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# Linkage analysis of the 5q31–33 candidate region for asthma in 240 UK families

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*Atopy and asthma are complex genetic diseases resulting from the interactions of a number of genetic and environmental factors. We had previously reported allelic association between the IL9 marker on chromosome 5q31–33 and atopy. In order to further investigate the role of susceptibility genes on 5q31–33 in the development of atopy and asthma we have studied 240 UK families comprising 131 families selected at random, 60 multiplex families with affected sib pairs, and 49 single proband nuclear families. Polymorphic markers on 5q31–33 were genotyped and both single and multipoint linkage analysis was undertaken using the BETA program. We have used both affection status and quantitative scores for atopy and asthma for phenotypic variables, combining data into scores for asthma and atopy. The strongest suggestion of linkage using multipoint analysis was centred around D5S410 with a maximum Lod of 1.946 at location 171.3 cM and a standard error of 3.3 for the asthma quantitative score. There was no evidence of linkage with atopy, the atopy quantitative score or total serum IgE.* Genes and Immunity (2001) **2**, 20–24.

**Keywords:** *asthma; atopy; IgE; linkage; chromosome 5q*

## Introduction

Asthma is an atopic disorder characterised by activation and recruitment of eosinophils to the lung resulting in chronic swelling and inflammation of the airways. The processes leading to allergic inflammation are controlled by the Th2 lymphocytes, which secrete IL4 and IL5 leading to enhanced production of IgE by B cells and the generation and recruitment of eosinophils. Asthma, irrespective of its aetiology, exhibits strong genetic characteristics, heritability being estimated at 30–50%. Epidemiological studies have identified atopy as the strongest risk factor for the development of asthma, especially the expression of IgE to indoor allergens. Atopy and atopic disease also show strong familial clustering which contributes in part to the genetics of asthma.[1]

There are several genes on chromosome 5q that may be important in the development or progression of inflammation associated with atopy and asthma including the cytokines IL-3, IL-4, IL-5, IL-9, IL-12(β-chain), IL-13, interferon regulatory factor-1 (IRF-1) and GM-CSF.[2] There are also number of other genes in this cluster which may play a role in the development of asthma or its pathogenesis, including colony stimulating factor 1 receptor (CSF1R), acidic fibroblast growth factor (FGF1), and the $\beta_2$-adrenergic receptor (ADRB2). ADRB2 is one of several genes on 5q31–33 in which polymorphism may effect the response to common asthma treatment.[3–6] Other examples include the corticosteroid receptor (GRL),[7] and leukotriene $C_4$ synthase.[8]

Given the large number of candidate genes for both atopy and asthma in the 5q31-q33 region a number of studies have previously investigated the role of this genetic region in susceptibility to atopy and asthma.[9–14] In order to investigate this further, we have genotyped 10 markers on chromosome 5 in a large UK Caucasian sample of 240 families comprising 1216 individuals extensively phenotyped for atopy and asthma. In addition we have used data from four additional markers previously typed within the population[15,16] and incorporated phenotype scores in the analysis in attempt to confirm previous findings of linkage to 5q31–33 and define the area of interest.

## Results and Discussion

The phenotypes used for linkage analysis were the qualitative traits affection status (AF) and atopy and the quantitative scores ATIc (atopic asthmatic score) and ASIc ('non-atopy' asthmatic score) and log total IgE (IGE). The results of non-parametric linkage analysis using the BETA program are presented in Tables 1 and 2. Table 1 shows the Lod scores and β values of single point linkage analysis. The highest single point Lods were seen between affection status and D5S421 (2.122) and between ASIc and D5S470 (1.163). No evidence of linkage was seen between any marker and ATIc, IGE, or atopy.

Table 2 shows the results of multipoint linkage analysis. Suggestive evidence for linkage can be seen between the marker D5S410 and the ASIc (Lod 1.935) telomeric to the Th2 cytokine cluster. There is a second peak of linkage evident between D5S421 at the centromeric end of

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**Table 1** Results of single-point linkage analysis using BETA

| *Marker* | *Map position (cM)* | *AF* | | *ASIc* | | *ATIc* | | *IGE* | | *Atopy* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Lod* | *β* | *Lod* | *β* | *Lod* | *β* | *Lod* | *β* | *Lod* | *β* |
| D5S421 | 138.7 | 2.122 | 0.528 | 0.255 | 0.064 | 0.875 | 0.091 | 0.271 | 0.052 | 0.384 | 0.140 |
| D5S642 | 153.4 | 0.862 | 0.541 | 0.052 | 0.046 | 0.294 | 0.086 | 0.064 | 0.035 | 0.235 | 0.156 |
| D5S666 | 154.21 | 0.407 | 0.295 | –0.003 | –0.007 | 0.215 | 0.060 | 0.284 | 0.067 | 0.312 | 0.147 |
| IL4 | 154.2 | 0.075 | 0.138 | –0.049 | –0.036 | 0.000 | 0.002 | 0.177 | 0.056 | –0.089 | –0.084 |
| IL9 | 156.5 | 0.015 | 0.044 | 0.136 | 0.044 | –0.002 | –0.004 | –0.031 | –0.017 | –0.062 | –0.052 |
| D5S393 | 158.0 | 0.550 | 0.258 | 0.510 | 0.089 | 0.173 | 0.038 | 0.007 | 0.008 | –0.002 | 0.008 |
| D5S399 | 159.9 | 0.065 | 0.102 | 0.037 | 0.025 | 0.013 | 0.013 | –0.035 | –0.021 | –0.049 | –0.054 |
| D5S658 | 161.5 | 0.097 | 0.116 | –0.001 | –0.004 | –0.011 | –0.011 | –0.132 | –0.037 | 0.001 | 0.005 |
| D5S436 | 166.6 | 0.184 | 0.143 | 1.404 | 0.143 | –0.017 | –0.012 | 0.015 | 0.011 | 0.102 | 0.064 |
| D5S210 | 166.61 | 0.058 | 0.097 | –0.106 | –0.042 | –0.057 | –0.026 | –0.041 | –0.023 | –0.111 | –0.078 |
| ADRB27 | 168.70 | 0.016 | 0.099 | 1.132 | 0.409 | –0.018 | –0.028 | 0.237 | 0.102 | –0.097 | –0.193 |
| D5S470 | 172.7 | 0.167 | 0.167 | 1.163 | 0.153 | 0.255 | 0.060 | 0.008 | 0.010 | 0.308 | 0.147 |
| D5S410 | 176.3 | 0.756 | 0.360 | 0.543 | 0.114 | 0.548 | 0.110 | 0.103 | 0.040 | 0.823 | 0.252 |
| No. families | | 81 | | 238 | | 238 | | 238 | | 167 | |
| No. Sibpairs | | 112 | | 623 | | 623 | | 631 | | 314 | |

Results are presented as the Lod and Beta score for each marker for the five phenotypic traits AF, ATIc, ASIc, IGE and atopy. Map locations are shown for each marker in centimorgans (cM) from the p telomere.

**Table 2** Results of multi-point linkage analysis using BETA

| *Marker* | *Map position (cM)* | *AF* | | *ASIc* | | *ATIc* | | *IGE* | | *Atopy* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Lod* | *β* | *Lod* | *β* | *Lod* | *β* | *Lod* | *β* | *Lod* | *β* |
| D5S421 | 138.7 | 1.736 | 0.448 | 0.144 | 0.045 | 0.812 | 0.082 | 0.0125 | 0.033 | 0.138 | 0.077 |
| D5S642 | 153.4 | 0.466 | 0.209 | 0.230 | 0.051 | 0.127 | 0.030 | 0.069 | 0.022 | 0.014 | 0.021 |
| D5S666 | 154.21 | 0.383 | 0.188 | 0.155 | 0.041 | 0.041 | 0.017 | 0.045 | 0.018 | 0.006 | 0.014 |
| IL4 | 154.2 | 0.470 | 0.208 | 0.105 | 0.034 | 0.080 | 0.023 | 0.056 | 0.019 | 0.005 | 0.014 |
| IL9 | 156.5 | 0.005 | 0.021 | 0.083 | 0.030 | –0.001 | –0.003 | –0.033 | –0.015 | –0.018 | –0.024 |
| D5S393 | 158.0 | 0.042 | 0.060 | 0.143 | 0.039 | 0.000 | 0.000 | –0.067 | –0.033 | –0.035 | –0.032 |
| D5S399 | 159.9 | 0.032 | 0.053 | 0.275 | 0.054 | –0.030 | –0.014 | –0.253 | –0.041 | –0.004 | –0.044 |
| D5S658 | 161.5 | 0.255 | 0.150 | 0.266 | 0.054 | –0.053 | –0.019 | –0.176 | –0.034 | –0.001 | –0.007 |
| D5S436 | 166.6 | 0.114 | 0.098 | 0.706 | 0.087 | –0.231 | –0.038 | 0.007 | 0.007 | 0.031 | 0.031 |
| D5S210 | 166.61 | 0.114 | 0.098 | 0.922 | 0.100 | –0.311 | –0.044 | 0.015 | 0.010 | 0.019 | 0.025 |
| ADRB27 | 168.7 | 0.124 | 0.114 | 1.686 | 0.149 | –0.200 | –0.040 | 0.062 | 0.022 | 0.058 | 0.047 |
| D5S470 | 172.7 | 0.432 | 0.206 | 1.860 | 0.152 | 0.075 | 0.024 | 0.141 | 0.032 | 0.338 | 0.113 |
| D5S410 | 176.3 | 0.500 | 0.232 | 1.935 | 0.165 | 0.084 | 0.028 | 0.154 | 0.036 | 0.518 | 0.146 |
| No. families | | 81 | | 238 | | 238 | | 238 | | 167 | |
| No. Sibpairs | | 112 | | 623 | | 623 | | 631 | | 314 | |

Results are presented as the Lod and Beta score for each marker for the five phenotypic traits: AF, ATIc, ASIc, IGE and atopy. Map locations are shown for each marker in centimorgans (cM) from the p telomere.

the marker panel and affection status (Lod 1.736). There is no evidence of linkage between any of the markers and total serum IgE or atopy. The maximum lod for ASIc is 1.946 at a map position of 171.29 cM.

The first reported linkage of atopy with chromosome 5 was by Marsh *et al*.[9] In this study of 11 large Amish pedigrees, selected on the basis of serum IgE to common allergens in at least one child, there was linkage between five markers located on chromosome 5q31 and total serum IgE with the linkage being centred around the IL-4 locus. However, no linkage was seen between antigen-specific IgE and the same markers. Further evidence for the role of the Th2 cytokine cluster on chromosome 5q was provided by Meyers *et al*.[10] Using a similar phenotype, they reported linkage between several markers on 5q31–33 and total IgE in 92 Dutch families. Further segregation analysis of these families provide evidence for a second major locus regulating serum IgE levels unlinked to that on 5q31–33.[17] In addition a positive linkage between several markers on 5q and BHR was also seen in this population,[13] with the strongest evidence for both phenotypes being centred around the $\beta_2$ adrenergic receptor. Further to this, Doull *et al*[16] identified a possible association with total IgE and the IL-9 locus, but not with BHR in a UK random population. In 68 Japanese families ascertained through asthmatic children a positive linkage has also been found between asthma and atopy and gene markers in or near the IL-4 and Il-9 genes and D5S393 on chromosome 5q31–33.[18] In addition to candidate gene studies, The Collaborative Study on the Genetics of Asthma (CSGA), using a genome-wide search, also found linkage between asthma and 5q31–33 in Caucasian families[19] and also between 5q31-q33 and specific IgE responses to HDM in African-American families.[20] In contrast however two other large genome scans of Caucasian subjects have failed to find any strong evidence of

linkage between 5q31–33 and any atopy or asthma phenotypes.[21,22]

Other groups of investigators have been unable to detect evidence of linkage between polymorphic markers on chromosome 5q31–33 and IgE or BHR in large atopic families in Minnesota[23] and in 119 sibling pairs recruited from an Australian population, using the polymorphic marker D5S399.[24] Laitinen *et al*,[12] using 16 polymorphic markers spanning 5q31–33, failed to detect any linkage to either serum IgE level or asthma in 157 families from Finland. Another study examining association between 11 polymorphic markers spanning the region 5q31.1–33.1 and total serum IgE and BHR traits also did not detect any associations between these markers and either IgE or BHR.[11]

The inconsistency of these results may be attributable to several factors including differences in populations such as ethnicity and ascertainment of the families studied, differences in definition of phenotype and differences in the relative power of studies due to sample size. However, it remains that the linkage between 5q31 and asthma and atopy phenotypes is one of the most reproduced of all the possible linkages reported along with chromosome 12q.[25–27] Based on the evidence of 5q31–33 linkage studies, a number of recent studies have begun to identify polymorphisms in candidate genes at this locus and examine possible association of these polymorphisms with disease traits.[1,28]

One of the main difficulties in comparing results amongst these studies has been the multiplicity of methodologies used for assessing phenotype. For example atopy can be defined as specific IgE responses, total serum IgE levels, the presence of positive skin prick tests, the presence of an allergic disease such as hay fever, eczema or asthma or any combination of the above. Asthma has been measured both in terms of a doctor's diagnosis of reversible bronchoconstriction, or measurement of bronchial hyperresponsiveness in response to several agents by several methods. This holds especially true for the 5q31–33 candidate region as studies have suggested that there may be more than one candidate gene in this region, one predisposing to atopy (generation of Th2 responses to allergens) and another predisposing to the development of asthma (targeting of that Th2 response to the respiratory tract with concomitant chronic remodelling and repair of the epithelium and mesenchyme). Therefore we have used scores for both atopy and asthma that provide quantitative traits for non-parametric linkage and association analysis.[27]

The finding of a locus affecting asthma telomeric to the Th2 cytokine cluster in this study is consistent with data from other populations. Previously it has been shown that there is a locus affecting heritability of bronchial hyperresponsiveness on 5q31–33 independent of a locus affecting IgE and atopy in a Dutch family sample.[13] However unlike many other studies, in our population there was little evidence of linkage between atopy or total serum IgE levels and any marker on 5q31–33. The linkage between affection status and D5S421 is surprising given that it is 30 cM away from IL-4. However the sample size for this phenotypic trait is much smaller than that for the quantitative score (~3 times smaller).

The inconsistency of the results of linkage between 5q31–33 in different studies may be attributable to differences in populations, such as ethnicity and ascertainment of the families studied and/or differences in definition of phenotype. One approach to resolving problems of sample size and differences in phenotyping between studies is COAG (The Consortium On Asthma Genetics). This is a multi-centre collaborative initiative initiated in June 1998 with the limited goal of determining what could be learned about the genetics of asthma by pooling information from different studies, each of which had published its principal results, to allow combined analysis of the data. The initial focus of COAG will be to undertake a combined analysis of linkage data from several studies including this one on chromosome 5q31–33.[29]

In conclusion the results of this study support the hypothesis that the 5q31–33 region is linked to susceptibility to asthma, however there was no apparent linkage of measures of atopy in this population. Further typing of both STS markers and polymorphisms in candidate genes within this region will be needed to further understand this linkage and how it relates to both the atopy and asthma phenotypes.

## Patients and methods

### Sample collection

Initially random families were recruited from the Southampton area without reference to asthma or atopy.[30] The parents of 1800 children between 11 and 14 years of age randomly selected from the practice lists of all children registered with GPs in the Southampton area were sent a questionnaire inquiring about their general health and the number of children in their family. The questionnaire was open-ended and specifically did not mention atopic diseases. All families with three or more children were contacted to determine their willingness to take part in further studies. A total of 131 agreed to take part. Subsequently, a further 60 families were recruited through asthma probands with affected sibs via hospital-based clinics, and 49 single proband nuclear families were selected without regard to family history.[27] The studies were approved by the Southampton University Hospitals Joint Ethical Committee with children giving informed verbal consent, and parents giving informed written consent. This sample yielded a total of 240 families comprising 626 sib pairs from 237 informative families (at least two genotyped sibs) for the quantitative score and 114 affected sib pairs from 81 informative nuclear families.

### Clinical data collection

Each family member completed a structured written questionnaire on atopic symptoms and diseases derived from the International Union Against Tuberculosis and Lung Disease (IUALTD) questionnaire.[31] The written questionnaire was supplemented by a video questionnaire specifically on wheeze during exercise, nocturnal symptoms, and symptoms occurring at rest.[32] All subjects in each cohort were assessed for the following measurements. Skin-prick testing for 14 common allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, feathers, egg white and yolk, cow's milk, mixed grass, mixed trees, *Alternaria*, horse, *Aspergillus*, and *Cladosporium*, with a negative (saline) and a positive (histamine) control. The perpendicular diameters of each wheal were recorded. Bronchial reactivity to histamine was measured using the Yan technique[33] and FEV1 was

measured. Blood samples were taken for the measurement of serum total IgE levels and specific IgE levels to the following allergens: house dust mite (Der p 1, Der p 2), dog (Can f 1), cat (Fel d 1), *Alternaria* (Alt a 1), and timothy grass (Phl p 5). The serum total IgE was measured by enzyme-linked immunosorbent assay. Specific IgE antibodies were measured using the Magic Lite system.

**Molecular methods**

DNA was extracted using a simple salting out procedure[34]. Ten polymorphic markers spanning the q31–33 region of chromosome 5 were genotyped using the polymerase chain reaction (Table 1). Oligonucleotide primers, one of each pair labelled with one of four fluorescent dyes, were synthesised by Oswel. The PCR mix contained labelled and unlabeled primers (25 ng each primer), 10× buffer (Perkin–Elmer), magnesium chloride (Perkin–Elmer), deoxynucleotide triphosphates (Promega), water and Taq DNA polymerase (Perkin–Elmer), and approximately 40 ng of template DNA in a total reaction volume of 10 μl. The PCR conditions were optimised for each marker. PCR cycling was performed using an Omnigene Hybaid Thermocycler (Hybaid Ltd, UK) and a tetrad DNA engine (MJ Research Ltd, MA, USA). Data for four other markers previously typed were included in the linkage analysis, the $(CA)_n$ repeqat markers IL4 and IL9[16] and the single nucleotide polymorphisms R16G and Q27E of the ADRB2 gene.[15] Groups of markers were pooled together for multiplexing. 0.5 μl internal lane size standards (GeneScan 500-TAMRA, PE Biosystems), 1.66 μl of formamide and 0.33 μl of loading dye were mixed with 1/10 volume of the pooled PCR products. After denaturation at 94°C for 3 min, samples were loaded onto 36 cm 6% polyacrylamide gels and electrophoreses carried out in 1x TBE for 3–4 hours using a 373 Stretch DNA sequencer (Applied Biosystems, Foster City, CA, USA). For semi-automated data collection and analysis, 672 Genescan Collection software (version 1.1) was used. DNA fragment analysis software Genotyper Version 1.1 (Applied Biosystems) was used for semi-automated allele size assignment in base pairs.

**Statistical methods**

*Construction of quantitative scores:* Quantitative phenotype scores were derived for asthma and atopy using methods previously described.[27] Briefly, we defined asthma affection as a positive response to the question: 'Have you ever had asthma?' The qualitative trait of atopy was defined as a positive skin prick test (>3 mm above saline control) and/or a positive specific IgE (>1.43 SU/ml) and/or a positive Total IgE. Positive Total IgE was defined by the clinical guidelines in use in Southampton General Hospital with >81 IU/l for those aged 16 and above. And the following cut-offs for children: 1 year olds ⩾29, 2–5 year olds ⩾52, 6–7 ⩾56, 8–10 ⩾63, 11–12 ⩾44.8, 13–14 ⩾70, 15 ⩾75 IU/l. All analysis for atopy was also done using a standard value of 100 IU/l as a positive for all subjects but this made no difference to the results.

The questionnaire data were reduced to two variables by taking the first principal component of the written (WZ) and video (VID) questions. The histamine challenge (BHR) data were summarised as the slope of the dose–response. Spirometry was expressed as the ratio of predicted to observed $FEV_1$ (RFEV). The four summary variables (WZ, VID, BHR, and RFEV) were then adjusted for age and sex within ascertainment. Each of the four variables was then normalised by rank transformation.[35] Total serum IgE, specific IgE, skin prick, and written questionnaires on hay fever and eczema were used as indicators of atopy. Questionnaire responses were coded as integers monotonic on expected risk for eczema and hay fever. The questionnaire data were then reduced to two variables by taking the first principal component of the eczema (EZ) and hay fever (HF) questions. IGE is the natural logarithm of the total serum IgE. RAST is the natural log of the specific IgE summed across each allergen. SP is the principal component of the size of the wheals to 14 allergens, which were measured as major and minor axes.

From these variables atopy and asthma scores were constructed so as to better differentiate between atopy and asthma. For this we considered all traits related to both atopy and asthma (Affection status, WZ, VID, BHR, and RFEV, EZ, HF and RFEV), the first principal component was taken as atopy and the second as asthma. This gives a score for asthma that represents asthma without atopy. It gives higher scores to individuals with high values for the phenotypic variables related to asthma (ie, AF, WZ, VID, BHR and RFEV) and at the same time low values for the phenotypic variables related to atopy (ie, EZ, HF, IGE, RAST and SP). Conversely individuals with high values for both atopy and asthma related traits have negative scores.

*Linkage analysis:* Both single point and multipoint weakly-parametric (sib-pair) linkage analysis was undertaken using the BETA program.[36] The BETA program considers allele sharing proportions for 0, 1 and 2 alleles identical by descent as a function of one single parameter (β) and has been shown to be more powerful than alternatives such as MAPMAKER/SIBS. Linkage analysis was undertaken using both qualitative and quantitative traits. Traits analysed were the qualitative traits affection status (AF) and atopy and the quantitative scores ATIc (atopic asthmatic score) and ASIc ('non-atopy' asthmatic score) as well as log total IgE (IGE).

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