

Linkage and association of atopic asthma to markers on chromosome 13 in the Japanese population

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Chromosome 13 contains several candidate genes for asthma and atopy, and markers on this chromosome have been shown to be linked to phenotypes of atopy or asthma in two genome-wide searches. We conducted a linkage study for atopic asthma using markers spanning the whole of chromosome 13 in Japanese families ascertained through asthmatic children and examined associations of atopic asthma with markers where linkage was suggested. Data were analysed using MAPMAKER/SIBS for the multipoint lod score (MLS) analysis and SIB-PAIR for the transmission disequilibrium test (TDT). Three peaks which exceeded a lod score of 1.0 were observed (MLS 2.4 between D13S175 and D13S217, MLS 2.0 between D13S153 and D13S156, and MLS 1.4 between D13S285 and D13S293). The global TDT for atopic asthma was significant for the marker D13S153 ($P = 0.0065$) and the 96 bp allele of D13S153 was preferentially transmitted to atopic asthma-affected children ($P = 0.0009$, Bonferroni correction 5% = 0.0037, 1% = 0.00072). These findings indicate that genes on chromosome 13 may play an important role in the development of atopy or asthma across various populations.

INTRODUCTION

Atopy, expressed clinically as asthma, atopic dermatitis and/or rhinoconjunctivitis, is characterized by a genetic predisposition for generating IgE antibodies against common environmental allergens. Since family and twin studies have demonstrated an involvement of genetic components in the development of atopy and asthma (1,2), much work has been done to find the responsible gene(s). Using the candidate gene approach, linkage of atopy, asthma and/or asthma-associated phenotypes to chromosomes 5q31.1–q33 (IL-4 gene cluster) (3,4), 6p21 (HLA and TNF α) (5), 7q35 (TCRB) (6), 11q13 (high affinity IgE receptor) (7–11), 12q15–q24 (IFN γ , etc.) (12,13), 14q11 (TCRA) (14,15) and 16p12 (IL-4 receptor α chain) (16,17) has been described. Several other loci linked to atopy and asthma have also been suggested through genome-wide linkage studies (18,19). Although linkage results should

be replicated to be credible, only a small number of genes or regions have been replicated in separate population samples for asthma and atopy genes. Chromosome 13 contains several candidate genes for asthma and atopy, such as RANTES, STAT5a, endothelin receptor type B and chemokine receptor 7 (20) (information available at World Wide Web site <http://www.ncbi.nlm.nih.gov/genemap/>). Also, markers on this chromosome have been shown to be linked to phenotypes of atopy or asthma in two genome-wide searches (18,19). In this study, we conducted a linkage study for atopic asthma using markers spanning the whole of chromosome 13 in Japanese families ascertained through asthmatic children and, subsequently, examined associations between atopic asthma and markers where linkage was suggested. Since atopy/asthma is a complex disease in which a number of genes and environmental factors are involved, factors such as threshold inheritance, locus heterogeneity, epistasis, gene–environment interactions, time-dependent expression of the gene, etc. make it difficult to find atopy/asthma-associated genes (21). To overcome these difficulties, we selected as homogeneous as possible atopic asthma-affected sib pairs, with respect to childhood onset, strong mite sensitivity and ethnic origin.

RESULTS

Figure 1 shows the results of the multipoint lod score (MLS) for atopic asthma as a qualitative trait with markers on chromosome 13 using MAPMAKER/SIBS (22). Three MLS peaks which exceeded lod score 1.0 were observed (MLS 2.4 between D13S175 and D13S217, MLS 2.0 between D13S153 and D13S156 and MLS 1.4 between D13S285 and D13S293).

The program SIB-PAIR (23) was used to perform multiallelic forms of the transmission disequilibrium test (TDT) for the markers located next to the three MLS peaks in atopic asthmatic children. In the absence of linkage and allelic association between the chosen marker locus and the trait, one expects each allele of a heterozygous parental genotype to be transmitted with equal likelihood to the proband. Multiallelic TDT tests for symmetry in the full $n \times n$ transmission table, where n is the number of alleles at the locus (24). The null hypothesis is tested with Ewens χ^2 (global TDT; Table 1) (13). The global TDT for atopic asthma was significant for the marker D13S153 ($P = 0.0065$). Further TDT analysis, allele-by-allele,

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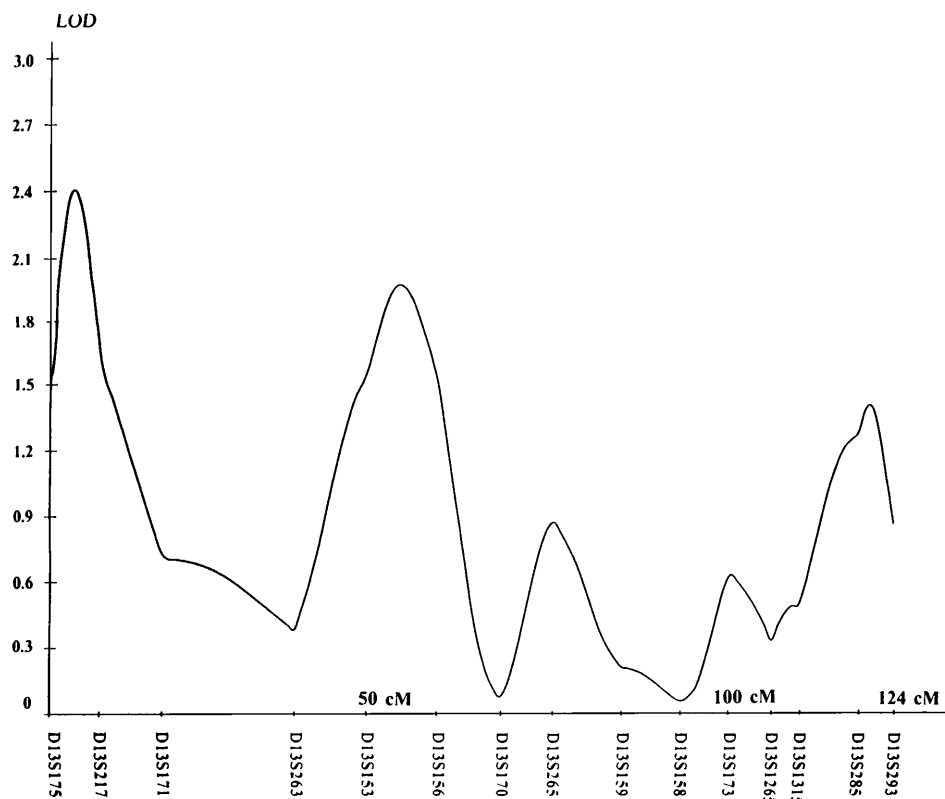


Figure 1. MLS for mite-sensitive atopic asthma on chromosome 13

Table 1. TDTs for mite-sensitive atopic asthma and markers on chromosome 13

Locus	No. of allele	Global TDT <i>P</i> -value for asthma ^a
D13S175	6	0.23
D13S217	11	0.27
D13S153	15	0.0065
D13S156	8	0.95
D13S285	15	0.24
D13S293	5	0.58

^aCorrected *P*-value for the multiple analysis is 0.0083 at the significance level of 0.05.

revealed that the 96 bp allele of D13S153 was preferentially transmitted to atopic asthma-affected children ($P = 0.0009$, Bonferroni correction 5% = 0.0037, 1% = 0.00072). Conversely, the 94 bp allele of D13S153 was transmitted less frequently to atopic asthma-affected children than would be expected by chance ($P = 0.0005$, Bonferroni correction 5% = 0.0037, 1% = 0.00072).

DISCUSSION

The present study suggests that loci responsible for the development of mite-sensitive atopic asthma are located near the marker D13S153 on chromosome 13. A genome-wide search conducted by Cookson and colleagues in a British population of 80 families (172 sib pairs) showed significant linkage between atopy and the marker D13S153, with a *P*-value

<0.001 (18). This linkage was confirmed in another set of families, recruited from clinics in the UK. On the other hand, another genome-wide search by the Collaborative Study on the Genetics of Asthma (CSGA) in the USA suggested linkage between markers on 13q21.3–qter and asthma in the Caucasian population (19). The region 13q21.3–qter contains the markers D13S285 and D13S293. Although the CSGA paper did not provide details of the markers which showed evidence of linkage with the trait of asthma, our data suggest that an asthma-associated gene(s) which is common to both Caucasian and Asian asthmatics might be located in this region.

Evidence for both linkage and association with atopic asthma was observed with marker D13S153, the same marker for which Cookson and colleagues found linkage to atopy in a British population (18). The gene signal transducer and activator of transcription 5a (STAT5a) is located within 1 cM distance from D13S153 (20). Asthma is characterized by recurrent episodes of airway obstruction, increased bronchial hyper-responsiveness and airway inflammation. An increased number of eosinophils is observed in asthmatic airways, and several studies have shown that the number of eosinophils is associated with abnormalities of FEV1, airway reactivity and clinical symptoms of asthma (25–27). Cytokines such as IL-3, IL-5 and GM-CSF are known to prolong eosinophil survival and to enhance their activity, resulting in progression of the inflammation of asthmatic airways (28). IL-5 receptor activation upon stimulation of IL-5 results in tyrosine phosphorylation of intracellular substrates, including two Janus kinases (Jak1 and Jak2) and STAT5 (29). Recent studies have shown that Jak1, Jak2 and STAT5 are particularly important in IL-5-mediated eosinophil responses (30). Therefore, STAT5a, as

a signal transducer of IL-5, is considered to be another candidate asthma-associated gene. Furthermore, evidence for linkage and association between D13S153 and the atopic phenotype were observed in both a British and our Japanese population. Since IL-5 has an enhancing effect on IL-4-induced IgE synthesis (31), it is possible that STAT5a may play some role in the development of atopy.

This study presents further evidence for a role of genes on chromosome 13 in the aetiology of asthma and atopy. Although the exact genes and nucleotide variants influencing atopy and asthma remain to be discovered, this study suggests that these genes may play an important role in the development of atopy and asthma across various populations.

MATERIALS AND METHODS

Subjects

Probands of the families studied were asthmatic children visiting the Paediatric Allergy Clinic of the University Hospital of Tsukuba. A full verbal and written explanation of the study was given to all family members interviewed and 86 families (375 members including 172 atopic asthmatic children) gave informed consent and participated in this study. Informed consent for subjects younger than school age was given by their parents. The mean age of the probands and their siblings was 11.2 years (range 3–29); the mean age of the parents was 41.1 years (range 28–72). The families examined in this study are, in part, the same ones that participated in our previous study (4).

Each family member was questioned regarding allergic symptoms and underwent a physical examination by paediatricians. Asthma was diagnosed in subjects according to the criteria of the National Institutes of Health, USA, with minor modifications (32). Patients had to show the two following characteristics: (i) two or more episodes of wheezing and shortness of breath during the past year; (ii) reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment. Patients treated with systemic steroids were excluded from this study. Since wheezing is often associated with viral respiratory infection in young children (33), subjects >3 years old were evaluated for the asthma phenotype. Young adult patients included in this study had been suffering from chronic asthma since childhood. The diagnosis of asthma in this population was confirmed by physicians or paediatricians. Total serum IgE levels and specific IgE levels to house dust mite, *Dermatophagoides farinae* (Df), were determined by the Pharmacia CAP System (Uppsala, Sweden). Atopy was defined by the presence of either or both of the following: a total serum IgE level more than one standard deviation above the geometric mean for the normal Japanese population and/or raised specific serum IgE levels to Df (RAST score >2). From the families enrolled we selected the families which had more than two asthmatic children with Df RAST score >3. These affected sib pairs and their parents were subjected to genetic analysis (65 affected sib pairs).

We analysed linkage using the affected sib pair method to avoid misdiagnosing any subjects as 'falsely non-affected'. The affected sib pair method applied to a large number of small families also offers an advantage because they are likely to be more representative of the population than a small number of large families. Also, analysing childhood onset asthmatics in

genetic studies may be advantageous since early onset forms of the disease are more likely to be genetic in origin as opposed to being merely a correlate of ageing or exposure to environmental stimuli (21). Since there is as yet no readily applicable and validated biomarker for airway inflammation and asthma, definition of an asthma phenotype largely depends on patient symptoms and the definition of asthma may vary in different studies. The diagnosis of asthma in our study was based on the questionnaire, but all of the asthma diagnoses were confirmed by their doctors. We excluded children <3 years old since children of this age often wheeze with viral respiratory tract infections (33). Allergic sensitization to house dust mite is important for the development of asthma in children, because high levels of IgE antibodies against the mite antigen show significant correlations not only with a clinical history of asthma, but also with a provocation test result with this antigen (34–36). More than 90% of child asthmatics are atopic and sensitized to house dust mite. However, non-atopic asthma exists in child asthmatics, who might have developed asthma under the influence of different pathophysiological mechanisms. Therefore, we selected mite-sensitive atopic asthmatics for our affected sib pair analyses to ensure homogeneity.

Molecular methods

DNA was extracted from peripheral blood leukocytes collected in EDTA. We selected 15 microsatellite markers listed in the Genome Data Base (GDB; <http://www.gdb.org>) on chromosome 13 with high heterozygosities (>0.70) and separated from each other by ~10 cM (D13S175, D13S217, D13S171, D13S263, D13S153, D13S156, D13S170, D13S265, D13S159, D13S158, D13S173, D13S1265, D13S1315, D13S285 and D13S293). The region of interest was amplified by PCR with a primer pair for each microsatellite marker. Forward primers were labelled with either 6-FAM, HEX or TET phosphoramidites (Custom Oligo Service, Tsukuba, Japan and Applied Biosystems, Foster City, CA). The markers were divided into two groups, considering the allele size ranges for the loci amplified by each primer pair, and labelled with appropriate phosphoramidites, so that the PCR products generated by each primer pair in the same group could be pooled and detected in one gel lane. The PCR reaction cocktail contained 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.0–1.5 mM MgCl₂, 0.1% Triton X-100, 200 µM each deoxynucleotide triphosphate (dNTP), 0.6 U AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, CT), 5 µM each primers and ~40 ng of template DNA in a total volume of 10 µl. PCR was performed in a 96-well plate using a programmable thermal cycler (GeneAmp PCR system 9600; Perkin Elmer) at 94°C for 5 min, followed by 35 cycles at 89–94°C for 15 s, then at an annealing temperature of 55°C for 15 s, an extension of 72°C for 30 s, followed by 72°C for 10 min. Electrophoresis was carried out using an ABI PRISM model 377 DNA Sequencer equipped with Genescan software. The PCR products of the same group were mixed in an appropriate ratio and diluted in sterile distilled water to a final dilution ratio of 1:10–1:20. Aliquots of 3.5 µl of loading cocktail, prepared by mixing 2.5 µl formamide, 0.5 µl blue dextran (50 mM EDTA, 50 mg/ml blue dextran) and 0.5 µl size standard (GS-500 TAMRA), were added to 1.5 µl of pooled PCR products and heated at 95°C for 5 min and then kept chilled. An aliquot of 2 µl of this mixture was loaded on a 4% polyacrylamide gel and run for 2–3 h. Analysis was performed using the Genescan software as described by the

manufacturer. Gel lane tracking and sizing of the ROX-labelled size standard peaks was checked manually for all lanes. Genescan Analysis v.2.02 was used for semi-automatic 'allele calling' as described by the manufacturer. Alleles were defined as the highest two peaks within the expected allele range and checked manually for all lanes.

Statistical analyses

To detect linkage and allelic association, multipoint non-parametric linkage analysis was performed using the computer program MAPMAKER/SIBS v.2.0 (22). The program SIB-PAIR was used to perform multiallelic forms of the TDT (23).

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