Analysis of Chromosome 5q31–32 and Psoriasis: Confirmation of a Susceptibility Locus but no Association with SNPs within SLC22A4 and SLC22A5

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We have previously reported a region on chromosome 5q as a possible susceptibility region for psoriasis. This cytokine cluster-rich region has also been suggested as a susceptibility locus in other autoimmune or inflammatory diseases including Crohn's disease (CD) and rheumatoid arthritis (RA). Three specific single-nucleotide polymorphisms (SNPs) have been reported to associate with RA and CD and to change the functional activity of two organic cation transporters, solute carrier family 22 member 4/5 (SLC22A4) and (SLC22A5). In this study, we have analyzed these SNPs for an association with psoriasis. We have also performed a denser linkage analysis of this region with an additional 31 microsatellite markers. We were not able to detect any association with any of the three SNPs analyzed. However, our linkage result supports the involvement of this region in the etiology of psoriasis. We obtained a peak non-parametric linkage value of 3.1 for marker D5S436 in a subgroup of patients with joint complaints. This result supports the findings in another study of psoriasis patients originating from Iceland in which the authors obtained a peak logarithm of the odds score of 2.6 for marker D5S2090, only 2 Mb from D5S436. This suggests a psoriasis susceptibility locus on chromosome 5q32 that is involved in the arthritic phenotype of the disease.

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INTRODUCTION

Psoriasis is a chronic inflammatory skin disease that affects approximately 2–5% of the Caucasian population (Nevitt and Hutchinson, 1996) and around 0.3% in the Mongoloid population (Yip, 1984). The most common form, psoriasis vulgaris, is characterized by red scaly plaque caused by the abnormal proliferation and differentiation of epidermal keratinocytes and the infiltration of inflammatory cells, in particular activated T cells (Barker, 1991). Psoriasis is strongly associated with an inflammatory form of arthritis called psoriatic arthritis. It affects between 10 and 40% of all psoriatic patients (Gladman, 1994). Crohn's disease (CD) is another disease with a markedly increased prevalence of psoriasis (Yates *et al.*, 1982; Lee *et al.*, 1990).

As with other complex diseases, the development of psoriasis is caused by a combination of both genetic and environmental factors. The search for a contributing genetic factor has revealed several regions across the genome with significant linkage values. The psoriasis susceptibility 1 locus on chromosome 6p21 is the most consistently replicated of these. This locus is estimated to account for 30–50% of the genetic contribution to psoriasis (Trembath *et al.*, 1997). In a genome scan, our group has previously identified candidate susceptibility regions on chromosomes 3q21, 3p21–23, 5q31, and 15q11 (Samuelsson *et al.*, 1999). On chromosome 5q31, marker D5S816 gave an non-parametric linkage (NPL) value of 2.22 in the total family material and increased to NPL = 2.45 in a subgroup stratified for joint complaints (Samuelsson *et al.*, 1999). After application of the information content term *prediction score* (Knutsson, 2002) to the whole genome scan, chromosome 5q was one of the regions that was a natural target for denser genotyping (Figure 1).

Overlapping genetic locations of loci for different autoimmune diseases have been known for several years. The distal region on chromosome 5q has been suggested to harbor susceptibility loci for autoimmune or inflammatory diseases such as CD, rheumatoid arthritis (RA), and asthma (Grunig *et al.*, 1998; Kauppi *et al.*, 2001; Rioux *et al.*, 2001; Tokuhiro *et al.*, 2003; Peltekova *et al.*, 2004). Three single-nucleotide polymorphism (SNPs) located in this region have been reported to alter the functional activity of two organic cation transporters, solute carrier family 22 member 4/5 (SLC22A4) and (SLC22A5). One of the SNPs (rs3792876) has been associated with RA (Tokuhiro *et al.*, 2003). The other two SNPs (rs1050152, rs2631367) form a haplotype, which has been associated with CD (Peltekova *et al.*, 2004). These

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Abbreviations: CD, Crohn's disease; NPL, non-parametric linkage; OR, odds ratio; RA, rheumatoid arthritis; SLC22A4/5, solute carrier family 22 member 4/5; SNP, single-nucleotide polymorphism

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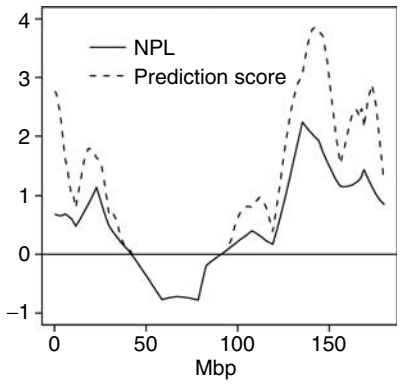

Figure 1. NPL values and prediction score values from our genome scan study on chromosome 5.

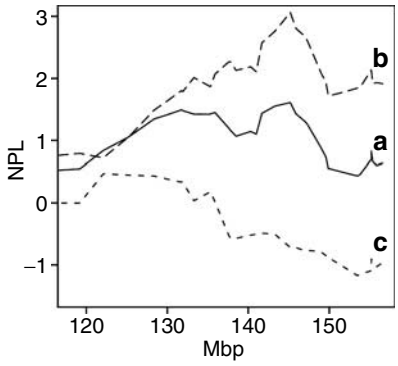

Figure 2. Result of linkage analysis of chromosome region 5q31–32. (a) Total family material (114 families), (b) families with joint complaints (55 families), and (c) families without joint complaints (59 families).

genes, SLC22A4 and SLC22A5, are located just less than 4 Mb upstream from the microsatellite marker that generated the peak NPL value on chromosome 5 in our genome-wide scan (D5S816).

Recently, another report on linkage to this region was presented. Karason *et al*. (2005) obtained a logarithm of the odds score of 2.6 for marker D5S2090 in a scan of female psoriasis patients. This marker maps within 17 Mb of rs3792876 associated with RA and 12 Mb from marker D5S816.

Taken together, there are several reasons why we believe further investigation of the 5q region is of great importance in psoriasis. They are the results of our genome-wide scan, the logarithm of the odds score of psoriasis patients originating from Iceland, and the association of this region with other inflammatory diseases. Additionally, this region harbors a cytokine gene cluster and other immunologically active genes that are good candidate genes for psoriasis.

The aim of this study was therefore to refine the linkage analysis in this region with a denser set of microsatellite markers. In addition, we wanted to perform an association analysis on the three SNPs with reported functional activity in RA and CD.

RESULTS

Linkage analysis

When calculating the *prediction scores* of our genome scan, the maximum predicted NPL value was 3.9 on chromosome 5q (Figure 1). This was one of the highest predicted NPL values in the entire genome scan. Linkage analysis was performed on a total of 114 multiaffected families for 34 markers within this region. The families were further stratified into two groups, one group of 55 families with joint complaints and one group of 59 families without joint complaints. The result of the unstratified family set gave a maximum NPL value of 1.6 for marker D5S436, whereas in the subgroup with joint complaints, the NPL value reached 3.1 ($P=0.001$) for the same marker (Figure 2). Of individual families in the group with joint complaints, 59% had an NPL value >0 and 40% had an NPL value >1. The 95% confidence interval for the peak position in the subgroup spanned between marker rs1050152 and D5S2112. In the subgroup without joint complaints, there were no NPL values above 1 (Figure 2). The difference in NPL value at marker D5S436 between the two subgroups was significant ($P=0.003$).

We have also performed an NPL analysis only including affected females. In contrast to Karason *et al*., we did not observe an increase in the NPL value (data not shown).

Stratification in our previous genome scan generated an NPL score of 2.64 at marker D3S1551, located at psoriasis susceptibility 5 on chromosome 3q21, for the subgroup with joint complaint (Samuelsson *et al*., 1999). In order to investigate correlation between these loci, we calculated the family-wise NPL-values at D5S436 and D3S1551 in the joint complaint set.

There was a slight degree of negative correlation ($r=-0.315$, $P=0.037$).

Association analysis

The SNPs, rs3792876, rs1050152, and rs2631367, were all analyzed separately, as well as in haplotypes of two to three markers per haplotype. The analysis was performed on both the total family material of 264 families and on the subgroup of 102 families with joint complaints. Neither analysis produced any significant association results.

Power calculations

The smallest detectable attributable fraction, that is, the proportion of cases caused by a particular exposure, and the corresponding odds ratio (OR) required for a power of 80% under four genetic models are shown in Table 1. Under most models, an OR of approximately 2 would be sufficient for all SNPs.

DISCUSSION

In a typical genome-wide scan of a genetically complex disease such as psoriasis, several genomic regions suggestive of a susceptibility locus are usually identified. To start confirmational studies of all candidate regions at the same time is both time and resource consuming, since not all of them will represent true disease loci. It is therefore necessary

Table 1. Attributable fraction (AF) and corresponding odds ratio (OR) required for a power of 80%

	Additive	*Multiplicative*	*Recessive*	*Dominant*
rs3792876				
AF	0.15	0.14	0.06	0.15
OR	2.2[1]	2.0[1]	15.4	2.2
rs1050152				
AF	0.41	0.36	0.20	0.57
OR	1.8[1]	1.6[1]	2.3	3.0
rs2631367				
AF	0.45	0.40	0.22	0.65
OR	1.9[1]	1.6[1]	2.3	3.6

[1]Odds ratio for heterozygotes.

to choose the peak regions with which to begin. If we rely solely on the peaks to determine continued research, interesting regions may be overlooked if information is incomplete owing to low heterozygosity, failed genotyping, or low marker density. A low score can therefore be owing to poor information, rather than the absence of linkage. Current measures of information, such as information content graphs, have the disadvantage of not combining the informativeness with the obtained score. A more desirable approach is to extrapolate the outcome of incomplete data on the basis of the observed data, to be able to compare the levels of information in a linkage context. This is the simple yet appealing idea of *prediction scores*. When we applied this evaluation tool to data from our genome scan, the chromosome region 5q31–32 was indicated as having low NPL values owing to low information content.

We therefore decided to perform a denser linkage analysis on chromosome 5q31–32. By slightly increasing the family material and analyzing a denser set of microsatellite markers, we obtained an increase in the NPL score from 2.45 to 3.1 in the subgroup with joint complaints. This NPL value is not surprising considering the size of the family material and the fact that this is probably a minor psoriasis locus compared with the major histocompatability complex region (psoriasis susceptibility 1). The peak NPL score of the joint complaint subgroup together with the significant difference in NPL value between the two subgroups indicate an arthritic involvement of the region around the peak marker D5S436 in psoriasis patients. In the total family material, the NPL value decreased from 2.22 to about 1.6. This emphasizes the importance of stratifying patients according to phenotypic criteria, which has also been underlined recently by Karason *et al.* (2005).

The psoriasis susceptibility 5 locus on chromosome 3q21 also associates with joint complaint. In order to investigate a possible epistasis or locus heterogeneity between these two loci, we calculated the family-wise NPL values at D5S436 and D3S1551 in the joint complaint set. We found a negative correlation between the NPL values indicating possible locus heterogeneity for the two loci.

Interestingly, marker D5S436 is only 2 Mb from marker D5S2090, which yielded an logarithm of the odds score of 2.6 in a scan of female psoriasis patients originating from Iceland (Karason *et al.*, 2005). D5S436 also lies within 5 Mb from the highest linkage score in the pooled analysis of European celiac disease families (Babron *et al.*, 2003) and 13.5 Mb from the SNPs that associate with CD or RA. This genetic overlapping of all these autoimmune/inflammatory diseases indicates that this cytokine gene-rich 5q region might harbor a gene involved in a common pathway leading to autoimmunity.

In this study, we have also analyzed two SNPs within the gene SLC22A4 and one SNP in the promoter of the gene SLC22A5, which were previously described by Tokuhiro *et al.* (2003) and Peltekova *et al.* (2004). The frequency of the SNP that gave significant association under a recessive model on patients with RA originating from Japan is, however, considerably lower in the Caucasian population (0.09 vs 0.33 and 0.008 vs 0.11 for homozygotes). Hence, an OR of 15.4 (Table 1) would be required for the association to be detectable in our study using a recessive model. Assuming the OR in Tokuhiro *et al.* (OR = 1.98) power is lacking in our material. However, considering the low allele frequency, we regard it unlikely that this would be a disease-involved polymorphism in a complex genetic disease such as psoriasis.

Our data was also compared with the study on patients with CD (Peltekova *et al.*, 2004). When applying the OR of rs1050152 and rs2631367 presented by Peltekova *et al.* (OR 2.56 for heterozygotes), the power of our material is larger than or equal to 97% for both SNPs under additive and multiplicative models. These models seem to be the likely models considering the reported OR (OR 2.56 for heterozygotes, OR 5.14 for homozygotes).

Needless to say, this does not totally exclude the genes SLC22A4 and SLC22A5 from involvement with psoriasis. However, our lack of association to rs3792876, rs1050152, and rs2631367 is in line with another study performed on psoriatic arthritis patients (Butt *et al.*, 2005) and with two studies performed on Canadian Caucasian RA patients (Newman *et al.*, 2005) and RA patients originating from the United Kingdom (Barton *et al.*, 2005).

To summarize, our results support a susceptibility region for psoriasis on chromosome 5q32, probably involved in the arthritic phenotype of the disease. Our data also indicate that the three functional SNPs that associate with RA or CD are not involved in psoriasis susceptibility in our population.

MATERIALS AND METHODS

Study population

Ascertainment of families were through membership register of the Swedish psoriasis association (Inerot *et al.*, 2005). For the linkage analysis, we used 114 nuclear families with unaffected parents available for genotyping and at least two affected children (481 individuals, 168 affected sib pairs). Of these, 86 families (366 individuals, 134 affected sib pairs) had previously been analyzed in

our genome scan. In order to confirm our previous findings for this locus, we divided the families based on joint complaints in affected individuals (giving 55 families in this group compared to 44 in the genome scan) or not (59 families compared to 42 in the genome scan). The association analysis was complemented with an additional set of 152 trios. Of these, 47 had and 52 did not have joint complaints as defined previously. For 53 simplex families, the status was unclear and hence was not used in the stratified analysis. In all, we used 264 families, of which 102 had joint complaints. The stratification criteria for joint complaint were based on self-reporting by probands in simplex families. Participating individuals in multiplex families was examined for joint involvement by one dermatologist. More detailed information about the ascertainment of families and stratification criteria is available elsewhere (Samuelsson *et al.*, 1999; Inerot *et al.*, 2005). The collection of all samples was made with informed consent and was approved by the local ethics review committee and all the studies were performed in accordance with the requirements of the Declaration of Helsinki Principles.

Prediction scores

Prediction scores (Knutsson, 2002) make predictions for partially uninformative affected sib-pair families by extrapolating the outcome of incomplete data on the basis of observed data; in other words, re-calculating scores conditioned on the existing data. In regions where the degree of information is low, but available data provide some support for linkage, the scores increase considerably. The prediction scores are not affected in regions with complete information and can never be smaller than the corresponding NPL score. Note that the prediction score should *not* be regarded as a test statistic but as a guideline for identifying regions worth the effort of further genotyping to increase information. The calculations are performed in a script utilizing ibd-sharing probabilities generated by Genehunter v.2.1 (Kruglyak *et al.*, 1996).

Markers and genotyping

A total of 31 microsatellite markers and three SNPs were analyzed (Table S1). The markers are spaced over a region of 37.4 Mb around the peak marker from the genome scan, D5S816 (Samuelsson *et al.*, 1999). Polymerase chain reactions were performed in a total volume of 10 μl, containing 25 ng of genomic DNA, 7.5 pmol of each primer, and 1 × AmpliTaq Gold® PCR Master Mix (Applied Biosystems, Foster City, CA). Polymerase chain reaction amplifications were carried out on a Gene Amp® PCR System 9700 (Applied Biosystems) with an annealing temperature of 55°C, or with a touch-down interval between 60 and 50°C. Genotyping was performed on an ABI3730 using GeneMapper software (Applied Biosystems).

Genotyping of the three SNPs was performed with either Taqman SNP Genotyping Assays or Custom Taqman SNP Genotyping Assays from Applied Biosystems. All Taqman reactions were prepared according to the manufacturer's instructions and detection was performed on an ABI7900HT sequence detection system instrument (Applied Biosystems).

Statistical analysis

Linkage analysis was performed with Allegro v.1.2c (Gudbjartsson *et al.*, 2000). Association analysis was calculated using the family-based association test (Rabinowitz and Laird, 2000; Horvath *et al.*, 2004). Confidence interval for the position of an NPL score was determined by bootstrap sampling. The difference in NPL score between two groups was assessed by a permutation test. Correlation between NPL values was used to test for possible epistasis or locus heterogeneity between two loci. In addition, linkage analysis was carried out on female patients only.

Power calculation

The power of the family-based association test statistic was assessed with PBAT 3.0 (Lange *et al.*, 2004; Steen and Lange, 2005) using simulation. Pedigree structure, number of missing founder genotypes, and founder allele frequencies identical to the observed data set were used, along with a population prevalence of 3%. Power was computed under four inheritance models: additive, multiplicative, recessive and dominant, and the least detectable attributable fraction, respectively OR at 80% power was reported. A type I error rate of 0.05 was used in all calculations.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1: Marker information

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