Association Analysis of Cystatin A and Zinc Finger Protein 148, Two Genes Located at the Psoriasis Susceptibility Locus PSORS5

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Psoriasis is a multifactorial hereditary skin disease. The searches for causative DNA variations have generated several susceptibility loci, but at present, the gene(s) involved has not been identified. In this article, we investigated whether cystatin A, an upregulated gene in psoriatic plaques and located at chromosome 3q21, is the disease-causing gene at the psoriasis susceptibility locus PSORS5. We also investigated association to a second gene located in this region, zinc finger protein 148. The two genes have been sequenced in a small case/control set in search for SNP markers, followed by family-based association analysis using the transmission disequilibrium test. We did not detect association with either of the genes.

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Psoriasis vulgaris is a common, inflammatory disease of the skin. Manifestation is characterized by hyperproliferation that ultimately leads to red, scaly plaques. The prevalence is 2% to 3% in individuals from Northern Europe and at present there is no cure and treatment is limited to relieve symptoms. Psoriasis vulgaris is classified as type I or type II depending on age of onset of patients (Henseler and Christophers, 1985; Henseler, 1997). As judged by twin- and large population-based genetic studies, at least psoriasis vulgaris type I seems to have a strong genetic component, but no causative gene product(s) has been identified. At present it is generally accepted that the disease is both multifactorial and genetically heterogeneous. Many groups have pursued the search for causative gene mutations using linkage analysis in large family cohorts. Several candidate susceptibility loci have been reported, of which the PSORS1 locus in the HLA region on chromosome 6p is the only consistently replicated in independent studies (Elder et al., 2001). The PSORS5 locus on chromosome 3q21 (OMIM 604316) was first identified by linkage analysis in a Swedish family cohort (Enlund et al., 1999; Samuelsson et al., 1999). Subsequent studies using SNP markers and association analysis revealed association with a specific 5 SNP-marker haplotype, extending over 90 kb (Hewett et al., 2002). Nevertheless, the identification of this haplotype has not, to the best of our knowledge, led to identification of a causative DNA variation, despite the intragenic location of the haplotype in the gene SLC12A8 (Gene Bank No. NM_024628). Therefore, we have also investigated association with the gene zinc finger protein 148 (ZNF148, Gene Bank No. NM_021964). ZNF148 is located less than 100 kb from the 5' end of SLC12A8 and the nearest SNP in the associated haplotype (see Fig 1). ZNF148 has been shown to be involved in regulation of T cell receptors (Wang et al., 1993). Because the chromosome 3q21 region where PSORS5 is located has been reported as a susceptibility locus for other immune-mediated complex diseases such as rheumatoid arthritis (Cornelis et al., 1998) and atopic dermatitis (Lee et al., 2000), we speculated that the overlapping linkage reports might be due to mutations in the same gene with a function in immune response regulation.

Another approach in the search for causes of psoriasis is to study global gene expression in psoriatic plaque and normal skin (Bowcock et al., 2001; Oestreicher et al., 2001). Altered gene expression has by such studies been reported for a number of genes of which some are located at chromosomal regions identified as harboring psoriasis susceptibility loci. One example is the cysteine protease inhibitor cystatin A (CSTA, Gene Bank No. NM_005213).

Abbreviations: CSTA, cystatin A; TDT, transmission disequilibrium test; ZNF148, zinc finger protein 148
located at the PSORS5 locus (see Fig 1). CSTA has been shown to have an increased expression level in psoriatic plaques (Bowcock et al, 2001). We therefore wanted to investigate whether we could detect association between CSTA and a Swedish psoriasis family set.

To identify single-nucleotide polymorphisms, coding regions both of genes and of the promoter region of CSTA were sequenced in 11 patients carrying the associated haplotype and 11 healthy blood donors. Identified SNPs were then genotyped, and preferential transmission of specific alleles was analyzed by the transmission disequilibrium test (TDT).

## Results and Discussion

Two novel SNPs were found in CSTA and ZNF148, respectively (see Table I). In CSTA, the first SNP is located in the promoter region and the second in exon 3. This SNP leads to an amino acid exchange, threonine → methionine. In ZNF148, both SNPs are intronic. We genotyped these four novel SNPs and one additional SNP located in exon 9 of ZNF148 (dbSNP Accession No. rs 1053738). After genotyping we analyzed for distorted transmission of specific alleles using the TDT. No association was detected for any of the SNPs. We also stratified families according to transmission of the haplotype associated with affected children and repeated the TDT analysis. The results obtained were similar with no association (see Table II).

During the past decade the search for genetic changes underlying human disease has progressed substantially. By use of linkage analysis in large family pedigrees, mutations underlying monogenic diseases have been identified. The same approach has then been used on common diseases with a multifactorial origin. Nevertheless, localizing mutations in diseases with complex genetic background has turned out to be a more difficult task. By combining linkage and association studies, candidate regions can be decreased in size but so far few disease-causing DNA variations have been identified. This is the case with the PSORS5 locus on chromosome 3q21. By use of SNP markers and TDT analysis an associated 5 SNP-marker haplotype could be identified (Hewett et al, 2002). Although the haplotype is contained within a single gene, SLC12A8, all associating SNPs are intronic. This is fully compatible with a disease-causing function, but to define this, extensive functional analyses are required.

We have in this study focused on two genes located in a broader region defined by allelic association to microsatellite markers (Enlund et al, 1999). The first gene we investigated is CSTA. In a global gene expression study, increased expression of CSTA mRNA was detected in psoriatic skin compared to healthy skin. This suggests that CSTA have an important function in skin. Any mutation that disrupts the normal function might then contribute to the psoriatic phenotype. The other gene we have investigated is ZNF148. Our speculation of a role for this gene in psoriasis etiology is based on the location of the gene at PSORS5 and the function of ZNF148 as a regulator of T cell receptor expression. We identified novel SNPs in both genes by sequencing a small population-specific case/control set and genotyped these in a larger family cohort. No allele showed any tendency of preferential transmission from parent to affected child. Similar results were seen after repeating the analysis with families transmitting the associated haplotype only. Although we cannot rule out the existence of disease-causing mutations in distal regulatory regions, neither CSTA nor ZNF148 seems to be the genetic determinant of PSORS5.

In conclusion, we have identified two novel SNPs in CSTA and two in ZNF148. After association analysis in a large family set showing linkage and association to the PSORS5 locus, no preferential transmission of a specific allele could be detected. It is therefore unlikely that any of the two genes harbor the genetic cause of psoriasis at the PSORS5 locus.

## Materials and Methods

### Family set

Patients used for SNP discovery are from the family cohort used in the original linkage studies. They were all affected with psoriasis vulgaris and joint involvement and carry the risk haplotype at the PSORS5 locus previously shown to associate with psoriasis (Hewett et al, 2002). Healthy individuals recruited among blood donors in Gothenburg, Sweden, were used as controls.

Families used in the association study were included in the original localization of PSORS5 (Enlund et al, 1999). All probands have at least one parent born in the geographically selected region and both parents were available for genotyping. The collection of all samples was by informed consent and approved by the local ethical review committee.

### Table I. Sequence of SNaPshot primers used for genotyping

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>Primer sequence</th>
<th>Primer length (bp)</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTA SNP1</td>
<td>Promoter, −235 bp</td>
<td>5′-CCATGACTCTCGTACTTGCCCAAC/T-3′</td>
<td>22</td>
<td>NA</td>
</tr>
<tr>
<td>CSTA SNP3</td>
<td>119 bp into exon 3</td>
<td>5′-(A)10CTCAAAAAACAGGATGACGAGTCAGTCA/C/T-3′</td>
<td>58</td>
<td>Thr → Met</td>
</tr>
<tr>
<td>ZNF148 SNP2</td>
<td>65 bp 3′ of exon 2</td>
<td>5′-(A)10TGGATGCCTGGTGTTACACAGA/G-3′</td>
<td>31</td>
<td>NA</td>
</tr>
<tr>
<td>ZNF148 SNP3</td>
<td>179 bp 3′ of exon 3</td>
<td>5′-(A)12CTGTTGGTTTATGATGAAATGATCATGA-T-3′</td>
<td>40</td>
<td>NA</td>
</tr>
<tr>
<td>ZNF148 SNP9</td>
<td>119 bp into exon 9</td>
<td>5′-(A)23GAAGTACCAGAGGTCACCCCG/A-3′</td>
<td>49</td>
<td>No aa exchange</td>
</tr>
</tbody>
</table>

*Poly(A)*-tails of various lengths were added to primers in order to enable automatic size discrimination.

**NA**, not applicable.

cSequence for ZNF148 SNP3 primer is shown in antisense direction as used in the study.

*dbSNP Accession No. rs 1053738.*