

SPECIAL ARTICLE

# Ethnic Differences in Genetic Susceptibility to Atopy and Asthma

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Asthma and the associated syndrome of atopy are often familial, and both are believed to result from the interaction of genetic and environmental factors. Twin studies have indicated that both asthma and atopy are largely regulated by genetic factors.<sup>1,2</sup> Although the inheritance of multifactorial traits such as asthma and atopy is complex, recent advances in molecular genetics make it now possible to contemplate the genetic analysis of such complex traits. The availability of new technology has spawned growing interest in the genetics of asthma and atopy, with a proliferation of genetic studies on asthma (for recent reviews, see references 3-6).

Two different approaches may be used in the analysis of any complex genetic disease: the candidate gene approach, or a genome-wide scan. In the candidate gene approach to disease gene identification, genes encoding proteins known or suspected to be involved in the pathogenesis of a particular disease are studied. The role of

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**SUMMARY** The genetics of asthma and atopy is complex, but can be approached by studies of both candidate genes and mapping of susceptibility loci. Genetic factors conferring susceptibility to disease may vary among ethnic groups. We present our experience with some candidate gene studies for asthma and atopy and susceptibility locus mapping for linkage to chromosome 5q.

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such candidate genes may be assessed by examining association of genetic variants (polymorphisms) of these genes. Since cytokines such as interleukins 4 and 13, and tumor necrosis factor, have been implicated in the pathogenesis of atopy and asthma,<sup>7</sup> the genes for these cytokines and receptors are candidate genes for atopy and asthma. Genetic polymorphisms of these genes have been investigated both by other researchers as well as ourselves.

In complex diseases, the genetic factors influencing individual susceptibility to disease may vary in different ethnic populations. The genome-wide scan approach has been used in the US Collaborative Study on the Genetics of Asthma (CSGA) where different susceptibility loci for asthma and atopy were identified.<sup>8</sup>

In this review, we discuss our own studies examining the association of genetic polymorphisms conferring susceptibility to atopy and asthma, as well as preliminary data from linkage studies to chromosome 5q31-32.

## Interleukin-4 receptor

IL-4 acts through its receptor (IL-4R), a 140 kDa dimer made up of a  $\gamma$  chain which is common to several cytokine receptors and a unique  $\alpha$  chain which actually binds to its specific ligand. An A to G substitution at position 1902 leading to a change in the amino acid encoded at position 576 from

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glutamine (Q) to arginine (R) (denoted as was identified and shown to affect IL-4 signaling function.<sup>9</sup> In the same study, the R576 genetic variant (arginine instead of glutamine at codon 576) was reported to segregate with atopy.

However, another group found no evidence of association between atopy and the R576 allele.<sup>10</sup> Both these studies were done with Caucasian subjects. In a local study, we determined the frequency of R576 in relation to atopy in the three main ethnic groups in Singapore.<sup>11</sup> In the course of this study, we developed a simple allele-specific polymerase chain reaction (AS-PCR) protocol for genotyping this marker. Our method requires only genomic DNA and is faster and easier to perform than the reverse transcriptase-PCR (RT-PCR) methods used in the two earlier studies.

In our rapid PCR screen for the mutation which obviates the need for sequencing, allele-specific primers which can distinguish between the wildtype A and the mutant G allele were designed to amplify a region flanking the polymorphic site. Sequence of the A-specific primer was 5'-CGG CCC CCA CCA GTG GCT AGC A-3' and the G-specific primer was 5'-CAG CTG CAG CCC CCG TCT CGG CCC CCA CCA CTG GCT ATA G-3'. The anti-sense primer used was IL4RA for both alleles (sequence 5'-AAT GAG GTC TTG GAA AGG CTT ATA-3').

We genotyped a total of 228 individuals (101 controls, 127 atopics) for Q576R. Our results showed a slight overall increase in the frequency of the R576 allele and genotypes containing R576 for atopics compared to normal controls ( $p = 0.05$ ). However, when the comparison is done within each

**Table 1** Association of tumor necrosis factor genotypes with asthma

Genotypes	1.1	1.2	2.2	p-value
<b>TNF-308</b>				
<b>Malays</b>				
Asthmatics (38) <sup>1</sup>	34	4	0	0.358
Controls (36)	35	1	0	
<b>Chinese</b>				
Asthmatics (67)	49	18	0	0.734
Controls (151)	115	36	0	
<b>LT<math>\alpha</math></b>				
<b>Malays</b>				
Asthmatics (38)	7	20	11	0.354
Controls (35)	4	15	16	
<b>Chinese</b>				
Asthmatics (66)	13	38	15	0.902
Controls (153)	30	84	39	

<sup>1</sup>Number in parenthesis indicates total number of subjects studied. The alleles at risk according to Mofatt and Cookson<sup>2</sup> are allele 2 for TNF-308 and allele 1 for LT $\alpha$ .

race, only the Malay population showed slight significance.

In the studies reported by Hershey *et al.*<sup>9</sup> and Grimbacher *et al.*,<sup>10</sup> the allele frequency for R576 is 10%. In our study, only the Chinese has about the same frequency (between 8.5% and 13.7%) for this allele. The frequency is much higher in the other two ethnic groups. For Indians, it is between 14 and 19%. Its frequency is highest for Malays at 25% Malay controls and 44.8% for atopics.

Our data seemed to corroborate that of Grimbacher *et al.*<sup>10</sup> that there is little association between the R576 allele and atopy. Given the large difference in the frequency of R576 between the different racial groups and the comparable incidence for atopy, it is unlikely that this allele is an important determinant for atopy or has a dominant influence as proposed by Hershey *et al.*<sup>9</sup>

#### Tumor necrosis factor

Mofatt and Cookson<sup>12</sup> have

previously reported an association between specific haplotypes of tumor necrosis factor (TNF) and asthma, with the TNF- $\alpha$ -308\*2 and LT $\alpha$  NcoI\*1 allelic variants found more commonly in asthmatics. Albuquerque *et al.*<sup>13</sup> also found association of TNF haplotypes with childhood asthma, but identified the alleles at-risk as TNF- $\alpha$ -308\*1 and LT $\alpha$  NcoI\*2. In a recent study, we have found a lack of association between these variants and asthma in both Malays and Chinese in Singapore.<sup>14</sup>

We compared the frequency of each of the two alleles of each cytokine in asthmatics and non-asthmatic controls. A total of 219 Southern Chinese and 73 Malay unrelated subjects were studied. We found no association of the at-risk alleles as defined by Mofatt and Cookson<sup>12</sup> (Fisher's statistic of between 0.20 and 2.35 and  $p$ -values of between 0.354 and 0.902) with the asthma phenotype (Table 1). There was no association of TNF-308\*2/LT $\alpha$  NcoI\*1 haplotype with asthma in either race. For Malays, the fre-

quency for TNF-308\*2 was extremely low although the incidence and severity of asthma in Malays is comparable to Chinese.<sup>15</sup> Comparison of both the at-risk allele frequencies between asthmatics and controls in both race groups was not significantly different ( $Z$ -scores between 0.274 and 1.484,  $p > 0.05$ ).

The failure to confirm the findings of Mofatt and Cookson<sup>12</sup> indicates that the susceptibility genes for asthma are probably different in our population. Another possible factor is the difference in recruitment, as their 413 subjects were from 88 nuclear families while all our subjects are unrelated. Of note, another study on Caucasians by Albuquerque *et al.*<sup>13</sup> not only failed to confirm the association, but identified different at-risk alleles for each of the biallelic polymorphisms. The influence of TNF genetic variants in asthma may be difficult to dissect as it may be one of various cytokine interactions.

#### Other cytokine gene polymorphisms

Polymorphisms in the  $\beta$ -adrenergic receptor have been described in association with both nocturnal asthma<sup>16</sup> and susceptibility to bronchodilator desensitization.<sup>17</sup> Based on animal studies, it has also been speculated that interleukin-9 (IL-9) may be an important candidate gene for asthma,<sup>18</sup> but a T113M polymorphism in the human IL-9 gene showed no association with either serum IgE levels or asthma.<sup>19</sup> As preliminary studies in our local population revealed that the allele frequencies of both these genetic polymorphisms were not sufficiently informative for meaningful analysis (unpublished), these were not pursued further.

#### Susceptibility loci for atopy and asthma

Another approach in the analysis of genetic diseases is that of positional cloning, where linkage to loci on various chromosomes is examined. While positional cloning has been successfully employed in single gene defects, it is more challenging in polygenic diseases. However, recent advances have made it feasible to attempt analysis of complex disorders, and studies have identified candidate susceptibility loci for atopy and asthma on several chromosomes. We have been studying chromosome 5q31-32 as a potential susceptibility locus for asthma and atopy in our population. Our preliminary findings among Singaporean Chinese families with affected sibpairs suggest that there may be a susceptibility locus for both asthma and atopy that has not previously been reported in other races.<sup>20</sup> Comparison of Chinese, Malay and Indian sibpairs also suggests that there are differences in susceptibility loci among these races. However, the numbers are small for the non-Chinese families (unpublished).

In the US Collaborative Study on the Genetics of Asthma (CSGA) potential inter-ethnic differences in susceptibility loci for asthma and atopy were identified.<sup>8</sup> Evidence for linkage was noted to 6 novel regions: 5p15 and 17p11.1-q11.2 in African-Americans, 11p15 and 19q13 in Caucasians, and 2q33 and 21q21 in Hispanics. These observations suggest that the number and relative importance of asthma susceptibility genes may vary between ethnic groups, which in turn point to a need for assessment of genetic factors in different populations.

#### Conclusion

The genetics of asthma and atopy is clearly complex. Furthermore the interplay of immunologic, environmental and other factors in addition to genetic ones makes the dissection of the genetic component an even more challenging one. At least some of the genetic factors are likely to vary among ethnic populations, and such variation must be taken into consideration in the total management of patients with asthma and atopy, particularly when genetic discoveries lead to pharmacological treatments for asthma. The study of ethnically diverse populations would contribute important information on the genetic susceptibility to asthma and atopy, both in terms of defining 'universal' susceptibility genes, as well as determining ethnic-specific loci.

In studying the genetics of asthma and atopy in Singapore, we have the advantage of access to a multi-racial population living in a small country where environmental factors are fairly uniform, and may therefore be able to make a useful contribution to the understanding of the genetics of asthma and atopy. Understanding the underlying genetic mechanisms of these common diseases could have widespread health benefits such as the identification of genetic variants that influence immunological responses, delineation of targets for gene therapy, or the development of "customized" pharmacological therapy for patients based on their genotypes.

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