

Lack of linkage between chromosome 5q23–33 markers and IgE/bronchial hyperreactivity in 67 Scottish families

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Abstract

Background Raised serum immunoglobulin E (IgE) and bronchial hyperreactivity (BHR) are risk factors for the expression of the asthma phenotype. Previous studies have reported evidence for linkage between these traits and markers on the 5q23–33 cytokine gene cluster. **Objective** To test for linkage between total serum IgE/BHR and microsatellite markers which map to the 5q23–33 region in an ethnically distinct cohort of families from Aberdeen, Scotland.

Methods We performed a linkage study between five polymorphic markers (spanning the chromosome 5q23–33 region) and total serum IgE and BHR traits. A cohort of 67 families, who were recruited originally to study the natural history of wheeze, were clinically characterized and genotyped for D5S404, IL4, IRF-1, IL9, D5S436 markers. Linkage analyses were performed using the nonparametric Haseman-Elston algorithm for the quantitative trait log IgE, and the nonparametric LOD score (NPL-score) of the GENE-HUNTER package for the qualitative traits serum IgE and BHR.

Results The results of the nonparametric linkage analysis using either the Haseman-Elston algorithm or NPL-score were consistent and showed no evidence for linkage with IgE. There was also no evidence for linkage between the BHR traits (at cut-off values of PD20FEV₁ < 8 mmol and 16 mmol) and any of the tested five microsatellite markers.

Conclusions This study presents evidence against the presence of a gene with a major effect on total serum IgE or BHR in the 5q23–33 region, in this ethnic group.

Keywords: 5q, bronchial hyperreactivity, IgE, IL4, IL9, IRF-1, microsatellite analysis, .

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Introduction

The human chromosome 5q23–33 region has been a particular focus of genetic analysis of asthma and atopy traits, as it contains several cytokine and other candidate genes with an established role in the control of serum IgE and the inflammation underlying asthma. These include cytokines IL3, IL4, IL5, IL9, IL12B, IL13, the interferon regulatory factor (IRF-1), granulocyte macrophage colony stimulating

factor (GM-CSF), A receptor for the macrophage colony stimulating factor (CSF-R1), b₂-adrenergic receptor, CD14, and leukotriene C4 synthase.

Linkage between markers which map to this region and total serum IgE was initially reported by Meyers *et al.* [1] in 84 families from the Netherlands, and by Marsh *et al.* [2] who reported linkage between markers which map to the 5q31–33 region and total serum IgE in the Pennsylvanian Old Order Amish families. Various other studies, for and against a role for 5q23–33 region in the modulation of the asthmatic phenotype, have subsequently been published and are detailed in the discussion section.

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In this study, we examine the linkage of the 5q31–33 region to IgE and BHR in an ethnically distinct Caucasian population from Aberdeen, Scotland.

Materials and methods

Subjects

Probandes were selected from individuals originally recruited in the 1960s to study the natural history of wheeze [3]. At that time, 121 individuals with a clinical diagnosis of asthma (defined as 'recurrent dyspnoea of an obstructive type without other demonstrable cause') and 167 individuals with a clinical diagnosis of wheezy bronchitis (defined as 'wheeze occurring only in the presence of infection') were identified in a larger community sample of 2511 school children aged 10–14 years. Twenty-five years later, all accessible individuals within these groups, together with a comparison group of 167 individuals selected from the nonwheezing group within the original study population, were studied [4].

Probandes were selected on the basis of both their original classification and subsequent outcome 25 years later in order to identify those in whom natural histories were clearly different. Three groups were identified: 20 individuals with childhood onset asthma, continuing symptoms in early middle age and atopy (group 1); 30 with childhood wheezy bronchitis and no symptoms in early middle age (group 2); and 29 nonatopic probandes without symptoms in either childhood or middle age.

Sixty-nine probandes and their families (18 group 1, 24 group 2, and 27 group 3) agreed to participate (87.3%). One proband had divorced and remarried, and both his current and previous spouse and children from both marriages were examined. From the others, one spouse died of nonrespiratory illness, one spouse refused to participate, and six spouses could not be contacted following divorce or separation.

Each family member completed a written respiratory questionnaire which included questions derived from the MRC and the American Thoracic Society respiratory questionnaires [5]. These included questions covering atopic and asthmatic symptoms. Skin prick testing was carried out by the method of Hendrick [6], using HDM (*D. Pteronyssinus*), mixed grass, and cat fur (Dome Hollister Steir, Spokane, Washington, USA). A positive skin prick test was defined as a weal (3 mm larger than the negative saline control). Spirometric tests were carried out using a wedge spirometer (Vitalograph, Buckingham, UK) with subjects standing. Nose clips were not used. Bronchial reactivity to histamine was measured using the method of Yan [7].

Blood samples were collected for measurement of total serum IgE, specific IgE (HDM, grass pollens, and mixed trees), and for DNA extraction. The total serum IgE was

measured by an enzyme-linked immunosorbent assay (ELISA), and specific IgE (Pharmacia Cap System, Pharmacia, Uppsala, Sweden) to *D. pteronyssinus*, grass pollens, and mixed moulds were measured using a radioallergosorbent assay (RAST).

The principal phenotypes analysed in this study were total serum IgE and BHR. The total IgE level in this population was skewed, and was therefore log transformed to normalize its distribution and subsequently analysed as a quantitative trait [8]. The effects of age, sex and smoking covariates on total serum IgE levels in this population were examined prior to the linkage analysis. This population did not represent a continuous distribution of age but was divided into probandes, spouses, siblings, and the parents of probandes and spouses. Within the three generations the mean loge IgE were 3.67 IU/mL (95% CI 3.66–3.98) in the grandparents ($n = 119$), 3.67 IU/mL (95% CI 3.44–3.90) in middle aged ($n = 213$), and 4.02 IU/mL (95% CI 3.66–4.39) in children ($n = 93$), respectively, which was not significantly different (Kruskal-Wallis test for equality of populations (Chi square = 2.38, 2DF, $P = 0.31$). Regression of total IgE on age showed only a borderline association and a low correlation across the whole population studied (regression; coefficient for age = -0.008 , CI -0.017 – 0.0005 , $P = 0.07$). We chose not to age correct the IgE level in view of the small value of regression coefficient for age even though the significance of this association was borderline. The effect of sex on log IgE levels was examined using one-way analysis of variance and showed no significant correlation ($F = 0.61$, $P = 0.44$). Furthermore, regression of log total IgE levels against smoking history showed no significant correlation ($P = 0.368$). On the basis of these findings, the age, sex and smoking covariates were not used to modify log total IgE levels prior to either the segregation or the linkage analysis. Total serum IgE was also analysed as a qualitative trait. In this trait, individuals were labelled as either affected or not affected according to whether their total serum IgE levels were higher or lower than 100 IU/mL.

Bronchial hyperreactivity to histamine in an individual was defined as a 20% decline in FEV₁ when challenged with less than the specified threshold provocation dose of inhaled histamine (PD₂₀). In this study, and in keeping with Meyers *et al.* [1], BHR was analysed using two categorical traits (PD₂₀ FEV₁ < 8 μ mol, and PD₂₀ FEV₁ < 16 μ mol).

Molecular methods

Peripheral venous blood, collected in EDTA-containing tubes, was obtained from all individuals. During blood sample collection, one family failed to attend, and only the proband was available from another family. The remaining 67 families comprised 580 individuals (286 males), but blood samples were provided by only 427 individuals. DNA

Table 1. Details of the microsatellite markers analysed in this study. The microsatellites were selected on the basis of previous reports of linkage or association [1,17,27,31]. Interleukin 4 (IL4) gene exon 2 microsatellite was particularly uninformative displaying low heterozygosity with one common allele and four rare alleles.

Locus	Cytogenetic location	Genetic distance (cM)*	Heterozygosity	Number of alleles	Allele size range (bp)
D5S404	5q23–31	11	0.74	8	175–195
IL4	5q23–31	3.5	0.22	5	236–250
IRF-1	5q23–31	5.5	0.66	6	255–275
IL9	5q23–32	16	0.83	11	110–130
D5S436	5q31–33	–	0.85	13	234–254

was extracted using the phenol-chloroform method. All individuals with available DNA (427) were genotyped for microsatellites D5S404 (forward primer 5'CTGGAGATGT AATGCTGTGC, and reverse primer 5'GATCACCACATT CCACCTAAT, allele size range of 175–195 bp), IL4 (second intron, forward primer 5'TGCACCTGGGCAA CAGTTTA, and reverse primer 5'GTTGGATGGACTTGG AGATT), IRF-1 (seventh intron, forward primer 5'TCAT CCTCATCTGTTGTAGC, and reverse primer 5' ATGGC AGATAGGTCCACCGG), IL9 (fourth intron, forward primer 5'AGGCTTTCTCTAATGCAGAG, and reverse primer 5'GGTGGTTGACCTCAAATTGG), and D5S436 (forward primer 5'TATGTGCCAGGCATTACGCT and reverse primer 5'GTCTCCACCCACAGCAGG), spanning the chromosome 5q23–33 region (Table 1, Fig. 1). The primers' sequence of these markers were obtained from GDB, and purchased from Oswel (University of

Southampton). PCR amplification of DNA of each subject was performed in five separate reactions (one for each microsatellite). Reactions were performed in 96-well microtitre plates in a 20- μ l volume using a GeneE Thermal Cycler (Techne, Cambridge, UK). The PCR master mix of each reaction constituted 100 ng of DNA, 20 pmol of each primer, 2 μ l 10 $\times$ buffer (Promega Cambridge, UK), MgCl₂ (Promega), 3 μ l of 1 mmol dNTP mix (300 μ mol of each dNTP) (Promega), 0.2 μ l Taq polymerase (5 U/ μ l) (Promega) and sterile dH₂O to make up a total PCR mix volume of 20 μ l. The amount of MgCl₂ used for the amplification of the D5S404, D5S436, and IL4 markers was 1.2 μ l (final concentration of 1.5 mmol), for the IL9 marker 1.1 μ l (1.38 mmol) and for the IRF-1 marker was 2.5 μ l (3.13 mmol). The PCR cycles comprised: DNA denaturation segment of 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55.6 °C for 30 s and 72 °C for 1 min, with a final extension segment of 72 °C for 10 min. Following PCR amplification, 1–2 μ l of the PCR products for the five markers of each individual were pooled (the volume used depended on the relative intensity of PCR products as observed on a 2% agarose gel). To this mixture, 5 μ l of sterile dH₂O was added, thus diluting the PCR products of each marker by around 1 in 10. 0.5 μ l of this mixture was combined with 1 μ l formamide/EDTA-loading buffer and 1 μ l of TAMRA (500) internal sizing standard. Electrophoresis of the PCR products was performed on an Applied Biosystems Gene Scanner (ABI prism model 377). The final solution was heated at 95% for 2 min prior to loading on a 6-m urea/4.25% acrylamide gel, and run on 36-cm well to read plates for 2 h using module 36C-2400. PCR products from one control DNA were loaded in the first and last lanes of all runs. Consistency in the control DNA allele sizing among different gels was examined, which confirmed accuracy of typing.

Haplotypes were determined by using the GENEHUNTER software, to identify double crossovers that may reveal erroneous data [9]. The analysed microsatellite markers' best order, the genetic distance in centimorgans (cM) between the flanking markers and the corresponding two point LOD scores were measured using the Vitesse engine

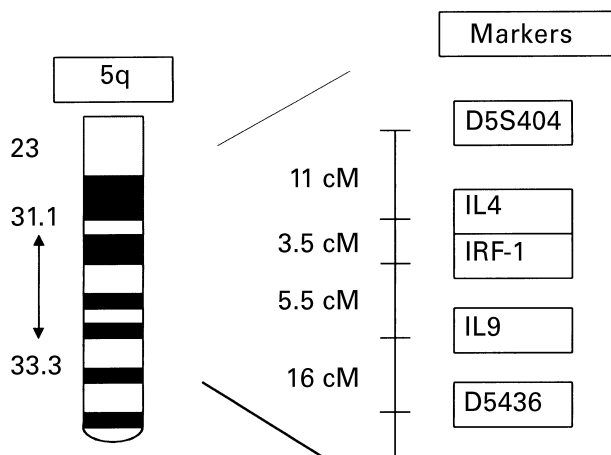


Fig. 1. Diagram of chromosome 5q23–33 region showing the relative position and order of the markers used in this study. The (sex-averaged) genetic distance in cM and the order of the markers are calculated from the study population using the two point LOD score approach of the Vitesse routine of the GAS program. IL4; interleukin 4, IRF-1; interferon regulatory factor 1, IL9; interleukin 9.

routine of the Genetic Analysis System (GAS, Alan Young, University of Oxford, 1993–95). This gave similar order to that recorded in the CEPH families [10–12]. The markers' order and distance as produced in this data-set have subsequently formed the fixed map for the multipoint analysis.

Linkage analysis

Due to the uncertainty encountered in specifying the precise inheritance parameters including the penetrance and the gene frequencies for IgE and BHR, the nonparametric linkage analysis was primarily used. The analysis was performed using the GAS and GENEHUNTER software packages.

The Haseman-Elston algorithm was employed to perform nonparametric linkage analysis between the quantitative trait log IgE and the five microsatellite markers using the identical-by-descent (IBD) sib-pair information combined with interval mapping [13]. In this method siblings sharing marker alleles near the quantitative locus are more likely to have similar quantitative values than nonsharing siblings. Under reasonable genetic model, the mean value of the quantitative trait difference between siblings should decrease as the fraction of alleles shared increases. A slope is fitted using least-squares, using allele sharing as independent variable and square of trait difference as the dependent variable. A significantly negative slope would indicate linkage. Data analysis was performed using the SIBIHE routine of GAS. This routine combines the Elston-Haseman algorithm with sib-pair interval mapping. This is a multipoint method in which information from adjacent markers is used to infer missing or ambiguous allele sharing. The sharing probabilities are calculated first at loci with definite known sharing status. Thereafter, estimated sharing at ambiguous intercrosses is calculated. Subsequently, unknown sharing loci are interpolated from the above information.

Further nonparametric linkage analyses for total serum IgE and BHR as dichotomous traits were conducted using

the nonparametric LOD-score (NPL-score) approach built in the GENEHUNTER software package. This analysis differs from the SIBIHE approach described above. It allows estimating the IBD allele sharing in all affected relatives in a pedigree rather than just sib-pairs. It handles multigeneration families (i.e. more than two generations), and extracts maximum information from pedigrees of average size, which is ideal for analysing the Aberdeen families (families size ranges from 4 to 14 members). It tests whether the affected relatives share alleles IBD more often than expected under random segregation. The statistic reported (NPL-score) will represent the deviation from Mendelian expectation observed, and will roughly follow the normal distribution. This approach may have a power comparable to that of the LOD-score parametric method under the correct model [17]. This is an important feature, because the NPL-score approach requires neither the consideration of multiple model of inheritance (thus avoiding corrections of multiple testing), nor advance knowledge of the correct model of inheritance (thus avoiding problems of parameters misspecification).

We considered using the two above described approaches in analysing these families (the SIBIHE routine of GAS and the NPL-score of GENEHUNTER) complementary. The SIBIHE routine has the advantage of using log IgE as a quantitative trait, but it breaks pedigrees into small nuclear families studying only sib-pairs. The NPL-score method, on the other hand, uses total serum IgE as qualitative trait (the package is not designed to handle quantitative traits), but it handles clinical and genotype data better, by using all affected family members in large pedigrees.

Results

Clinical and pedigree data

Sixty-seven pedigrees were included in the linkage analysis (64 extended pedigrees and three nuclear families). These comprised a total of 580 individuals (286 males, 49%) and

Table 2. Description of the distribution of $\log_{10}$ IgE (with corresponding total IgE value in IU/mL) across different subsets of the study population. The probands represent three groups: asthmatic, wheezy bronchitic, and normal subjects recruited in relatively similar proportions. S.E.; standard error, CI; confidence interval.

Category	Cases	Mean $\log_{10}$ IgE (IU/mL)	SE	95% CI
Parents of Probands & Spouses	119	1.60 (40)	0.07	1.46–1.73
Probands	64	1.64 (44)	0.10	1.43–1.85
Spouses	56	1.48 (30)	0.09	1.31–1.65
Siblings of probands	93	1.63 (43)	0.08	1.48–1.78
Children	95	1.75 (56)	0.08	1.59–1.91
All subjects	427	1.63 (43)	0.04	1.56–1.70

Table 3. The Haseman-Elston multipoint regression of the quantitative locus IgE against IBD allele sharing. Regression by the least-square fitting formula: $D = M * Sh + C$, where D is the difference in sib's log IgE values, Sh is the alleles shared. $M < 0 = >$, increased sharing tend to reduce D . Intercept is value Sh satisfying $0 = M * Sh + C$. In this analysis a total of 123 sib-pairs used with 121 degrees of freedom. The genetic distance in recombination fraction between the markers is estimated from the genotype data in these families using the Vitesse Engine Routine of GAS. *Recombination fraction for D5S404. The results provide evidence against linkage between log IgE and the analysed markers.

Location (θ)*	Locus	M	C	Intercept	P-value
0.00	D5S404	0.19	0.54	-2.8	0.86
0.11	IL4	0.25	0.5	-2.0	0.94
0.14	IRF-1	0.23	0.52	-2.2	0.94
0.18	IL9	0.16	0.58	-3.6	0.87
0.28	D5S436	0.35	0.4	-1.1	0.99

259 sib-pairs. The number of subjects per family ranged from four to 14, with a mean of 8.66 per family.

The total serum IgE values were available for 427 individuals. The mean levels of log₁₀ IgE across different subsets of this population is shown in Table 2. Total serum IgE levels > 100 IU/mL (affected group) were observed in 137 individuals (32.1%). The percentage of affected offspring were 63.6% if both parents had high IgE, 54.4% if only the father was affected, 26.1% if only the mother was affected, and 22.9% if both parents had IgE values within the normal expected range. This gave a strong association between father-child affectedness ($P < 0.00001$), but not between mother-child affectedness ($P = 0.5$).

Bronchial reactivity was measured in 412 individuals, in which 122 (29.6%) and 96 (23.3%) showed a positive reaction at a PD₂₀ FEV₁ < 16 μ mol and 8 μ mol, respectively. Using the cut-off level of PD₂₀ FEV₁ less than 16 μ mol, the percentage of affected offspring were 66.7% if both parents were affected, 40% if only the father was affected, 32.5% if only the mother was affected, and 30.3% if both parents were nonaffected, with nonsignificant but slightly stronger trend of paternal transmission. A total of 71 individuals (12.2%) were labelled as asthmatics. The percentage of the asthmatic offspring were 50% if both parents were asthmatic, 30.8% if only the father was asthmatic, 20% if only the mother was asthmatic, and 12.6% if both parents were nonasthmatic, again with evidence of stronger paternal transmission. Out of 412 individuals with available total serum IgE and bronchial reactivity data, there were 53 subjects (12.9%) who had both high IgE (> 100 IU/mL) and BHR at PD₂₀ FEV₁ of < 16 μ mol.

Linkage analysis for total serum IgE

In the first instance, linkage analysis was performed using the SIBIHE routine of the GAS program, which employed

the Haseman-Elston regression algorithm for the quantitative trait log₁₀ IgE against sib-pair IBD allele sharing combined with interval mapping. The data is presented in Table 3. A total of 123 sib-pairs were used in the analysis. No evidence for linkage was observed with any of the tested markers.

The results of linkage analysis using the multipoint NPL-score approach of the GENEHUNTER package are presented in Table 4. It produced results consistent with the Haseman-Elston approach, showing no evidence for linkage with any of the markers analysed.

Multipoint linkage analysis for BHR

The result of the multipoint linkage analysis for the categorical traits BHR at PD₂₀ FEV₁ < 8 μ mol, and PD₂₀ FEV₁ < 16 μ mol is presented in Table 5. The data show no evidence for linkage between either trait and any of the tested markers.

Table 4. Multipoint linkage analysis for the total IgE qualitative trait using the cut-off level of 100 IU/mL. The analysis was performed using the NPL-score approach of the GENEHUNTER package [9]. Specific functions included activation of the 'all' option which allows using all affected pedigree members, deactivation of the single option allowing the employment of the multipoint analysis. The NPL-score and the corresponding P-value for each locus is given.

Location (cM)	Locus	NPL-Score	P-value
00.00	D5S404	-0.33	0.70
12.40	IL-4	-0.08	0.57
16.05	IRF-1	-0.11	0.56
21.88	IL-9	-0.17	0.60
41.16	D5S436	-0.38	0.73

Table 5. Multipoint linkage analysis for the qualitative locus BHR at the cut-off values PD₂₀ FEV₁ < 8 µmol, and PD₂₀ FEV₁ < 16 µmol. See Table 4 for data presentation description.

Location (cM)	Locus	PD ₂₀ FEV ₁ < 8 µmol		PD ₂₀ FEV ₁ < 16 µmol	
		NPL-score	P-value	NPL-score	P-value
00.00	D5S404	−0.34	0.77	−0.46	0.80
12.42	IL-4	0.15	0.37	−0.10	0.57
16.05	IRF-1	0.16	0.36	−0.11	0.58
21.88	IL-9	0.33	0.23	0.09	0.43
41.16	D5S436	0.01	0.48	0.13	0.40

Discussion

This study provides evidence against the presence of a gene in the 5q23–33 region with a major effect on total serum IgE or BHR in this cohort of families from Aberdeen, Scotland. The results presented in this study conflicts with the previous reports of linkage of these phenotypes to this region by Meyers *et al.* [1], which we aimed to replicate. The two studies have similarities and differences. The recruitment criteria were different in the two studies. The Dutch families were recruited through a parent with asthma, whereas the Aberdeen families recruitment included asthmatics, wheezy bronchitics, and normal probands in roughly equal proportions. Both populations were of Caucasian origin. The traits definition utilized by both studies were consistent. The prevalence of high total IgE (>100 IU/mL) and BHR (PD₂₀ FEV₁ < 16 µmol) in the Aberdeen families were 32.1% and 23.3%, respectively, compared to 44% and 40%, respectively, in the Dutch families. Segregation analysis in the Dutch families showed that total serum IgE is significantly controlled by genetic factors. It also produced a recessive best-fit inheritance model with a polygenic component, estimating low IgE allele frequency as $L = 0.41$ and a polygenic component of 0.26 [1]. Segregation analysis for the total serum IgE in the Aberdeen families produce similar results, in which we observed a significant modulation of total serum IgE by genetic factors (data not shown). We found that the dominant, recessive, and polygenic genetic models all fitted the data better than the sporadic model. The polygenic component was more significant than the pure recessive model, but the combined recessive and polygenic models fitted the data best, although the polygenic component remained strong and accounted for about 0.44 of the phenotype variance. In this recessive polygenic model the estimated frequency for the low allele L was 0.39.

Five microsatellite markers were used in this study which were selected on the basis of previous reports of linkage or association. The heterozygosity of the markers varies between 0.22 and 0.86. The genotyping of multiple markers

has allowed analysis using the multipoint approach. Because the clinical criteria for a normal or abnormal IgE level may not reflect whether a given family member is a gene carrier, analysis of this variable as a quantitative trait was important. Non-parametric analysis was performed for total serum IgE as a quantitative trait (using Haseman-Elston multipoint regression algorithm of the quantitative trait log₁₀ IgE against IBD allele sharing) and as a qualitative trait (using the NPL-score method of the GENEHUNTER program), and for the BHR using the qualitative traits only. Attempts to use BHR as a quantitative trait was complicated by the non-normal distribution of all measures of BHR examined including the dose response slope. Whilst the Haseman-Elston algorithm utilized information from all sib-pairs with available IgE, the nonparametric test of the GENEHUNTER software extracted the IBD allele sharing information from all affected relatives, giving more complete linkage information, as compared to the standard affected sib-pair analysis. The results of linkage analysis using both of these methods were consistent and gave evidence against linkage between total serum IgE and the 5q23–33 region. Furthermore, we also performed parametric analysis for total serum IgE, using parameters obtained from the segregation analysis performed on these families. The results have again showed no evidence for linkage with any of the tested five markers, although they did not exclude the region as the LOD scores were generally around the level of zero (data not shown). A formal assessment of the power of this study to detect linkage using the parametric approach was not readily computable due to the level of polygenic inheritance, but the high frequency of the putative deleterious allele in this population would make the power limited if it were true.

The observation that segregation analysis for IgE within the present families showed similar results to those of Meyer *et al.* [1], yet the inability to replicate genetic linkage for IgE and BHR is of interest. The Dutch families were identified as atopic adult asthmatics with a history from childhood, whereas in the present study symptomatic

probands were equally distributed amongst those with persistent symptoms from childhood, transient childhood symptoms, and no symptoms in childhood. This is reflected in the lower population prevalence of IgE above 100 IU/mL in the present population. This might have limited the power of our study because of the limited number of affected individuals in our population and therefore type II error can not be excluded as a cause of lack of linkage. The effects of proband identification is likely to influence genetic linkage and closer definition of phenotypes used in identification of families may provide greater consistency in future studies. Of interest is the finding of stronger paternal rather than maternal transmission for high IgE. This contrasted with previous reports of stronger maternal transmission of atopy reported by Cookson *et al.* [14]. This may again be related to pattern of proband identification.

The replication of linkage reports in complex diseases has proven to be controversial [15]. The presence of genetic heterogeneity is often given as a possible reason for failure to replicate findings. The linkage to heterogeneous traits such as log IgE and BHR, where multiple genes interact to express the phenotype, will normally only be found fortuitously in samples which contain an exceptionally high proportion of individuals or families influenced by that particular gene. Genes expressed by exposure to different allergens may also confound heterogeneity. Simulation experiments have shown that, in these circumstances, many studies may be necessary before replication occurs. In addition, the power to detect linkage in multigenic diseases is very limited, so that several hundred families may be necessary to detect linkage to a gene affecting one third of the subjects with the disease [16]. Therefore the negative result of our study does not exclude the presence of genes on the 5q31–33 region which modulate total serum IgE and BHR. Indeed, evidence for a role played by this region in the genetics of asthma has, thus far, been reported in several different populations. These include; 84 families from the Netherlands (linkage of total IgE to D5S436) [1,17,18], 11 extended pedigrees from the Pennsylvania Old Order Amish families (linkage of total serum IgE to IL4) [2], 79 caucasians families studied in the Collaborative Study on the Genetics of Asthma [19], 68 Japanese families (linkage of atopy/asthma to IL4, IL9, and D5S393) [20], Hutterites American families who are a religious isolate of European ancestry (linkage of asthma to 5q23–31 in 292 individuals) [21], 121 nuclear Australian Caucasian families (linkage of total and specific serum IgE and blood eosinophils count to 5q31–33) [22], 246 Caucasian American sib-pairs (linkage of circulating blood eosinophil to D5S658 on 5q31–33) [23], and in a large Caucasian American kindred with familial eosinophilia (linkage of familial eosinophilia to D5S642 on 5q31) [24]. In addition to the above linkage studies, numerous other association studies have

also implicated various candidate genes which map to the region in the modulation of the asthmatic phenotype (listed in the Asthma Gene Database: <http://cooke.gsf.de>).

Conversely, in addition to our study, linkage between the 5q cytokine cluster region and various definitions of the asthma phenotype was not observed in numerous other populations. These include: four large Caucasian American pedigrees [25], 80 Caucasian families from Busselton Western Australia [26], 130 random Caucasian families from Southampton, UK (although this study reported an association between BHR and IL9 marker) [27], 119 Caucasian sib-pairs from Australia [28], an isolated founder Finnish subpopulation comprised of 157 nuclear families [29], and 97 German families [30].

In conclusion, our study showed no evidence for linkage between markers which map to the 5q23–33 region and total serum IgE or BHR in an ethnically distinct population from Aberdeen, Scotland. However we can not exclude type II error. These results further underline the observed difficulty in replicating linkage reports in complex traits. The publication of negative reports of linkage, however, is required to maintain the balance of literature by avoiding bias towards the selection of positive results.

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