

Ethnic Differences in Genetic Susceptibility to Atopy and Asthma in Singapore

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Abstract

Introduction: Asthma and atopy are complex traits, the phenotypes being influenced by the interaction of both genetic and environmental factors. In view of the growing evidence for inter-ethnic differences in genetic susceptibility to complex diseases such as asthma and atopy, we have assessed genetic factors for these diseases in the local population. **Methods:** The genetic analysis of asthma and atopy is being approached by candidate gene analysis as well as linkage studies for mapping of susceptibility loci. We reviewed published studies, which include data from our population on some candidate genes, as well as our preliminary data on susceptibility locus mapping for linkage to chromosome 5q31-33. **Results:** In local studies of the interleukin-4 receptor and tumour necrosis factor polymorphisms, we failed to find associations with genetic variants previously implicated to be associated with asthma and atopy. Evaluation of markers on chromosome 5q31-32 identified novel loci for susceptibility to asthma and atopy in addition to loci already described by others. **Conclusion:** Both the published literature and our own findings indicate that there are inter-ethnic differences in genetic susceptibility to asthma and atopy.

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Introduction

Twin studies have indicated that both asthma and atopy are largely regulated by genetic factors.^{1,2} However, the genetics is complex, involving an interaction of multiple susceptibility genes and environmental factors. Despite this complexity, recent advances in molecular genetics make it now possible to contemplate the genetic analysis of such traits. Indeed, in recent years there has been a proliferation of genetic studies on asthma.³⁻⁶

In complex diseases, the genetic factors influencing individual susceptibility to disease may vary in different ethnic populations. The US Collaborative Study on the Genetics of Asthma (CSGA) identified different susceptibility loci for asthma and atopy among Caucasians, African Americans and Hispanics.⁷ Work done among the Afro-Caribbean⁸ and Japanese⁹ also underscore the differences between races. We believe that it is important to determine the different susceptibility genes that may influence the development of asthma and atopy in our local population. Singapore's multi-racial population makes it possible for us to study both genes that may be universally important in the pathogenesis of asthma/atopy, as well as

ethnic-specific genes that influence disease to varying degrees in different groups.

There are essentially two different approaches to disease gene identification. In the candidate gene approach, genes encoding proteins known or suspected to be involved in the pathogenesis of a particular disease are studied. Genetic variants in the candidate genes are evaluated, comparing affected individuals to those unaffected. In the second approach, that of positional cloning, markers on chromosomes are used to track segregation of haplotypes with disease, and extensive physical mapping is performed using large pedigrees. For a complex disease such as asthma, however, physical mapping for genes involved would be more difficult than for single gene defects.

In this review, we discuss various studies evaluating possible candidate genes for their roles in asthma and atopy, as well as data from linkage studies mapping susceptibility loci for asthma and atopy. In the candidate gene approach, cytokines are popular candidate genes for asthma and atopy because of their implication in the pathogenesis of these conditions.

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Candidate Genes

Asthma and atopy involve complex pathways involving many cytokines. As a result, there is a plausible case for the importance of many candidate genes. Among them, interleukins 4 and 9, and tumour necrosis factor, have been implicated in the pathogenesis of atopy and asthma.¹⁰ Genetic polymorphisms of these candidate genes have been extensively investigated both by other researchers as well as locally.

Interleukin-4 Receptor

IL-4 acts through its receptor (IL-4R), consisting of a γ chain which is common to several cytokine receptors and a unique α chain which binds to its specific ligand. Hershey et al¹¹ have recently reported an association between atopy and a gain-of-function polymorphism in α subunit of the IL-4R gene on chromosome 16. An A to G substitution at position 1902 resulted in a change in the amino acid encoded at position 576 from glutamine (Q) to arginine (R). This allele resulted in enhanced signalling of the IL-4 receptor and increased CD23 expression. In that 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 such an association.¹² Both these studies were done with the Caucasian subjects. In a local study, we determined the frequency of R576 in relation to atopy in the 3 main ethnic groups in Singapore.¹³ 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) method used in the two earlier studies.¹¹⁻¹³

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 was done within each race, only the Malay population showed slight significance.¹³

Both Hershey et al¹¹ and Grimbacher et al¹² reported the allele frequency for R576 as 10%. In our study, the frequency was comparable in the Chinese but much higher in the other two ethnic groups. For the Chinese, the frequency for this allele was 14% for controls and 12% for atopics. For Indians, it was 19% in both groups. Its frequency was highest for Malays; 20% for controls and 45% for atopics.

Our results in the three ethnic groups in Singapore show there is little association between the R576 allele and atopy. The 3 racial groups have comparable incidences for atopy,¹⁴ yet there was a large difference in the frequency of

R576 between them. It is therefore unlikely that this allele is an important determinant for atopy or has a dominant influence as proposed by Hershey.

Another genetic variant of the IL4R- α has also been implicated in atopy among the Japanese. Mituyasu et al¹⁵ found that homozygotes for the Ile50 allele polymorphism in the extracellular domain of the α chain of the interleukin-4 receptor complex (IL4-R α) were in excess among the atopics. This led the authors to conclude that IL4-R α is a major atopy locus. We evaluated the frequency of the Ile50Val genotype in relation to atopy in the 3 main ethnic groups in Singapore but found no significant association of the Ile50/Ile50 variant of IL-4R α with atopy in our population.¹⁶

Tumour Necrosis Factor

Tumour necrosis factor (TNF- α) is a potent pro-inflammatory cytokine. Polymorphisms in the TNF gene complex result in variations in the level of secretion of this factor. Mofatt and Cookson¹⁷ have previously reported an association between specific haplotypes of tumour necrosis factor (TNF) and asthma. The TNF- α -308*2 and LT α NcoI*1 allelic variants were found more commonly in asthmatics. Albuquerque et al¹⁸ also found association of TNF haplotypes with childhood asthma, but identified the alleles at-risk as TNF- α -308*1 and LT α NcoI*2 instead.

We recently compared the frequency of each of the two alleles in asthmatics and non-asthmatic controls. A total of 219 Southern Chinese and 73 Malay unrelated subjects were studied. We found a lack of association between these variants and asthma in both Malays and Chinese in Singapore.¹⁹ There was no association of TNF- α -308*2/LT α NcoI*1 haplotype with asthma in either race.

As in the work on polymorphisms in IL-4R, we again failed to confirm the findings of other groups, underlying the probability that the susceptibility genes for asthma and atopy are 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 were unrelated. Albuquerque and co-authors' work on Caucasians¹⁸ failed to confirm the association found by Moffat and Cookson, instead they identified different at-risk alleles for each of the 2 allelic polymorphisms.

Interleukin-9

Animal studies have shown the importance of interleukin-9 (IL-9) in asthma, and it has been speculated that IL-9 may be an important candidate gene in humans.²⁰ Laitinen et al²¹ have studied a T113M polymorphism in the human IL-9 gene and showed no association with either serum IgE levels or asthma. We did a preliminary study of our local

population and did not find the allele frequencies of IL-9 genetic polymorphisms sufficiently informative for meaningful analysis (unpublished), hence, this was not pursued further.

Other Cytokine Gene Polymorphisms

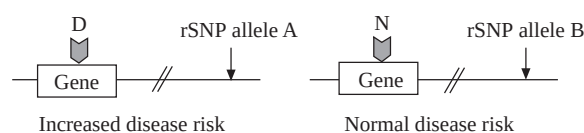
Polymorphisms in the β_2 -adrenergic receptor have been described in association with both nocturnal asthma²² and susceptibility to bronchodilator desensitisation.²³ Our preliminary work on the Arg16Gly polymorphism in the Chinese population shows a Gly16 allele frequency of 45.6%. Its relevance to drug treatment and disease susceptibility in our population remains to be investigated.

Other candidate gene polymorphisms that have been studied include those of interleukin-10,²⁴ β subunit of the high-affinity receptor for IgE,²⁵ T-cell receptor,²⁶ and the lipopolysaccharide marker CD14.²⁷ To date, these have not been studied in the local population.

Susceptibility Loci for Asthma and Atopy

Positional cloning for susceptibility genes has been employed in the study of asthma and atopy, with several genome-wide scans performed.^{7,28,29} Of interest, potential inter-ethnic differences in susceptibility loci for asthma and atopy were identified in the US Collaborative Study on the Genetics of Asthma (CSGA)⁷ and other studies (summarised in Table I). In the CSGA study, 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. This suggests that it is important to assess the genetic factors for these diseases in different populations, as the number and relative importance of susceptibility genes may vary between ethnic groups.

(A) Linkage disequilibrium-based association analysis



(B) Direct association analysis

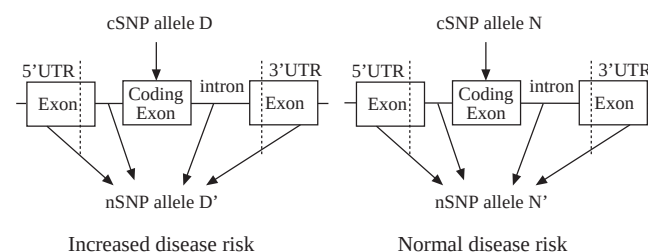


Fig. 1. Approaches to association analyses. (A) Linkage disequilibrium (LD)-based association analysis. D represents disease sequence while N represents normal sequence. This approach relies on random single nucleotide polymorphism (SNP) alleles (represented by rSNP allele A or B), which may span over a large genomic region, to exhibit linkage disequilibrium with the disease. LD-based association analyses test for presence of a higher frequency of rSNP A in affected individuals. (B) Direct association analysis. Here the SNP occur in the candidate disease-associated genes. The SNP can be found either in the coding regions (represented by cSNP allele D or N) or in the non-coding regions (represented by nSNP allele D' or N'). Direct association analyses test for presence of a higher frequency of cSNP D or nSNP D' in affected individuals.

In Singapore, we have embarked upon a study of chromosome 5q31-33 as potentially containing susceptibility genes for asthma and atopy (unpublished). This region is rich in candidate genes, including cytokines (IL-3, IL-4, IL-5, IL-9, IL-13), glucocorticoid receptor, fibroblast growth factor and β_2 -adrenergic receptor. We genotyped 373 individuals in 88 Chinese families (125 and 119 sib-pairs for atopy and asthma, respectively), focusing on 19 polymorphic microsatellite markers spanning 41 centiMorgan along chromosome 5q. There was significant

TABLE I: SUMMARY OF LINKAGE STUDIES REPORTED IN VARIOUS POPULATIONS

	Loci with significant evidence for linkage					Author and reference
	1p32	5q31-33	6p21-22	11q12-23	12q21-24	
Dutch		BHR, IgE			IgE	Meyers et al, ³⁰ Postma et al ³¹
Australian		IgE	IgE, Atopy	IgE, Atopy		Palmer et al, ³² Daniels et al ²⁸
Hispanic	Asthma					CSGA ⁷
Hutterite		Asthma			Asthma	Ober et al ²⁹
Japanese		Asthma, Atopy, IgE				Noguchi et al ⁹
Afro-Caribbean					Asthma, IgE	Barnes et al ⁸
African American		Specific IgE		Specific IgE		Hizawa et al ³³
Chinese		Asthma, Atopy				Shek et al (unpublished)

BHR: bronchial hyperactivity; CSGA: Collaborative Study on the Genetics of Asthma; IgE: serum immunoglobulin E levels

linkage of the asthma and atopy phenotypes with 3 markers, D5S2110, D5S2011, and D5S412 (P values between 0.001 and 0.00001). This suggests the presence of at least three candidate genes in the vicinity of these markers that determine increased susceptibility for asthma and atopy in our population.

Genetic variants of these candidate genes may, inherently or with interaction with specific environmental stimuli, enhance the allergic response. Comparison of Chinese, Malay and Indian sib-pairs also suggests that there are differences in susceptibility loci among these races. However, the numbers were small for the non-Chinese families.

Conclusion

Since the genetics of asthma and atopy are complex, a combination of approaches may well be required to dissect the genetic factors involved in their pathogenesis. Therefore, both the candidate gene approach and the physical mapping approach may yield useful information. 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. This could have widespread health benefits such as the identification of genetic variants that influence the phenotype, identification of targets for gene therapy, or the development of new pharmacological therapy for patients.

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REFERENCES

- Duffy D L, Martin N G, Battistutta D, Hooper J L, Matthews D. Genetics of asthma and hayfever in Australian twins. *Am Rev Respir Dis* 1990; 142:1351-8.
- Hanson B, McGue M, Roitman-Johnson B, Segal N L, Bouchard T J, Blumenthal M N. Atopic disease and immunoglobulin E in twins reared apart and together. *Am J Hum Genet* 1991; 48:873-9.
- Borish L. Genetics of allergy and asthma. *Ann Allergy Asthma Immunol* 1999; 82:413-24.
- Bleecker E R. Mapping susceptibility genes for asthma and atopy. *Clin Exp Allergy* 1998; 28 (suppl 5):6-12.
- Moffat M F, Cookson W O. Gene identification in asthma and allergy. *Int Arch Allergy Immunol* 1998; 116:247-52.
- Sandford A, Weir T, Pare P. The genetics of asthma. *Am J Respir Crit Care Med* 1996; 153(6 Pt 1):1749-6.
- A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). *Nat Genet* 1997; 15:389-92.
- Barnes K, Neely J D, Duffy D L, Friedhoff L R, Breazeale D R, Schou C, et al. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: Evidence from Afro-Caribbean and Caucasian populations. *Genomics* 1996; 37:41-50.
- Noguchi E, Shibasaki M, Arinami T, Takeda K, Maki T, Miyamoto T, et al. Evidence for linkage between asthma/atopy in childhood and chromosome 5q31-33 in a Japanese population. *Am J Respir Crit Care Med* 1997; 156:1390-3.
- Drazen J M, Arm J P, Austen K F. Sorting out the cytokines of asthma. *J Exp Med* 1996; 183:1-5.
- Hershey G K K, Friedrich M F, Esswein L A, Thomas M L, Chatila T A. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. *N Engl J Med* 1997; 337:1720-5.
- Grimbacher B, Holland S M, Puck J M. The interleukin-4 receptor variant Q576R in hyper-IgE syndrome. *N Engl J Med* 1998; 338:1073-4.
- Tan E C, Lee B W, Tay A W N, Shek L, Chew F T, Tay A H N. Interleukin-4 receptor variant Q576R: ethnic differences and association with atopy. *Clin Genet* 1999; 56:333-4.
- Goh D Y, Chew F T, Quek S C, Lee B W. Prevalence and severity of asthma, rhinitis, and eczema in Singapore schoolchildren. *Arch Dis Child* 1996; 74:131-5.
- Mitsuyasu H, Izuhara K, Mao X Q, Gao P S, Arinobu Y, Enomoto T, et al. Ile50Val variant of IL4R α upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 1998; 19:119-20.
- Tan E C, Lee B W, Chew F T, Shek L P C, Tay A W N, Tay A H N. IL4-R α gene Ile50Val polymorphism. *Allergy* 1999; 54:1005-7.
- Mofatt M F, Cookson W O C M. Tumour necrosis factor haplotypes and asthma. *Hum Mol Genet* 1997; 6:551-4.
- Albuquerque R V, Hayden C M, Palmer L J, Laing I A, Rye P J, Gibson N A, et al. Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. *Clin Exp Allergy* 1998; 28:578-84.
- Tan E C, Lee B W, Tay A W N, Chew F T, Tay A H N. Lack of association between asthma and TNF variants in Chinese and Malays. *Allergy* 1999; 54:1005-7.
- Nicolaides N C, Holroyd K J, Ewart S L, Eleff S M, Kiser M B, Dragwa C R, et al. Interleukin 9: A candidate gene for asthma. *Proc Natl Acad Sci U S A* 1997; 94:13175-80.
- Laitinen T, Kauppi P, Ignatius J, Ruotsalainen T, Daly M J, Kääriäinen H, et al. Genetic control of serum IgE levels and asthma: linkage and linkage disequilibrium studies in an isolated population. *Hum Mol Genet* 1997; 12:2069-76.
- Turki J, Pak J, Green S A, Martin R J, Liggett S B. Genetic polymorphisms of the β_2 -adrenergic receptor in nocturnal and non-nocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. *J Clin Invest* 1995; 95:1635-41.
- Tan S, Hall I P, Dewar J, Dow E, Lipworth B. Association between β_2 -adrenoceptor polymorphism and susceptibility to bronchodilator desensitization in moderately severe asthmatics. *Lancet* 1997; 350:995-9.
- Hobbs K, Negri J, Klennert M, Rosenwasser L J, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998; 158:1958-62.

25. Shirakawa T S, Dubowitz M, Dekker J W, Shaw A E, Faux J A, Ra C, et al. Association between atopy and variants of the β subunit of the high-affinity IgE receptor. *Nat Genet* 1994; 7:125-9.
 26. Moffat M F, Schou C, Faux J A, Cookson W O C M. Germline TCR—A restriction of IgE responses to allergens. *Immunogenet* 1997; 46: 226-30.
 27. Baldini M, Halonen M, Holy P, Martinez F D. A polymorphism in the 5'-flanking region of the CD14 gene is associated with circulating CD14 levels and total serum IgE. *Am J Cell Mol Biol* 1999; 20:976-83.
 28. Daniels S E, Bhattacharya S, Leaves J A, Young A, Hill M R, Faux J A, et al. A genome-wide search for quantitative trait loci underlying asthma. *Nature* 1996; 383:247-50.
 29. Ober C, Cox N J, Abney M, Di Rienzo A, Lander E S, Changyaleket B, et al. Genome-wide search for asthma susceptibility loci in a founder population. *Hum Mol Genet* 1998; 7:1393-8.
 30. Meyers D A, Postma D S, Panhuysen C I M, Xu J, Amelung P J, Levitt R C, et al. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994; 23:464-70.
 31. Postma D S, Bleeker E R, Amelung P J, Holroyd K J, Xu J F, Panhuysen C I M, et al. Genetic susceptibility to asthma—bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995; 333:894-900.
 32. Palmer L J, Daniels S E, Rye P J, Gibson N A, Tay G K, Cookson W O, et al. Linkage of chromosome 5q and 11q gene markers to asthma-associated quantitative traits in Australian children. *Am J Respir Crit Care Med* 1998; 6:1825-30.
 33. Hizawa N, Freidhoff L R, Ehrlich E, Chiu Y F, Duffy D L, Schou C, et al. Genetic influences of chromosomes 5q31-q33 and 11q13 on specific IgE responsiveness to common inhaled allergens among African American families. *J Allergy Clin Immunol* 1998; 102:449-53.
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