Editorial

Genetics of Asthma: The road ahead

In recent years, concerted efforts have been made towards understanding the genetic factors of asthma. Asthma is characterized by variable airflow obstruction and hyper-responsiveness of the airways, which is related to chronic airway inflammation and remodelling. Due to its increased prevalence in related individuals, it is considered to have a strong genetic component.[1–3] The patterns of asthma inheritance indicate that it is a complex genetic disorder which is not explained by simple mendelian inheritance. Such disorders are multifactorial in origin, and reflect the outcome of many processes linked to each other in a tangled web that is usually poorly understood. Asthma is no exception and this makes the study of genetics of asthma challenging.

The asthma syndrome, for it is not a singular disease, has a loosely defined phenotype as described above, which may result from one or more of many pathologies—allergic inflammation, increased smooth muscle contractility, mucous metaplasia, subepithelial fibrosis, neural hypersensitivity, etc. These are in turn interlinked and modulated by environment, further complicating the process. This is important in genetics because detection of a genotype-to-phenotype association is more difficult if the phenotype is ambiguous. This can be partially addressed by using restrictive inclusion criteria to create somewhat more homogeneous study groups. The association of genotype with the phenotype of interest can then be examined. In hypothesis-driven studies, we look for allele frequency differences of the candidate genes between affected (cases) and non-affected (control) individuals; or look for transmission disequilibrium of candidate gene allele(s) in affected and unaffected family members. Hypothesis-free studies such as genome-wide association (GWA) studies rely upon detection of linkage between genotyped markers in the entire genome with the phenotype. This is usually followed up by fine mapping and hypothesis generation. Each approach has its own set of advantages, with hypothesis-driven approaches being more sensitive and GWAs being more specific but requiring large sample sizes (in thousands) to be sufficiently powered. Limited positional cloning approaches are similar to GWAs but are smaller in scale.

At this time, over 100 genes have been identified and some have been consistently replicated in many populations across the globe.[1] However, most initial findings fail to replicate, sometimes even within the same populations where they were originally discovered.[4] This seems to be more frequent with candidate gene-based studies. In contrast, positional cloning approaches that test linkage in a relatively unbiased manner are more robust. Since candidate gene studies derive from our perception of the pathways involved in asthma, they have a limited ability to enhance our understanding. A large number of inflammation-related genes have been implicated in asthma through such studies. Prominent among them are IL-3, IL-4, IL-5, IL-9, IL-12b, IL-13, IFNg, iNOS, FC eRIb, etc.[5] Most of these influence T cell development/polarization towards Th1 or Th2 besides modulating other features such as recruitment of eosinophils, mast cells, neutrophils, etc. to the site of inflammation. These genes have been validated using candidate gene approaches in different studies and a number of functional polymorphisms have been identified. It is interesting to note that positional cloning-based approaches have additionally identified novel genes and

pathways that were previously not implicated in asthma such as ADAM33, DPP10, and GPRA.[1–3,6] Surprisingly, the first large scale GWA in asthma identified a region on chromosome 17q21 to be strongly associated with asthma, which was ultimately narrowed down to a gene ORMDL3 that did not have a known function.[7] Findings such as these have had a major impact on shifting the spotlight from inflammatory cells such as T-lymphocytes, mast cells and eosinophils to the epithelial mesenchymal trophic unit (EMTU), which seem to be pointing to many novel pathways.[8]

It is likely that genetic susceptibility to asthma would vary across geographical regions due to the impact of racial genetic diversity, environment and lifestyle. Dr Balaram Ghosh's group at our institution has taken the lead in studying genetic factors related to the risk of asthma in India. These have been largely limited to a northern Indian population and restricted to atopic asthma. A number of key candidate gene polymorphisms have been found to be associated with asthma susceptibility for this population, and have been reviewed elsewhere.[5] They have also recently identified inositol polyphosphate 4-phosphatase type I (INPP4a), a novel gene associated with asthma, using a combination of bioinformatics, positional cloning and functional studies.[9] There are very few studies on the genetics of asthma from India outside this group, and therefore independent validation in other Indian populations is desirable.

While genetic studies in asthma have contributed much to our understanding of the disease at a biological level, the identified risk factors are far from being clinically useful. Assuming a 5%–10% population prevalence of disease, and >5% allele frequency of the at-risk allele (<5% allele frequency would be a mutation and therefore not part of normal single nucleotide polymorphism genotyping); it can be readily calculated that typical risk odds of approximately 2 would provide a positive predictive value of 20% or less (see footnote). To achieve a positive predictive value of $\geq$0.6, the associated risk odds would have to be near 20, which is near impossible for a yet undiscovered common genetic variant. Thus, even if cost-effective genotyping were to be made available, the predictive values are too low for general screening. This is true for almost all complex diseases because the odds associated with any single risk gene are typically low. In theory, a combinatorial system using a panel of genes could be used to compensate for low individual predictive power, but will require extensive testing and validation. A more practical scenario may be the targeted screening of selected subsets where the predictive power may be higher.

Another potential application is to assess drug response based on an individual's genetic make up. However, these are also extremely context-dependent. One of the best examples is the genetic variation in response to beta-2 agonists (b_2-agonist), which are widely used as bronchodilators and recommended as first-line anti-asthma drugs. b_2AR is the key target of b_2-agonist drugs and is encoded by an intronless gene at 5q31-5q32 that has been linked to asthma. It has many polymorphisms and it is believed that these could be potential modifiers of asthma or might be responsible for inter-individual variation of responses to b_2-agonist drugs. One of these that leads to

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For a given population prevalence of disease (p%), and allele frequency (g%) the test statistics can be derived from the distribution:

	Disease present	Disease absent	
Risk allele present	x	g–x	g
Risk allele absent	p–x	(100+x–g–p)	
	p		100

Where x<p; x<g; g>5%

Odds ratio (OR) = x (100+x–g–p)/(g–x)(p–x); and x can be solved as a quadratic root of

$Ax^2 + Bx + C=0$ where A = (OR–1); B = ((1–OR)(g+p)–100); C = OR.g.p

Assuming the prevalence of asthma to be 10%; for odds of (2; 5; 10; 20) the maximum corresponding positive predictive values (x/g) would be (18%, 32%, 46%, 60%), respectively.

substitution of glycine for arginine in position 16 was found to associate with differences in response to therapy with salbutamol, a b_2-agonist. In one study from our institute, subjects homozygous for Arg16 were poor responders to salbutamol in terms of increase in forced expiratory volume at 1 second (FEV1), an established objective measure of lung function, in comparison to those homozygous for Gly16.[10] While this agreed with other studies that found poorer control of asthma in Arg16 homozygotes, it was contrary to some earlier studies where asthmatic children homozygous for arginine at codon 16 were shown to have significantly greater (>5-fold) bronchodilator response to albuterol than individuals homozygous for glycine residues, and the Arg16 variant was found to be more sensitive *in vitro*.[11] It is likely that the sub-sensitivity and poorer control of asthma in Arg16 homozygotes is a result of increased initial sensitivity coupled with chronic use.[12,13] While further study is necessary before coming to a firm conclusion regarding management, genotyping at this locus is of potential benefit to asthmatic patients.

One of the important challenges facing the field of genetics of asthma is that asthma is fundamentally heterogeneous in terms of molecular pathology, with a strong environmental component. These are further influenced by gene–environment interactions. Although larger and larger studies are being conducted, the problem remains unchanged. It was recently shown that the C159T polymorphism in the CD14 gene had opposite effects in rural and urban areas because of varying levels of endotoxin in the environment.[14] While the TT genotype was protective in urban populations with a low endotoxin load, it was associated with increased risk in rural communities where the endotoxin load was higher. To address such problems, it is necessary to subphenotype asthma and to interpret genetic data in the context of detailed phenotypic data as well as the environment. While it is now easy to genotype a large number of samples in previously unimaginable detail, simply increasing the sample size has never been and will not be a viable strategy as has been elegantly discussed elsewhere.[15] While a wealth of biological understanding is now within our reach we must make sure that it does not get lost in translation.

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