

A genome-wide linkage analysis of orchard grass-sensitive childhood seasonal allergic rhinitis in Japanese families

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Seasonal allergic rhinitis (SAR) is an inflammatory disease of the nose and eyes that follows sensitization to air-born pollens. We conducted a genome-wide linkage screening of 48 Japanese families (188 members) with orchard grass (OG)-sensitive SAR children (67 affected sib-pairs) in a farming community in central Japan where OG was planted for apple farming and OG pollen is a major cause of SAR. We used the GENEHUNTER program to performed nonparametric multipoint linkage analysis for OG-sensitive SAR as a qualitative trait and for log total serum IgE levels and OG-RAST IgE levels as quantitative traits. Genotyping data of 400 microsatellite markers suggested linkage of SAR to chromosomes 1p36.2, 4q13.3, and 9q34.3 ($P < 0.001$), linkage of serum total IgE levels to 3p24.1, 5q33.1, 12p13.1, and 12q24.2 ($P < 0.001$), and linkage of OG-RAST IgE levels to 4p16.1, 11q14.3, and 16p12.3 ($P < 0.001$). Weak evidence for linkage of SAR to 5q33.1 was also observed ($P = 0.01$). All these regions, with the exception of 9q34.3, have been previously reported to be linked to asthma and/or atopy. These data suggest that, although loci linked to SAR are likely to be common to asthma, a strong contribution by specific gene(s) to OG-sensitive SAR is unlikely.

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Introduction

Allergic diseases, such as asthma, allergic rhinitis (AR), and atopic dermatitis, are complex disorders, involving both environmental and genetic factors. Twin and family studies have confirmed the existence of a genetic predisposition in the development of allergic diseases,^{1,2} where a clear Mendelian pattern of inheritance has not been established. Several genomic loci influencing asthma and atopic phenotypes have been suggested through genome-wide linkage studies of a British/Australian population,³ different American populations including African-Americans, Hispanics, and Caucasian samples of asthmatic sib-pairs and extended families,⁴ a founder population among the Hutterites,^{5,6} German-Swedish populations,⁷ and Japanese families.⁸ Chromosomal regions containing candidate genes for atopy and asthma have also been investigated for linkage and association. Although the results of these studies were inconsistent, some loci were

common across different populations, suggesting a true linkage. Recently, a genome-wide linkage scan identified a novel susceptibility locus for atopic dermatitis, that has not been reported for asthma or atopy-related phenotypes.⁹

The development of seasonal allergic rhinitis (SAR), which is also referred to as hereditary allergic diathesis, is primarily dependent upon the degree of IgE antibody response to a specific pollen. It is suggested that IgE antibody responses to pollen may be controlled by genes associated with a particular HLA haplotype.^{10–13} Epidemiologic studies have suggested that SAR and asthma sometimes occur together in the same family, indicating that some overlap exists in the genetic factors that contribute to the development of the two diseases.^{14–16} However, there has been no reports of genome-wide screens for SAR susceptibility genes.

In central Japan, there is a farming community in which orchard grass (OG) pollens are a major cause of SAR, and 8.4% of randomly selected schoolchildren suffer from SAR.¹⁷ To clarify further the genetic bases of atopic diseases, we conducted a genome-wide linkage scan for OG-SAR among families living in this region. We think that these families were appropriate to evaluate genetic factors contributing to allergic rhinitis because they were living in a relatively homogenous area concerning OG pollen concentration.

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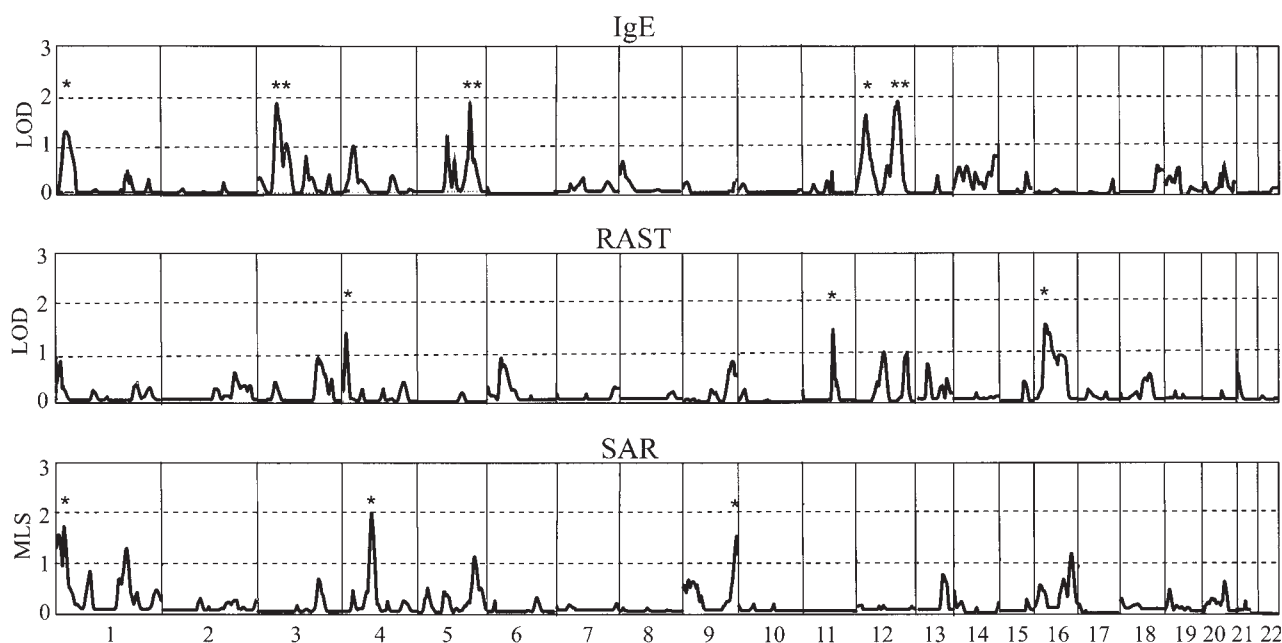


Figure 1 Linkage results for orchard grass-sensitive seasonal allergic rhinitis (SAR), orchard grass-specific IgE levels (OGRAST) and total serum IgE levels (IgE). Chromosomes are arranged by number from p-ter to q-ter with recombination distance in centimorgans on a linear scale. Peaks with significant and suggestive linkage are marked as ** and *, respectively.

Results

Genotyping 400 microsatellite markers in 188 members of 48 families revealed that three loci, 1p36.2 (D1S2667), 4q13.3 (D4S392), and 9q34.3 (D9S1826), satisfied the criteria for suggestive evidence for linkage to OG-sensitive SAR, and that three loci, 4p16.1 (D4S2935), 11q14.3 (D11S4175), and 16p12.3 (D16S3046), satisfied the criteria for suggestive evidence for linkage to OG-specific IgE levels (OG-RAST) titers. Three loci, 3p24.1 (D3S1266), 5q33.1 (D5S410), and 12q24 (D12S86), satisfied the criteria for significant evidence for linkage to log serum total IgE levels. The distributions of the maximum lod scores (MLS) for OG-sensitive SAR and lod scores for log serum total IgE levels and those for OG-RAST titers on autosomal chromosomes are shown in Figure 1 and Table 1.

Discussion

Although several genome-wide linkage studies of asthma and atopy have been reported, to our knowledge, this is the first report of a genome-wide linkage scan for SAR. The genomic loci with possible linkage to OG-sensitive SAR are 1p36, 4q13, and 9q34. Linkage of asthma to 1p36 in the Hutterites,⁶ and linkage of elevated IgE levels,¹⁸ and IgE specific to mites to 4p13.3¹⁹ have been reported. Taken together with the findings of the present study, the data indicate that suggestive loci linked to SAR are common to asthma and SAR. However, no report has documented linkage of atopy or asthma to 9q34. The question of whether linkage to 9q34 is specific to OG-sensitive SAR warrants further investigation because the SAR linkage findings are only suggestive, not significant.

Table 1 Regions with significant and suggestive linkage evidence

Chromosome	Position		Marker	SAR		RAST		IgE	
	Genetic	Physical		MLS	P	LOD	P	LOD	P
1p36.22	4.2 cM	5.8 Mb	D1S2667	1.70*	<0.001	0.59	0.03	1.30*	0.002
3p24.1	52.6 cM	33.65 Mb	D3S1266	0.00	>0.05	0.33	>0.05	1.85**	<0.001
4p16.1	14.0 cM	6.5 Mb	D4S2935	0.00	>0.05	1.47*	<0.001	0.00	>0.05
4q13.3	79.0 cM	81.5 Mb	D4S392	2.01*	<0.001	0.15	>0.05	0.00	>0.05
5q33.1	156 cM	168.1 Mb	D5S410	1.09	0.01	0.00	0.03	1.92**	<0.001
9q34.3	159.6 cM	137.5 Mb	D9S1826	1.52*	<0.001	0.83	0.02	0.20	0.02
11q14.3	91.5 cM	99.4 Mb	D11S4175	0.11	>0.05	1.38*	<0.001	0.19	>0.05
12p13.1	30.6 cM	15.47 Mb	D12S364	0.00	>0.05	0.01	>0.05	1.55*	<0.001
12q24.23	134.5 cM	128.4 Mb	D12S86	0.00	>0.05	0.09	>0.05	1.83**	<0.001
16p12.3	40.7 cM	32.8 Mb	D16S3046	0.01	>0.05	1.41*	<0.001	0.00	>0.05

**Significant and *suggestive evidence for linkage. P: empirical P value at the marker (uncorrected for multiple testing).

The lipocalin 1 gene is located near the D9S1826 marker on 9q34. Lipocalins are a group of extracellular proteins and are abundant in tears, nasal mucus proteins, and saliva.²⁰ They are thought to play a role in nonimmunologic defenses and in the control of inflammatory processes in oral and ocular tissues.²¹ If linkage of SAR to 9q34 is confirmed, the lipocalin 1 gene may be a good candidate for association with SAR.

In the present study, significant evidence for linkage of total serum IgE levels to chromosomes 3p24, 5q33, and 12q24 was observed. Linkage of atopy and total serum IgE levels to chromosome 5q31–q33 and 12q24 has been reported.^{8,22–29} Weak linkage of asthma to chromosome 3p24 was also reported in the Hutterites.⁶ Suggestive evidence for linkage of specific IgE against OG to 4p16.1, 11q14.3, and 16p12.3 was observed in the present study. Linkage of specific IgE against house dust mite to 4p16, 11q14, 16p12 has been reported.^{6,7,30} Therefore, the present study confirms previous findings of linkage of atopy to these chromosome regions. With the exception of 5q33, linkage of SAR to these loci was not suggested. These findings indicate that loci linked to total serum IgE levels do not strongly influence a genetic predisposition for SAR and vice versa.

Vaz and Levine³¹ found clear cut differences among inbred mouse strains in IgE antibody responsiveness to repeated injections of antigens. The difference in responsiveness was correlated with the H-2 genotype of the strain. Marsh *et al*³² have suggested that the enhanced IgE response to highly purified pollen allergens such as antigen E and Ra5 is associated with a particular HLA haplotype. In the present study, however, none of the SAR traits, OG-specific IgE levels, or total serum IgE levels showed positive linkage to 6p21, which is where the HLA genes are located.

We previously reported linkage of atopic asthma to chromosomes 4q35, 5q31–q33, 6p22–p21.3, 12q21–q23, and 13q14.1–q14.3 by a genome-wide linkage study of Japanese families.⁸ Among these regions, only 5q33.1 is linked commonly to asthma and SAR. Chromosome 5q31–q33 is linked to atopy and/or asthma and bronchial hyper-responsiveness.^{8,22–28} Thus, the weak but positive findings of linkage to 5q33 in the present study may reflect one of the loci common to asthma and SAR.

We should underline the limited power of our study because of a small sample size. It is possible that the HLA loci and other asthma loci putatively implicated by our linkage study of Japanese families do influence both asthma and SAR, but that linkage has not been detected because of the limited power of the present study.

Materials and methods

Subjects and families

Symptoms of SAR were assessed with a standard respiratory and allergy questionnaire in students at three elementary schools and one junior high school in Matsukawa, Japan. Families having two or more siblings with SAR were selected for participation in the study. Each family member was further questioned regarding allergic symptoms and underwent a physical examination by participating physicians. The diagnostic criteria for OG SAR included hay fever or persistent nasal symptoms, associated itching of the eyes or nose, and/or watering or

redness of the eyes, symptoms occurring regularly only between April and June, and positive prick test or radioallergosorbent test (RAST) reactivity to OG antigen (Torii, Tokyo, Japan). Other allergic symptoms such as asthma and atopic dermatitis were also evaluated by participating physicians. A full verbal and written explanation of the study was given to all family members interviewed. A total of 48 families (188 members) who gave informed consent participated in this study. These families were not the ones we previously examined for linkage to asthma.⁸

OG-specific IgE levels (OG-RAST) and total serum IgE levels were determined for all participants with the Pharmacia CAP System (Uppsala, Sweden). To achieve homogeneity of the SAR patients, only subjects with OG-RAST IgE levels higher than 3.5 U_A/ml were included as affecteds. Total serum IgE levels of the children and the parents were 830.8 ± 1161.6 IU/ml (mean $\pm$ s.d.) and 269.5 ± 559.0 IU/ml, respectively, and OG-RAST IgE levels for children and their parents were 33.18 ± 31.91 U_A/ml and 11.99 ± 23.97 U_A/ml, respectively.

Characteristics of the families examined in this study are listed in Table 2. No non-paternity in each family was confirmed by microsatellite marker analysis. Average age of the probands and their siblings was 14.1 years (range, 6 to 29) and that of the parents was 46.0 years (range, 35 to 59). Unaffected children of families in which parents' material was not available were included to estimate parents' genotypes. The present study was approved by the Committee of Ethics of the University of Tsukuba (Tsukuba, Japan).

Genotyping

DNA was extracted from peripheral blood leukocytes by the standard phenol extraction method. Genotyping was performed with the ABI PRISM[®] Linkage Mapping Set Version 2 (Applied Biosystems, Foster City, CA, USA). For higher resolution mapping of chromosome 1, 21

Table 2 Characteristics of the families

No. of children	123
Mean age (years)	14.1 $\pm$ 4.4
Sex ratio (M/F)	66/57
Total IgE (IU/ml)	830.8 $\pm$ 1161.6
OG-RAST IgE (U _A /ml)	33.2 $\pm$ 31.9
No. of affected children	110
(Allergic rhinitis regularly only between April and June and OG-RAST IgE levels higher than 3.5 U _A /ml)	
No. of affected children with conjunctivitis	70
No. of affected children with asthma	17
No. of affected children with atopic dermatitis	27
No. of affected SIB pairs	67
No. of parents	65
Mean age (s.d.)	46.1 $\pm$ 4.4
Total IgE (IU/ml)	269.5 $\pm$ 559.0
OG-RAST IgE (U _A /ml)	12.0 $\pm$ 24.0
No. of families	48
Family with	
No parent	13
One parent	3
Two parents	32

additional markers were used: D1S2845, D1S2660, D1S2633, D1S2694, D1S503, D1S2750, D1S2799, D1S433, D1S2658, D1S2851, D1S452, D1S2790, D1S242, D1S416, D1S2791, D1S212, D1S2640, D1S466, D1S2217, D1S2848, and D1S202. These additional markers were selected from those listed on the publicly available chromosome 1 genetic map at the Marshfield Medical clinic web site (http://www.marshmed.org/genetics/Map_Markers/maps/indexmap.html). The total number of markers analyzed were 400. The microsatellite markers were amplified by polymerase chain reaction (PCR) with the specific primer pair for each microsatellite marker as follows. 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 μ M each of deoxynucleotide triphosphates (dNTPs); 0.6 U AmpliTaq DNA polymerase (Perkin Elmer, NJ, USA); 5 μ M each of primers, and 20 ng of template DNA in a total volume of 10 μ L. PCR was performed in a 96-well plate with a programmable thermal cycler (GeneAmp PCR System 9600, Applied Biosystems). The amplification was accomplished with a 2-min primer cycle at 95°C, followed by 30 cycles consisting of denaturation at 94°C for 45 s, annealing at 52 to 65°C for 45 s, and extension at 72°C for 60 s followed by a final 7-min cycle at 72°C. Reactions for each marker were performed separately, and the products were multiplexed into size-specific sets prior to gel electrophoresis. PCR products then were multiplexed into panels by pooling on the basis of allele-size range and fluorescent label. Aliquots of the multiplexed samples were mixed with TAMRA-labeled GeneScan 500 (Applied Biosystems). Electrophoresis was carried out with an ABI PRISM model 3100 DNA Sequencer equipped with GeneScan™ software. Analysis was performed with GeneScan™ and Genotyper™ software (Applied Biosystems) as described in the manufacturers' manuals. Gel lane tracking and sizing of the TAMRA-labeled size standard peaks were checked manually for all lanes. Alleles were defined as the two highest peaks within the expected allele range and were also checked manually for all lanes.

Linkage analysis

Allele frequencies were estimated from the parental chromosomes with the SIB-PAIR program (version 0.99.9, <http://www.qimr.edu.au/davidd/davidd.html/>). Information about marker order and position was obtained from the Marshfield Medical Clinic web site. Multipoint non-parametric linkage analysis was performed with the computer program GENEHUNTER version 2.³³ Maximum lod scores (MLS) for SAR or lod scores of the EM Haseman Elston analysis for OG-RAST titers and serum total IgE levels were computed with the Estimate and Haseman-Elston subroutines in the GENEHUNTER program.

All possible sibling pairs were examined and were weighted to account for dependency. Linkage results were interpreted empirically by simulation analysis. Using all pedigrees and all genetic markers used in the actual analysis, we generated 1,000 unlinked replicates by the Simulate program (<ftp://linkage.cpmc.columbia.edu/software/simulate>) and conducted analyses with the same software (GENEHUNTER) used in the actual analysis. We calculated an empirical genome-wide significance level as the proportion of replicates for which MLS for SAR or lod scores of the EM Haseman Elston analysis for OG-RAST titers and serum total IgE levels

was greater than that obtained in the real data set. Suggestive and significant evidence for linkage were defined according to published criteria³⁴ as once random occurrence per genome screen for suggestive evidence and 0.05 times for significant evidence. The simulation procedure suggested that we would have expected to have obtained on average one multipoint MLS of 1.6 per genome scan in the absence of linkage. An MLS of 3 would have been expected only once in every 20 genome scans in the absence of linkage. Thus, MLSs of greater than 1.50 and 3.02 were interpreted as suggestive and significant in this study for SAR, respectively. In the same way, lod scores of greater than 1.11 and 2.03 for OG-RAST titers and those of greater than 0.95 and 1.76 for total IgE levels were interpreted as suggestive and significant, respectively.

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