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Genome-wide linkage analysis of allergic rhinoconjunctivitis in a Swedish population

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Summary

Background Allergic rhinoconjunctivitis is a common complex disorder characterized by itching and irritation in the nose, bouts of sneezing, watery rhinorrhoea, nasal congestion and itchy eyes with tears and swelling. Like other atopic disorders such as allergic asthma and atopic dermatitis, the development involves complex interactions of genes and environmental factors.

Objective The purpose of this study was to identify susceptibility loci for allergic rhinoconjunctivitis.

Methods We conducted a genome-wide linkage analysis using a non-parametric, affected-relative-pair method. The 250 families used were collected originally for an atopic dermatitis linkage study.

Results Three regions showed favour in evidence of linkage to allergic rhinoconjunctivitis: 3q13 (D3S1278: logarithm of odds ratio (LOD) = 1.64, $P < 0.003$), 4q34–35 (D4S1652: LOD = 1.49, $P < 0.005$) and 18q12 (D18S535: LOD = 1.94, $P < 0.002$). In addition, four regions showed weaker evidence in favour of linkage: 6p22–24 (D6S1959: LOD = 1.39, $P < 0.006$), 9p11–q12 (D9S1118: LOD = 1.15, $P < 0.02$), 9q33.2–34.3 (D9S915: LOD = 1.29, $P < 0.01$) and 17q11.2 (D17S1294: LOD = 1.13, $P < 0.02$). In single-point analysis, one locus on chromosome 3 close to marker D3S1278 reaches the suggestive level (LOD = 2.28, $P < 6 \times 10^{-4}$) while one on chromosome 17 close to marker D17S921 almost reaches this level (LOD = 2.17, $P < 8 \times 10^{-4}$, Table 3).

Conclusion Our results support the linkage to allergic rhinoconjunctivitis on 3q13, 6p23–p24 and 9q34.3 shown in previous investigations.

Keywords affected sib pairs, allergic rhinoconjunctivitis, atopic disorders, genome-wide linkage analysis

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Introduction

Allergic rhinoconjunctivitis is a common disorder characterized by itching and irritation in the nose, bouts of sneezing, watery rhinorrhoea, nasal congestion, itchy eyes with tears and swelling around the eyes [1]. Epidemiological studies indicate an increasing trend of allergic rhinoconjunctivitis over the past 20 years, especially in

the industrialized world. The same is true for other atopic diseases such as atopic dermatitis and allergic asthma [2–8]. Together, these three disorders have a high rate of co-existence, 50–80% of atopic dermatitis patients also having asthma and allergic rhinoconjunctivitis [9]. The development of the atopic diseases involves both environmental and genetic factors and is considered to be multi-factorial. Twin and family studies indicate a genetic predisposition [10–12]. In allergic rhinoconjunctivitis, genetic factors contribute with an estimated 75% while environmental factors contribute 25% of the risk [13].

In the search for the genetic components of the atopic disorders, several genome-wide linkage analyses have

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been performed. They have shown susceptibility loci influencing allergic asthma and atopic dermatitis, e.g. [14–18]. Despite inconsistencies, some loci are common among the studies. Most studies have focused on allergic asthma, IgE levels and/or atopic dermatitis, and to date only two genome-wide linkage analyses for allergic rhinitis (AR) have been published [19, 20].

To identify susceptibility loci influencing the development of allergic rhinoconjunctivitis, we conducted a genome-wide linkage analysis with 367 microsatellite markers. We used a non-parametric, affected-relative-pair method in families. The families were originally collected for linkage analysis of atopic dermatitis [18].

Material and methods

Patient material

The siblings in the present study form part of a larger patient material collected in Sweden as previously described [21]. Families were recruited during 1995–1997. Patient registers at the Departments of Dermatology, Karolinska Hospital and Danderyd Hospital, Stockholm, Sweden were used. Five thousand patients diagnosed with atopic dermatitis were identified and contacted by letter. Families with at least two affected siblings above 4 years of age were included. All participating patients gave their informed consent, and for children below 18 years of age consent was also obtained from their parents. The parents were included when possible, regardless of their atopic status. All siblings were evaluated regarding atopic dermatitis using the UK Working Party's Diagnosis Criteria [22–24]. All affected siblings also participated in a standardized interview covering different aspects of atopic diseases, including allergic rhinoconjunctivitis. In all affected siblings the occurrence of IgE antibodies to Phadiatop[®], a mixture of inhalant allergens (Pharmacia CAP System Phadiatop[®] FEIA, Uppsala, Sweden), were measured. The Phadiatop[®] was reported as either positive or negative. The siblings with a positive Phadiatop[®] and reporting the presence of allergic rhinoconjunctivitis in the interview were included in the linkage analysis (Table

1). The Karolinska Hospital Ethics Committee approved the study.

Genotyping

Genotyping was as described previously [18]. A control DNA was run on each gel. A total of 351 autosomal markers and 16 X-chromosomal markers were genotyped. The basis for the microsatellite markers was the Weber 6 screening set (Cooperative Human Linkage Center, Iowa City, USA) [25]. The marker maps were based on the Marshfield Medical Research Foundation (<http://research.marshfieldclinic.org/genetics/>). Mean heterozygosity for the autosomal markers was 0.82 and for the X chromosomal 0.71. The mean intermarker distance was 10.3 cM, covering the whole genome.

Statistical analysis

As the mode of inheritance is unclear, non-parametric, affected-relative-pair-based methods were used to detect linkage. The Allegro [26] software was used and the corresponding *P*-values were interpreted according to Lander and Kruglyak [27], as summarized by Nyholt [28]. For multi-point linkage analysis, logarithm of odds ratio (LOD) 2.19 (corresponding to $P \leq 7.4 \times 10^{-4}$) is regarded as suggestive level. All the families were weighted equally in a linear model. For the X chromosome the linkage analysis was performed with MAPMAKER/SIBS [29] as suggested by Nyholt [28]. *P*-values were estimated using $P(\text{LOD}) = 0.5 \times (\chi^2_1 > 2 \ln 10 \times \text{LOD})$. The suggestive level was defined as X-MLS (maximum LOD score) 3.06 (corresponding to $P \leq 7.4 \times 10^{-4}$).

Allele frequencies were estimated from all genotyped individuals using the zGenStat1.126 program (H. Zazzi, unpublished). All genotyped markers were checked for Mendelian incompatibilities using zGenStat1.126. Either incompatibility was resolved unambiguously or individuals and/or pedigrees were excluded from linkage analysis. To identify markers with allele dropout or other problems, the expected number of homozygote was calculated based on the estimated allele frequencies and

Table 1. Affected-relative pairs used in the following susceptibility regions

Chromosome	Number of marker	Number of families	A-S	A-H-S	P-O	Aunt/uncle-niece/nephew
3	30	241	214	24	263	10
4	21	79	102	9	121	5
6	22	250	252	25	302	35
9	15	76	94	5	114	5
17	18	82	105	9	123	9
18	12	214	224	18	265	27

A-S, affected sib pair; A-H-S, affected half-sib pair; P-O, affected parent offspring pair.

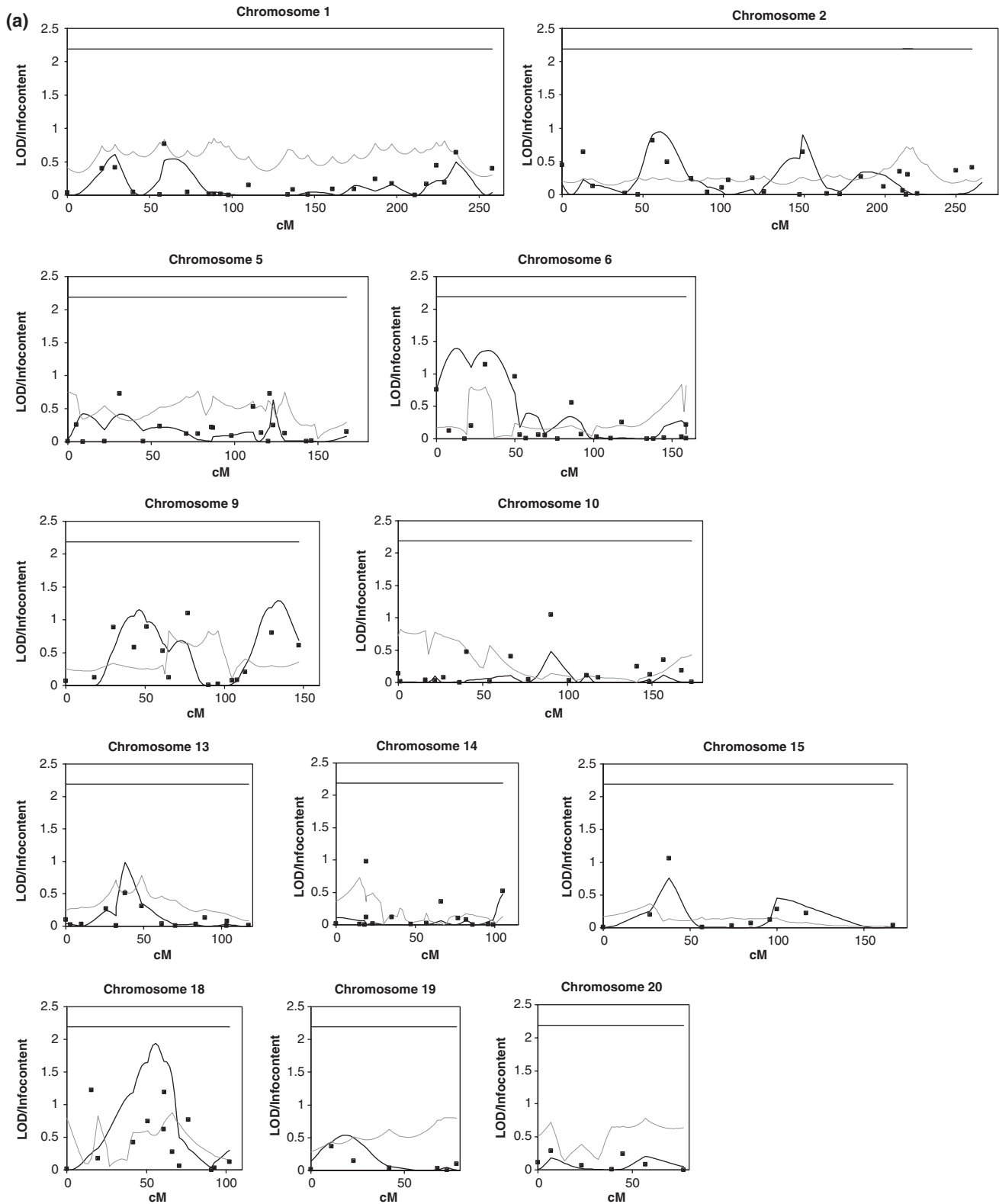


Fig. 1. Results of the multi-point and single-point linkage analysis for the allergic rhinoconjunctivitis phenotype. The logarithm of odds ratio (LOD) score is given on the Y-axis and the recombination distance in centimorgans (cM) from p-ter to q-ter on X-axis in linear scale. The linkage analysis was performed in Allegro for the autosomes and in MAPMAKER/SIBS for the X chromosome. The horizontal line indicates the suggestive level of linkage (LOD = 2.19, $P < 7.4 \times 10^{-4}$; and LOD = 3.06, $P < 7.4 \times 10^{-4}$ for X chromosome). The "■" indicated single-point LOD score, while the thick black line indicates multi-point LOD score and the thin grey line the information content.

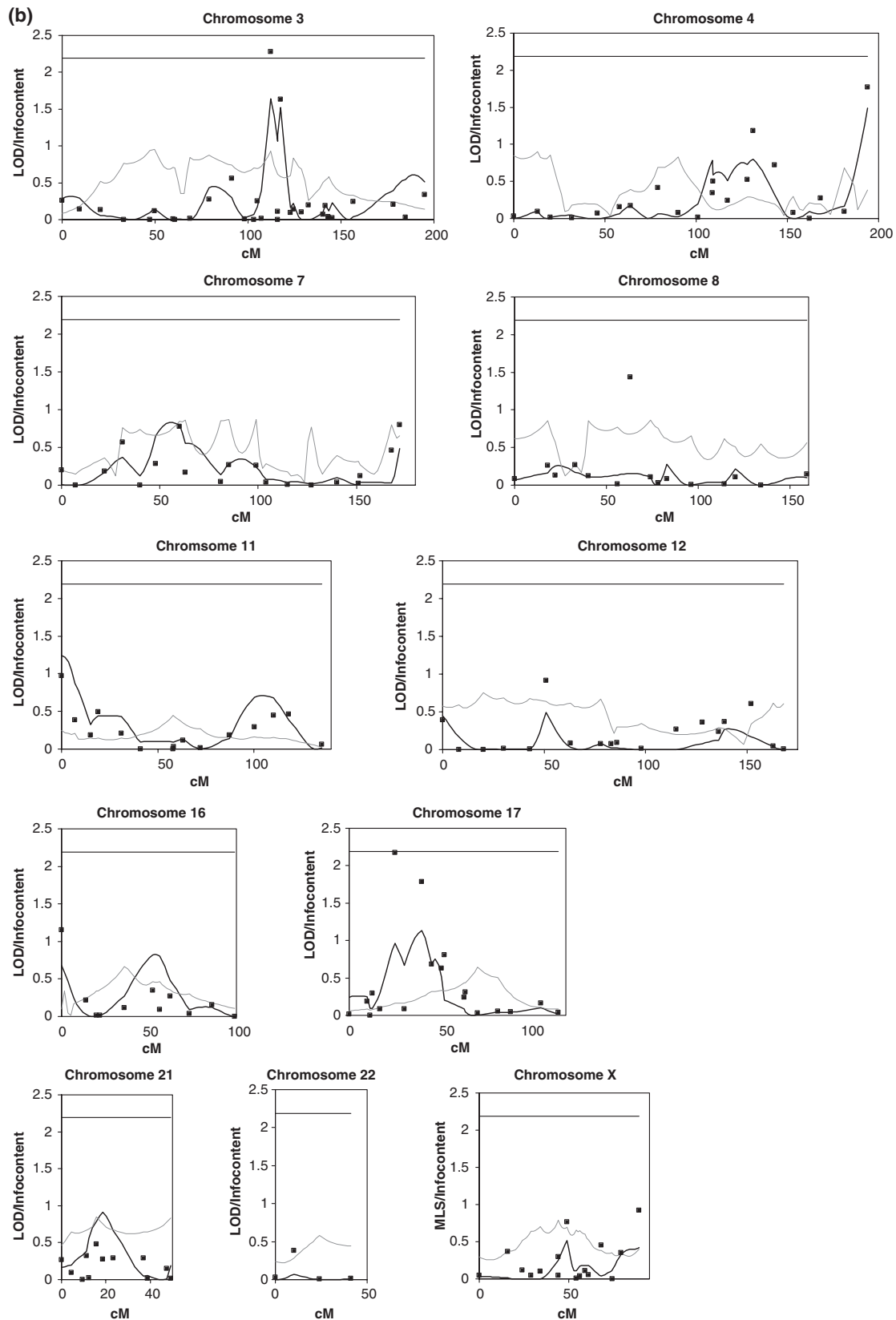


Fig. 1. Continued

compared with the observed numbers of homozygote using the Pearson's χ^2 test with zGenStat1.126 software (H. Zazzi, unpublished). Any marker showing significant deviation from expected homozygosity frequency ($P < 0.001$) was reanalysed or removed from the analysis.

Results

In the multi-point linkage analysis, evidence in favour of linkage was found to seven regions: 3q13 (D3S1278: LOD = 1.64, $P < 0.003$), 4q34–35 (D4S1652: LOD = 1.49, $P < 0.005$), 6p22–24 (D6S1959: LOD = 1.39, $P < 0.006$), 9p11–q12 (D9S1118: LOD = 1.15, $P < 0.02$), 9q33.2–34.3 (D9S915: LOD = 1.29, $P < 0.01$), 17q11.2 (D17S1294: LOD = 1.13, $P < 0.02$) and 18q12 (D18S535: LOD = 1.94, $P < 0.002$) (Fig. 1, Table 2). In single-point analysis, one locus on chromosome 3 close to marker D3S1278 reaches the suggestive level (LOD = 2.28, $P < 6 \times 10^{-4}$) while one on chromosome 17 close to marker D17S921 almost reaches this level (LOD = 2.17, $P < 8 \times 10^{-4}$, Table 3). All of the chromosomes were typed in at least 76 families. Additional typing was performed on some chromosomes, which explains the different numbers of families actually used for the linkage analysis (Table 1). The reason for

using different number of families is that the original genome-wide linkage analysis (for atopic dermatitis) was carried out in two steps, adding more families in the regions indicated linkage. Among the seven regions with evidence of linkage to allergic rhinoconjunctivitis, 250 families were used for chromosome 6, 241 families for chromosome 3, 214 families for chromosome 18, 82 families for chromosome 17, 79 families for chromosome 4 and 76 families for chromosome 9.

Discussion

We report a genome-wide linkage analysis with 367 microsatellite markers in up to 250 families affected with allergic rhinoconjunctivitis. When interpreting the results from such analysis, opinion differs regarding the thresholds for linkage. Lander and Kruglyak [27] give $P < 7.4 \times 10^{-4}$ as the threshold for suggestive linkage and $P < 2.2 \times 10^{-5}$ for significant linkage when allele-sharing methods in human sib pairs are used. According to these criteria, suggestive linkage means statistical evidence of one random occurrence in a genome-wide linkage analysis, while significant linkage is 0.05 random occurrences in a genome-wide linkage analysis. We have performed simulations to evaluate the results from the genome-wide linkage analysis and to obtain more precise significance levels in our material. The simulation was done for the phenotype atopic dermatitis. In the simulation, 921 of the 5000 simulations had at least one peak reaching the level for suggestive linkage. This is much less than the expected 5000 peaks, indicating that the threshold according to Lander and Kruglyak is too conservative for this study. On the other hand, we have not corrected for studying more than one phenotype and the simulation was carried out on atopic dermatitis and not the phenotype allergic rhinoconjunctivitis.

In our multi-point analysis seven regions gave evidence of linkage above LOD 1; 3q13, 4q34–35, 6p21–24, 9p11–q12, 9q33.2–34.3, 17q21 and 18q21.3. In addition one marker, D3S1278, on chromosome 3 reached the suggestive level for single-point analysis (LOD = 2.28, $P < 6 \times 10^{-4}$).

When performing linkage analysis in multi-factorial or complex diseases, one limitation is the sample size. A relatively small sample size can limit the power of detecting linkage in a genome screen and increase the possibility of type I experiential error (false positive) [30]. Increased power can be achieved by adding more affected relatives pairs to the study. Other factors such as statistical noise, epistasis, reduced or incomplete penetrance, phenocopies and genetic and environmental heterogeneity also make the gene mapping difficult [31]. Our patient material is fairly large, even if it diverges among the chromosome regions, as does the marker spacing. This has to be kept in mind when interpreting the results.

Table 2. Multipoint linkage analysis for rhinoconjunctivitis

Chromosome	Cytogenetic region	Map position (cM)*	Closest marker	LODP-value†
3	3q13.1	117	D3S1278	1.64 < 0.003
4	4q34–35	194.1	D4S1652	1.49 < 0.005
6	6p24–21	13	D6S1959	1.39 < 0.006
9	9p12–q11	46.2	D9S1118	1.15 < 0.02
9	9q33–34	133.8	D9S915	1.29 < 0.01
17	17q11.2	40.1	D17S1294	1.13 < 0.02
18	18q21.3	57.7	D18S535	1.94 < 0.002

*Position in centiMorgan (cM) of markers from p-ter to q-ter based on the Marshfield map (<http://research.marshfieldclinic.org/genetics>).

†P-values estimated using $P(\text{LOD}) = 0.5 \times (\chi^2_1 > 2 \ln 10 \times \text{LOD})$.

Table 3. Single-point linkage analysis for allergic rhinoconjunctivitis

Marker	Map position (cM)*	LOD	P-value†
D3S1278	129.73	2.28	< 6×10^{-4}
D3S1267	139.12	1.63	< 0.004
D4S1625	145.98	1.18	< 0.01
D4S1652	208.07	1.77	< 0.003
D6STNFA	63.41	1.14	< 0.05
D9S257	91.87	1.10	< 0.05
D17S921	36.12	2.17	< 8×10^{-4}
D17S1294	50.74	1.78	< 0.005
D18S843	28.1	1.23	< 0.001

*Position in centiMorgan (cM) of markers from p-ter to q-ter based on the Marshfield map (<http://research.marshfieldclinic.org/genetics>).

†P-values estimated using $P(\text{LOD}) = 0.5 \times (\chi^2_1 > 2 \ln 10 \times \text{LOD})$.

The susceptibility loci reported previously for allergic conjunctivitis are 1p36.2, 4q13.3 and 9q34.3 [20], 2q12-q33, 3q12, 4q24-q27, 5q12-q15, 6p23-p24, 12q13, 22q13, Xq21 [19]. Barnes et al. [32] had earlier reported the region 12q14-q24 as a region in favour of linkage to AR. Candidate genes in that region are IFNG and signal transducer and activator of transcription (STAT)6. The STAT6 gene encodes a signal transducer and activator of transcription 6, a key transcription factor involved in both IL-4 and IL-13-mediated biological responses such as allergy. A study of Japanese families indicated that a combination of STAT6 gene polymorphisms might be a useful marker for predicting allergic disease [33].

Our present results support three previously reported regions: 3q13, 6p23-24 and 9q34.3 [19, 20]. We are not aware of any candidate genes in the regions.

So far many susceptibility loci underlying allergic asthma, allergic rhinoconjunctivitis and atopic dermatitis have been published and almost all chromosomes now possess candidate regions. This picture may agree with expectations because of genetic heterogeneity, difference in diagnostic criteria, the effect of environmental factors in different places in the world, and e.g. gene-gene or gene-environment interaction.

In a former linkage study, we found six regions (3p22-24, 3q14, 13q14, 15q14-15, 17q21, 18q21) linked to atopic dermatitis in the same family material as was used in the current investigation [18]. In this material, 74% of the patients also had asthma and allergic rhinoconjunctivitis [21]. The present linkage study for allergic rhinoconjunctivitis did not show the same suggestive susceptibility regions as for atopic dermatitis. However, the peaks found in allergic rhinoconjunctivitis 17q21, 18q21 overlapped and were also linked in the atopic dermatitis genome-wide linkage analysis. The reason for the overlapping could be that most of our affected siblings exhibited more than one atopic manifestation. Narrowing the atopic phenotype can help to locate specific loci for allergic rhinoconjunctivitis, even though it can be difficult to collect sufficient allergic rhinoconjunctivitis patients without other atopic phenotypes. The same problem exists in distinguishing AR from allergic conjunctivitis, as 95% of allergic conjunctivitis patients also have AR. In spite of this, a goal for future study of allergic rhinoconjunctivitis would be to collect more specific phenotypes of allergic rhinoconjunctivitis with fewer other atopic manifestations.

In conclusion, we conducted a whole genome-wide linkage analysis to locate susceptibility regions for allergic conjunctivitis. Three hundred and sixty-seven highly informative microsatellite markers and non-parametric linkage analysis with affected-relative pairs were used. Seven candidate regions showed evidence of linkage to allergic rhinoconjunctivitis at $P < 0.05$. One locus marker,

D3S1278, reached suggestive level in single-point analysis ($\text{LOD} = 2.28$, $P < 6 \times 10^{-4}$). Our results support the linkage of regions 3q13, 6p23-24 and 9q34.3 reported by others.

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