

Original article

Genetic susceptibility to asthma and atopy among Chinese in Singapore – linkage to markers on chromosome 5q31–33

Background: Asthma and atopy are complex genetic traits, influenced by the interaction of multiple genes and environmental factors. Linkage of these traits to chromosome 5q31–33 has been shown in other populations, but has not been well studied in the Chinese. We studied linkage between asthma and atopy with markers on chromosome 5q31–33 in the Singapore Chinese. This region contains many candidate genes, including the cytokine gene cluster.

Methods: We recruited 88 Chinese families with at least two affected offspring, totaling 373 subjects, with 125 and 119 sib-pairs for atopy and asthma, respectively. All individuals were genotyped with 19 polymorphic microsatellite markers spanning a distance of 41 cM along chromosome 5q31–33. Affected sib-pair and multipoint linkage analysis was performed.

Results: There was evidence for linkage of the asthma and atopy phenotypes with three markers, D5S2110, D5S2011, and D5S412 (*P* values of 0.001 to 0.00001). Multipoint analysis further substantiated this (nonparametric linkage scores of 1.8–2.9). These findings suggest that susceptibility genes for asthma and atopy are found in this region in the Chinese.

Conclusions: This study has shown linkage of atopy and asthma to chromosome 5q31–33 in a heterogeneous Chinese population. These findings further substantiate the notion that chromosome 5q31–33 contains “universally” important susceptibility genes for these traits.

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There is overwhelming evidence from family and twin studies that both asthma and atopy are influenced by genetic factors (1, 2). The prevalence and severity of asthma and atopic diseases are on the rise in Western populations (3, 4) and also in Singapore (5). This phenomenon strongly suggests that environmental factors play an important role in disease development. Understanding the genetic factors would provide important clues into the gene–environment interactions that result in disease susceptibility. For example, a recent study showed that a polymorphism in the 5' flanking region of the CD14 gene was associated with soluble CD14 and total serum IgE levels in a North American Caucasian population (6).

Furthermore, in complex diseases, the genetic factors influencing individual susceptibility to disease may vary between 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). The study of ethnically diverse populations would contribute important information on the genetic susceptibility to asthma and atopy, in terms both of defining “universal” susceptibility genes and determin-

ing ethnic-specific loci. Many regions have been identified as possible candidate regions. Among them, the region on chromosome 5q31–33 has been extensively studied. In this study, we performed linkage analysis with polymorphic markers in the region of chromosome 5q31–33, as this region is rich in candidate genes (cytokine gene cluster, β -adrenergic receptor, glucocorticoid receptor, CD14, and fibroblast growth factor). We chose to study the Chinese because they form the major proportion of the population in Singapore. The Singapore Chinese belong mainly to three dialect groups (Cantonese, Hokkien, and Teochew), originating from the southern provinces of China. The similarity in the antigen profiles of HLA in these three ethnic groups suggests a close genetic makeup (8).

Material and methods

Family ascertainment

Families were selected that had a proband with asthma and a similarly affected sibling. Subjects were recruited from patients attending the Children's Clinic at the National University Hospital, Singapore, or from volunteers responding to publicity in the local

press. Families were identified between November 1995 and March 1999. Written informed consent was obtained from the families. The study was approved by the hospital's ethics committee.

Definition of asthma and atopy

Subjects were considered asthmatic if they had 1) physician-diagnosed asthma, and 2) at least two episodes of wheezing after the age of 3 years. This information was obtained by questionnaire. In children, the diagnosis of asthma had to be verified by a pediatrician. Those with a history of prematurity or other chronic lung diseases were excluded. Bronchial hyperresponsiveness, as defined by methacholine bronchoprovocation, was not included in this study for logistic reasons.

Atopy was present if an individual had one or more of the following: total serum IgE more than 2 SD above the mean for that age (9); positive specific IgE to the dust mite *Dermatophagoides pteronyssinus* (an important source of indoor allergens in the tropics) by the fluorescent allergosorbent test (FAST) ($\geq$ class 2/ $\geq$ 0.75 IU/ml), or skin prick test (wheal $\geq$ 3 mm greater than negative control).

Total serum IgE and *D. pteronyssinus*-specific IgE were measured by FAST (Biowhittaker, Maryland, USA). The skin test was done with extracts of the dust mite, *D. pteronyssinus* (Greer Laboratories, North Carolina, USA).

Genetic map

The map of the region of chromosome 5q is based on the Cedars genetic map (website <http://cedar.genetics.soton.ac.uk/pub>). Nineteen polymorphic markers spanning the 41-cM region from D5S404 to D5S422 were evaluated.

Molecular methods

Genomic DNA was extracted from either peripheral blood leukocytes or mouthwash epithelial cell samples with the QIAamp® (Qiagen, Inc, California, USA) blood and body fluid protocol, as outlined in the manufacturer's manual.

Genotyping was performed with the polymerase chain reaction (PCR) with fluorescence-tagged primers spanning microsatellite markers in the region of interest. PCR primer sequences were obtained from the genome database (GDB) or from published sources (10). The 5'-end of one primer in each set was tagged with fluorescent dye (5'-FAM), and PCR amplification was performed in 10- μ l volume reactions. The PCR cocktail contained 10 pM of each primer, 1 μ l of 10 $\times$ buffer (Boehringer-Mannheim, Germany) (containing 100 mM Tris-HCl, 15 mM MgCl₂, 500 mM KCl [pH 8.3]), 0.2 mM of each dNTP, and 0.5 U Taq DNA polymerase (Boehringer-Mannheim, Germany). The samples were denatured for 5 min at 95°C, followed by 30 cycles each of 45 s at 94°C and 30 s at 55°C, and a final extension for 10 min at 72°C. A volume of 1 μ l of the PCR reaction was combined with 18.5 μ l of deionized formamide and 0.25 μ l of GeneScan 350 Rox-labeled DNA size standard. The mixture was denatured at 95°C for 5 min and then put on ice.

The PCR products were analyzed in the ABI PRISM 310 genetic analyzer, and the PCR products were sized by the GeneScan® analysis software. GeneScan results were read independently by two persons who were blind to the clinical data. In cases of ambiguity, the typing was repeated. Accuracy of allele size calling was checked by calibration against DNA from CEPH (Centre pour l'Études des Polymorphismes Humaines) controls.

The following polymorphic markers spanning a reasonable distance around the cytokine cluster were typed: D5S404, D5S2110, D5S816, D5S393, D5S500, D5S2009, D5S2116, D5S658, D5S2011, D5S2010, D5S2099, D5S436, D5S2090, D5S410, D5S412, D5S422, and intronic polymorphisms within the interferon regulatory factor (IRF1), interleukin-9 (IL-9), and fibroblast growth factor-acidic (FGFA) genes.

Linkage study

We genotyped 373 individuals in 88 Chinese families. In these pedigrees, there were 125 and 119 sib-pairs for atopy and asthma, respectively, in most cases (except for 13 families) with complete sets of parents. When a parent was not available, parental alleles were inferred if possible from the genotypes of the siblings during linkage analysis.

The affected sib-pair analysis was done with the Genetic Analysis System (GAS), Version 2.0 (Alan Young, 1995, Oxford University), for most likely sharing. This method is based on sharing of marker alleles identical by descent (IBD) in sib-pairs and is not dependent on the genetic model from segregation analysis. A regression analysis by the Haseman-Elston method in GAS was also carried out for age-sex-adjusted log(IgE), a quantitative trait. In this method, the difference in log(IgE) within each sib-pair is squared and modeled by least-squares regression as a function of the number of alleles shared by sib-pairs at the marker locus. In the presence of linkage, sibs that are identical by descent for the marker would be expected to have similar IgE levels, and those not sharing marker alleles would be expected to have a bigger difference in their IgE levels.

Multipoint linkage analysis was carried out on asthma, atopy, and log(IgE) with the Genehunter computer package (11). The nonparametric linkage score generated is a measure of the IBD sharing among all affected pedigree members.

Results

The demographics and clinical characteristics of the families are summarized in Table 1. The effect of smoking on the families was small as only a small percentage (4.8%) of parents (all fathers) smoked, and one family (1.1%) had pets (a cat and birds). The phenotypes of atopy and asthma were highly correlated ($\chi^2 = 57.9$, $P < 0.00001$).

Physical map and heterozygosity index

The location of the 19 markers studied in chromosome 5q, the number of alleles detected in our population, and the derived heterozygosity indices are shown in Table 2. A relatively high degree of polymorphism was observed for all markers

Sib-pair analysis

We found evidence of linkage for asthma to three markers by sib-pair analysis (Table 3). These were loci D5S412 ($P = 0.00013$), D5S2110 ($P = 0.00027$), and D5S2011 ($P = 0.00005$). These three markers were similarly significant for atopy ($P = 0.00001$, $P = 0.001$, and $P = 0.00001$, respectively).

Other areas that showed some evidence of linkage with asthma were IL-9 ($P = 0.0067$) and D5S2010 ($P = 0.00052$). For atopy, there was modest evidence of linkage with IL-9 ($P = 0.004$).

When analysis was carried out for total log(IgE) as a quantitative trait by the sib-pair method developed by Haseman and Elston, significant linkage was found for marker D5S2011 ($P = 0.012$). In addition, linkage was also shown with IL-9, although this did not reach statistical significance ($P = 0.07$) (Table 4).

Table 1. Characteristics of population studied

Characteristics	Offspring <i>n</i> = 210	Parents <i>n</i> = 163
Male sex	58%	NA
Age (years)	1–36 (median 8)	23–52 (median 39)
Asthma	90.6%	22%
Atopy	87.3%	55.7%
Smoking	0	2.4% (all fathers)
Pet owners	1.1% of households	

Multipoint nonparametric linkage analysis

Multipoint nonparametric linkage analysis further substantiated the linkage between the three markers (D5S412, D5S2110, and D5S2011) with asthma and atopy (Fig. 1). However, the NPL scores were not as impressive as the *P* values obtained by sib-pair analysis. This was not unexpected, as the adjacent markers had very low LOD scores, and could reflect some error in genotyping. Moreover, we tested a total of 19 markers, and as these markers are not independent, type 1 error was increased by multiple testing.

Analysis for log(IgE) showed a peak at IL-9, consistent with the sib-pair analysis. However, the other peak at D5S2010 was not found to be significant by sib-pair analysis, although this marker is close (1.2 cM telomeric) to D5S2011, where linkage was found by the sib-pair method.

Discussion

Our study has reinforced the importance of the chromosome 5q31–33 region in the genetic influence

Table 3. Results of linkage analyses in affected pairs of siblings with asthma and atopy

Locus	Asthma				Atopy			
	No. of sib-pairs	Mean*	<i>P</i> value	LOD score	No. of sib-pairs	Mean*	<i>P</i> value	LOD score
D5S404	59	0.46	0.82	0.0	60	0.45	0.86	0.0
D5S2110	96	0.63	0.00027	5.1	89	0.62	0.001	4.2
IRF1	86	0.53	0.18	0.29	76	0.53	0.23	0.4
IL9	89	0.59	0.0067	2.2	73	0.61	0.004	2.6
D5S816	74	0.52	0.41	0.0	74	0.52	0.28	0.2
D5S393	72	0.51	0.096	0.8	72	0.51	0.43	0.8
D5S500	60	0.48	0.19	0.3	60	0.48	0.71	0.0
D5S2009	79	0.54	0.046	1.4	79	0.54	0.17	0.5
D5S2116	85	0.56	0.084	0.6	85	0.56	0.054	0.7
D5S658	75	0.57	0.01	1.6	75	0.57	0.036	0.8
D5S2011	84	0.67	0.00005	3.8	84	0.67	0.00001	4.4
D5S2010	68	0.60	0.00052	2.7	68	0.57	0.052	0.8
FGFA	12	0.54	0.7	0.0	12	0.54	0.34	0.1
D5S2099	81	0.50	0.62	0.0	79	0.50	0.5	0.0
D5S436	67	0.51	0.13	0.4	67	0.51	0.36	0.0
D5S2090	72	0.50	0.63	0.0	72	0.50	0.5	0.0
D5S410	65	0.51	0.32	0.1	65	0.51	0.4	0.1
D5S412	89	0.64	0.00013	5.3	89	0.64	0.00001	6.8
D5S422	43	0.45	0.82	0.0	43	0.45	0.81	0.0

* Proportion of alleles that were identical by descent.

Table 2. Position, number of alleles, and heterozygosity index of polymorphic microsatellite markers studied

Markers	Position from top of chromosome (cM)	No. of alleles	Heterozygosity index
D5S404	133.19	18	0.94
D5S2110	138.6	16	0.80
IRF1	140.43	8	0.76
IL-9	142.648	13	0.76
D5S816	142.743	10	0.76
D5S393	142.86	14	0.87
D5S500	144.453	12	0.71
D5S2009	145.218	8	0.50
D5S2116	146.378	13	0.76
D5S658	147.01	15	0.86
D5S2011	147.1	15	0.81
D5S2010	148.311	25	0.88
FGFA	148.521	6	0.68
D5S2099	151.664	7	0.61
D5S436	152.5	14	0.77
D5S2090	154.238	12	0.62
D5S410	163.279	7	0.69
D5S412	171.25	13	0.80
D5S422	174.37	19	0.93

* Heterozygosity calculated by formula $1 - \sum p_i^2$, where p_i is population frequency of i th allele.

of asthma and atopy. This report on the Chinese population substantiates the findings in several other populations. These have included studies in homogeneous (Amish) (10) and heterogeneous (North American, Australian, British, Dutch Caucasian, and Japanese) (7, 12–15) populations. There have also been reports of a lack of association with this region of chromosome 5 in other populations (16, 17). This supports the theory that different genetic factors influence these disease traits in different ethnic groups.

Our study focused on a 41-cM region along chromosome 5q and included 19 polymorphic markers. The data suggest that genes in this region determine increased susceptibility for asthma and atopy in the Chinese. The markers are possibly surrogates for neighboring candidate genes, and genetic variants of

Table 4. Results of linkage analysis in affected sib-pairs with log (serum total IgE)

Marker	Sib-pair analysis* for log(IgE) <i>P</i> values
D5S2110	0.47
IL9	0.07
D5S816	0.14
D5S393	0.15
D5S2116	0.14
D5S2011	0.012
D5S2010	0.46
FGFA	0.32
D5S436	0.37
D5S422	0.21

* Haseman–Elston regression analysis using computer package GAS.

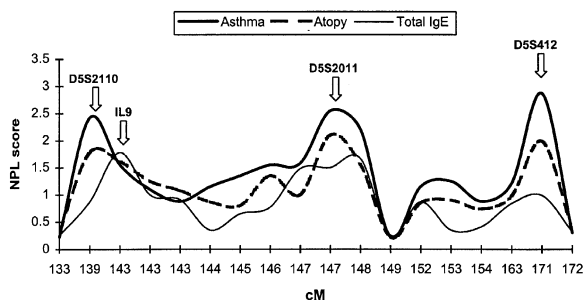


Figure 1. Multipoint nonparametric linkage analysis of asthma and atopy and log (IgE) to markers on chromosome 5q31–33 by Genehunter program.

these candidate genes may, inherently or by interaction with specific environmental stimuli, enhance the allergic response.

Specifically, significant linkage for asthma and atopy was demonstrated to marker D5S412 in our population. This marker is located near (mapped to approximately 0.35 cM apart) the gene for IL-12 β . It has been shown that nasal biopsies from patients with allergic rhinitis express reduced amounts of IL-12 (p40) and IL-12 β_2 receptor as compared to normal subjects (18). Since IL-12 is an essential cytokine for the induction of TH1 responses, a reduced capacity to produce this cytokine could lead to aberrant TH2 development. A functionally relevant polymorphism of IL-12 β may result in its reduced expression, leading to a susceptibility to atopy and asthma.

Similarly, marker D5S2110 is located near the cluster of genes encoding the Th-2 cytokines (IL-4, IL-5, and IL-13). Recently, it was reported that an IL-13 promoter gene polymorphism was associated with allergic asthma. This variant was also associated with increased altered regulation of IL-13 production and increased binding of nuclear proteins, indicating functional significance (19). Such functionally relevant polymorphisms may be important in our population.

The third marker, D5S2011, which was significantly associated with atopy and asthma in our population, is located near (approximately 1.5 cM from) the CD14 gene, and a polymorphism in the flanking region of this gene has recently been shown to be associated with total serum IgE (6).

Our study also documented linkage of IL-9 to serum total IgE. In fact, IL-9 has been shown to play a role in the pathogenesis of bronchial hyperresponsiveness in mice (20). Furthermore, IL-9 has been shown to play a role in the regulation of IgE synthesis in man (21). However, other investigators have not documented linkage to any specific polymorphic marker within the IL-9 gene (22). Our studies on this IL-9 biallelic marker in our population also failed to find it to be significant among the Chinese (unpublished).

This study is an attempt to assess the genetic susceptibility to asthma and atopy in the Singapore Chinese population. Importantly, it is part of the effort to define the genes responsible for asthma and atopy in different population groups. We have documented linkage with a chromosomal region rich in candidate genes. These encouraging results deserve further investigation into the putative genes, which will involve fine gene mapping and ultimately the identification of asthma genes.

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