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Absence of genetic linkage of chromosome 5q31 with asthma and atopy in the general population

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Abstract

***Background* – Clinical asthma is associated with increased serum total immunoglobulin E (IgE), atopy (skin prick test positivity to common aeroallergens), and bronchial hyperreactivity (BHR) to non-specific stimuli (positive methacholine challenge test). A region on chromosome 5q31–33 has been linked with increased total serum IgE and BHR. A study of the genetic linkage of this region with clinical asthma and atopy was therefore undertaken.**

***Methods* – A polymorphic microsatellite marker in chromosome 5q31 (D5S399) was studied in 119 sibling pairs recruited from the general population who shared asthma, atopy, and/or BHR. Based on our population distribution of 13 different alleles, it was expected that by chance alone sibling pairs would share on average 1.24 alleles and that a significant excess would indicate genetic linkage.**

***Results* – No evidence of linkage was found in 45 siblings concordant for asthma (shared alleles = 1.09, p = 0.95), in 103 sibling pairs with atopy (shared alleles = 1.18, p = 0.82), in 51 sibling pairs with BHR (shared alleles = 1.22, p = 0.62), or in 68 sibling pairs who shared atopy in the absence of BHR (shared alleles = 1.22, p = 0.61). A slight non-significant excess of shared alleles (1.44, p = 0.11) was observed in siblings who shared BHR without atopy.**

***Conclusions* – No evidence of genetic linkage of chromosome 5q31 with either clinical asthma or atopy was therefore detected in the population studied. Linkage between chromosome 5q and BHR needs further investigation.**

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The genetics of asthma has been inferred from linkage studies of atopy, bronchial hyperreactivity (BHR), and total serum IgE levels. These phenotypes show a high degree of heritability[1] but do not always coincide.[2 3] Independence, overlap, and interaction between different phenotypes confounds attempts to define specific genetic predisposition.

We have recently shown that the high affinity immunoglobulin E (IgE) receptor gene on chromosome 11q13 is linked with clinical asthma.[4] In our population this linkage was explained by BHR, but not atopy.[4] Chromosome 5q31–33 may be relevant to asthma as genetic variation in this region appears to influence total serum IgE levels and a number of candidate genes reside in this chromosomal vicinity.[5–7] Total serum IgE levels correlate with clinical asthma and allergy[3] but linkage studies of chromosome 5q with clinical asthma or atopy have not been reported.[8]

Methods

We used a polymorphic microsatellite marker studied previously[5] on chromosome 5q31 (D5S399) to examine linkage in sibling pairs recruited from the general population who shared asthma, atopy, and/or BHR. The selection of subjects and phenotypic determination were as described previously.[4] In brief, recruitment for screening was part of the European Community Respiratory Health Survey which has been described elsewhere.[9] We screened 4500 randomly selected young adults (aged 20–44 years) and a subgroup of 757 attended our respiratory function laboratory for detailed phenotype characterisation. Clinical asthma was defined using a validated questionnaire,[9] as wheeze or the use of asthma medications in the previous 12 months. Skin sensitivity (a weal of greater than 3 mm diameter) to common aeroallergens was used to define atopic status.[4] A methacholine challenge was used for bronchial provocation to determine BHR which was defined as a reduction in forced expiratory volume in one second (FEV_1) of 20% or more at a cumulative dose of less than or equal to 2 mg (10.2 μmol) of methacholine.[4] We selected individuals with asthma, atopy, or BHR and, by testing their siblings, we identified 119 affected sibling pairs who shared at least one of the phenotypes.

Genomic DNA was extracted from 10 ml samples of blood in EDTA by standard techniques.[4] The D5S399 PCR primers and amplification conditions were as described previously.[5] Allele sizes and genotypes were determined independently by two investigators (AK, ZYHW) who were unaware of the phenotype data. Linkage analysis was performed using the sibling pair method[10] based on the number of alleles shared by affected siblings in relation to that expected by chance alone. Linkage between the marker and phenotype was inferred when the number of alleles shared

Table 1 Allele sharing in groups of affected siblings

Concordance for	*No. of sibling pairs*	*No. of shared alleles*			
		Observed	*Expected*	*% excess*	*p value**
Asthma	45	49	55.9	−12.3	0.95
Atopy	103	122	127.9	−4.6	0.82
BHR	51	62	63.3	−2.1	0.62
Atopy alone	68	83	84.5	−1.7	0.61
BHR alone	16	23	19.9	15.7	0.11
Atopy with BHR	35	39	43.5	−10.3	0.88

BHR = bronchial hyperreactivity.
* Based on one sided test.

by affected siblings exceeded significantly that expected by chance. To clarify linkage of this marker with atopy or BHR alone, we also analysed sibling pairs who were concordant for atopy without BHR or concordant for BHR without atopy.

These studies were approved by the Alfred Hospital ethics review committee.

Results

We found 13 different D5S399 alleles in our population with a calculated heterozygosity of 81%. The sizes of alleles were assessed by specific molecular markers and DNA sequencing reactions run on each gel. The most common alleles were 115, 117, and 127 base pairs in length. Based on the observed distribution we expected that sibling pairs chosen at random would share, on average, 1.24 alleles. In 45 sibling pairs who shared clinical asthma we observed an average of 1.09 shared alleles ($p = 0.95$). In 103 sibling pairs characterised with atopy (table 1) we observed 122 shared alleles (1.18 shared alleles per pair, $p = 0.82$). Of these, 68 siblings were concordant for atopy without BHR and they shared 83 alleles (1.22 shared alleles per pair, $p = 0.61$). Concordance for BHR was found in 51 siblings in whom a total of 62 shared alleles were observed (1.22 shared alleles per pair, $p = 0.62$). In 16 sibling pairs concordant for BHR but not atopy we observed a slight excess of shared alleles (1.44 shared alleles per pair, $p = 0.11$). However, this excess was not statistically significant (table 1). Sibling pairs concordant for both atopy and BHR shared only 1.11 alleles on average ($p = 0.88$).

Discussion

This is the first report of a linkage study between asthma and atopy and chromosome 5q31. Previous studies have reported linkage of this locus with total serum IgE levels[5 6] and linkage with BHR[7] which has been disputed.[8] The association between serum IgE levels and asthma and allergy[3] led previous investigators to suggest that loci on chromosome 5q31 might also predispose to asthma. However, we were unable to demonstrate linkage between chromosome 5q31 and clinical asthma or atopy.

This negative result may reflect insufficient statistical power. Precise calculations of statistical power are not possible as they depend on specification of unknown parameters such as the contribution of the suspected locus to the disease, the genetic heterogeneity of the disease (number of implicated loci, respective effect of each locus, loci interactions), and the rates of recombination between the marker and the susceptibility locus. However, it is possible to estimate, for any given sample size, the percentage of excess allele sharing required to declare statistical significance at the (one sided) 5% level. For 16 sibling pairs an excess sharing 21.2% would be declared as statistically significant, whereas for 50 and 100 sibling pairs the excesses required are 12.1% and 8.6%, respectively. Other than siblings concordant for BHR alone, none of the sibling pair groups showed any excess of allele sharing.

The absence of linkage between chromosome 5q31 and atopy is not entirely unexpected as atopy may be more closely related to specific IgE antibodies than to total serum IgE levels.[11] Even where linkage of chromosome 5q31 with total IgE has been found, no linkage with IgE specific to common aeroallergens could be demonstrated.[5]

In an analysis of a subset of a Dutch study, chromosome 5q31–33 was linked with BHR.[7] The strongest linkage was seen for markers slightly more towards the end of the chromosome than D5S399. We found no evidence of linkage between D5S399 and BHR in 51 affected sibling pairs, but there was a 16% excess of shared alleles in 16 sibling pairs who shared BHR in the absence of atopy. Although not of statistical significance, this observation may be important as more distal markers might reveal stronger linkage to BHR. Detailed mapping of chromosome 5q is needed to examine the possibility that a gene affecting BHR is in the vicinity.

In conclusion, our findings indicate that in an Australian population chromosome 5q31 does not exert a substantial influence on the inheritance of asthma or on atopy. Linkage between chromosome 5q31 and BHR warrants further study.

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