able to move from a chromosomal region showing linkage to a close approximation of the position of the relevant gene on a physical map is extremely difficult. In view of this, a number of groups have tried an "inspirational" cloning approach, looking for genes potentially relevant to the disease in the chromosomal region of interest and screening those genes for mutations directly rather than using classical positional cloning approaches. With the increased information available from the genome projects (approximately 50% of genes are now either known or tagged in the cDNA and EST databases), the risk of missing important genes using this approach is reducing and will continue to do so as the databases become more complete.

Table 3 Candidate genes for asthma and related phenotypes

Chromosome	*Candidate gene*	*Association*	*Known polymorphisms*	*Reference*
5	IL-4	IgE	-590 C-T	21
	IL-9	IgE	M99T	22
	β_2AR	IgE, BHR, treatment response, nocturnal asthma	Codon 16, 27	23–29
6	HLA	Specific IgE	DR alleles	30
	TNFα	?Asthma	-308	20, 31
10	5-LO	Asthma	Promoter alleles 1–5	32
11	CC16	Asthma	?	33
	FcεR1β	Atopy, IgE, BHR	Codon 181, 183, 237	34, 35
14	TCRα	Specific IgE	?	36
16	IL-4α	Atopy	576 R	37, 39

CANDIDATE GENE APPROACHES

Candidate gene approaches have provided us to date with the best guesses currently available regarding genes likely to be important in determining aspects of the asthma phenotype. Nonetheless, this approach can only tell us about genes which we already know exist: by definition the approach will not discover novel genes whose products may be important in disease pathophysiology. A summary of the major candidate genes that have been studied is given in table 3.

For a candidate gene to be a viable candidate in asthma a number of criteria must be fulfilled.[7] Firstly, the gene product must be for a protein likely to be relevant to disease pathophysiology. Secondly, the gene must contain either within its coding or regulatory sequence variations which produce functional differences in the amount or structure of the protein. Thirdly, these structural variants must be related to the disease in association studies. Finally, ideally one would want to see linkage of the chromosomal region containing the

candidate gene to markers of the asthma phenotype in genome screens.

There have been a number of problems with association studies looking at candidate genes and these are important to note. Firstly, there is probably publication bias in the literature: it is much easier to publish a positive finding than a negative study and hence the true significance of some of the associations reported to date is unclear. Secondly, the design of candidate gene studies is critical. Many positive studies have reported relatively low levels of significance: if a number of different phenotypes have been studied and a number of structural variants in a candidate gene have been studied the possibility of false positives arising by chance is high. Thirdly, given the sample size used in many candidate gene association studies of allergic disease in the general population, polymorphisms which are likely to have a relatively small effect will only be detected if they exist with a high prevalence. Finally, population heterogeneity is frequently a problem with allelic association studies. For example, studies may pick a population of asthmatics taken from a hospital clinic and then use controls taken from a much less well characterised population (such as blood donors) which may not be age, sex, and racial group matched and which may also contain individuals with undiagnosed asthma. A number of groups including our own has taken the approach of acquiring large random samples from the population at large to try and get round these difficulties.

There is, despite these issues, consensus over a number of candidate genes. The role of the β_2 adrenoceptor in determining disease response and possibly also disease severity is now reasonably well accepted, although the possibility that this gene contributes to the initiation of asthma per se seems unlikely, the observed relationship between IgE levels, childhood asthma, and the codon 27 polymorphism probably being due to linkage disequilibrium with another gene controlling IgE in this region of 5q. A number of studies have focused on the Th2 cytokines and their receptors with several studies suggesting that interleukin-9, in particular, may be an important candidate. There is reasonable consensus that the high affinity IgE receptor FcεR1-β subunit on chromosome 11q is a strong candidate and the consistent linkage data from this region of the genome imply that, even if FcεR1-β is not critical, there must be another gene close by which is relevant to the development of atopy. Finally, two recent reports have implicated the IL-4Rα subunit as a strong candidate.[21–39]

Pharmacogenetics

A number of studies have examined the possibility that the response to treatment of patients with asthma is in part genetically determined. The best data in this area concerns β_2 adrenoceptor polymorphisms. It seems increasingly clear that individuals who are homozygous for the glycine 16 variant of this receptor (which downregulates to a greater extent than other forms of the receptor both in vivo and in vitro) show a reduced response following chronic dosing with β agonists.[27 29] About 35% of the Caucasian population have this genotype. Other preliminary data suggest that treatment response to 5-lipoxygenase inhibitors may be partly determined by a series of polymorphisms in the promoter region of this gene.[32] A number of groups have attempted to look at the molecular basis of glucocorticoid resistance in those asthmatics who seem to be less responsive to the effects of steroids; however, no clear genetic basis for this phenomenon has so far been identified.[38]

A number of important concepts arise from these studies. Firstly, there are marked racial differences in the prevalence of many polymorphisms. For example, the Glu 27 β_2 adrenoceptor polymorphism is much rarer in both the Japanese and black African populations than in Caucasians. This implies that different populations may respond differently to anti-asthma medication. Secondly, small phase II or phase III studies looking at the effect of a given medication may by chance include large numbers of individuals with one particular genotype and hence may underestimate or overestimate the effectiveness of a given drug. Finally, it seems likely that some individuals may benefit from being given treatment tailored by their genotype, although whether the effects of genotype are sufficiently large to warrant this approach remains to be confirmed.

Summary

The field of asthma genetics has evolved in a markedly short time into a research field which potentially can provide a new level of understanding on the pathophysiology of the disease. There is consensus over a number of candidate genes that are likely to be important in the disease and also in chromosomal regions likely to contain other novel genes. It is, however, clear that there is no single major genetic risk factor for the development of asthma and the development of the disease in an individual will depend upon the interaction of a number of genes of moderate effect with environmental factors.

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