Novel genetic association between the corneodesmosin (MHC S) gene and susceptibility to psoriasis


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Psoriasis is an inflammatory skin disease of unknown origin, but with a clear genetic component. The strongest genetic association has been found with the major histocompatibility complex (MHC) region, and specifically between susceptibility to familial early onset psoriasis and human leukocyte antigen (HLA)-Cw6. The basis of this association of the HLA-C locus with disease pathogenesis is, however, not clear, and it is possible that other genes, or a combination of genes, in the HLA region are of functional importance. The MHC S gene is expressed specifically in keratinocyte differentiation and, being located 160 kb telomeric of HLA-C, is a plausible candidate gene. We analysed the allelic distribution of two polymorphisms in the MHC S gene (at +619 and +1243) in a case-control association study. We could confirm a significant association between psoriasis and HLA-Cw6 [odds ratio (OR) = 7.75]. No association was found between disease (or any subtypes) and the MHC S gene polymorphism at position +619, despite its close proximity to HLA-C and the strong linkage disequilibrium between the loci. However, a significant trend with the rarer allele at MHC S (+1243) and psoriasis was detected in the overall data set (OR = 2.66; \( P = 2 \times 10^{-9} \)). This effect was most pronounced in the type 1a (early onset) psoriatics (OR = 3.43). Furthermore, homozygosity for the associated allele at MHC S (+1243) increased the risk of disease over that for carriage of HLA-Cw6 alone (OR = 9.38), suggesting that allele 2 of MHC S (+1243) provides an additional risk in psoriasis susceptibility. The strong association found here, coupled with the biological involvement of the MHC S gene product corneodesmosin in skin physiology, implicates this locus (or a haplotype across HLA-C and MHC S) in the impaired desquamation characteristic of psoriasis.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease with a prevalence ranging between 1 and 2% in the UK and northern European populations (1,2) to 0.1–0.3% in the Far East (3) and China (4). The skin lesion is characterized by epidermal hyperproliferation, abnormal keratinocyte differentiation and the presence of a lymphocytic inflammatory infiltrate (5). A genetic component of the disease was established originally in early family studies (6,7) and confirmed using twin studies (8,9), and it is likely that many genes are involved, interacting under environmental influences (10). Several loci have been proposed for involvement in psoriasis. The strongest evidence is for a locus (or loci) within the major histocompatibility complex (MHC) region (10,11). Evidence for a major determinant in this region has been reported and confirmed by several groups using both association methods (12–14) and linkage analysis (11,15,16). Other studies have reported evidence for linkage to 17q and 4q (17,18), and to 16q and 20q (11,16), but their contribution to disease susceptibility is probably weaker than the human leukocyte antigen (HLA) region. As the strongest candidate marker, the HLA-C gene has been well studied with respect to its involvement in the development of psoriasis (19,20). The strongest HLA-Cw6 association has been shown to be with the earliest onset disease type described here as type 1a, age at onset (AAO) = 0–20 years, which suggests that early onset psoriasis has a stronger genetic component (12,21). However, specific involvement of the HLA-Cw6 genotype in the disease pathogenesis has not yet been established. It is a fact that most association studies with Cw6 are based on serological data which may be inaccurate, and other candidate genes or loci within the MHC region cannot be excluded. A recent study supports the hypothesis that a major psoriasis-predisposing locus resides around the HLA-C region but is probably different from the HLA-C gene itself (15).

The MHC S gene initially was identified as a 2.6 kb cDNA from a human fetal skin library probed with genomic DNA derived from a yeast artificial chromosome (YAC) clone spanning the HLA-C region. This gene predicts a 486 amino acid protein which maps to a region ~160 kb telomeric of the HLA-C locus (22). It is expressed specifically in keratinocytes

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in the terminal phases of differentiation. Recently, amino acid sequencing of a partially purified 52–56 kDa protein, designated corneodesmosin, derived from fractionated human epidermis, revealed identity over 26 residues with the predicted amino acid sequence of the MHC S gene product (23). This result has been confirmed by the recent cloning and expression of the human corneodesmosin cDNA (24).

In view of the strong genetic association between MHC and psoriasis, the close proximity of the MHC S gene to HLA-C and the potential functional importance of the MHC S gene in keratinocyte differentiation, the MHC S gene was investigated for genetic association with this disease. Three polymorphisms in the MHC S gene with potentially functional significance were analysed at positions +619, +1240 and +1243 (Fig. 1) in 235 psoriatic patients and 374 matched healthy controls.

RESULTS

The allelic distribution of three markers, HLA-Cw6, MHC S (+619) and MHC S (+1243), was assessed in both the psoriatic and control populations (n = 235 and 374, respectively), as described in Materials and Methods. Table 1 shows the frequencies found for the rarer allele (allele 2) at each of these markers in the controls, the overall psoriatic population and the three age at onset subtypes. The assignment of alleles 1 and 2 was determined by their frequencies in the control population. It should be mentioned here for clarity later that for MHC S (+1243), the ‘rarer’ allele (allele 2) in the control population (frequency = 0.47) became the more common allele in the psoriatic population.

A dose effect was evident for the +1243 polymorphism in the MHC S gene where, in all subtypes and psoriasis as a whole, the homozygous odds ratio (ORhom) for allele 2 was approximately twice that of the heterozygous ORhet (Table 2). A χ² test for trend was therefore carried out for this locus. A test for carriage of allele 2 was used for the other two loci. Table 3 summarizes the types of test performed and the result of these analyses for each locus. A strong association was found between the HLA-Cw6 allele and all psoriatics (P = 8 × 10⁻⁴⁵, OR_Car = 7.75 [95% confidence interval (CI): 5.68,10.56]). When subdivided into AAO groups, the strongest association was seen in the type 1a population (P = 2 × 10⁻⁴⁴). This significance is slightly lower than for the total psoriatic group, probably due to a smaller population size; however, the OR for carriage indicates a stronger risk in type 1a than in the psoriatic population overall [OR = 15.16 (9.49,24.22)]. A significant trend was found between allele 2 at MHC S (+1243) and psoriasis (P = 2 × 10⁻⁸). Similarly to the results for HLA-C, this effect was strongest in the early onset type 1a psoriatics [P = 1 × 10⁻⁸, OR_Het = 2.87 (1.36,6.04), OR_Hom = 7.76 (3.61,16.67)]. The same directional trend was observed in the type 1b and type 2 psoriatics, but with lower risk as measured by OR (Tables 2 and 3). The result for type 1b was significant study-wide (P = 1 × 10⁻³) but, for type 2, the result was only nominally significant (P = 0.05). Analysis of the MHC S (+619) polymorphism showed no association with psoriasis either as a whole disease or with any of the subtypes. In an attempt to understand further the associations found with HLA-Cw6 and the trend found with allele 2 at MHC S (+1243) and psoriasis, composite genotype analysis was performed. The composite genotype combining 2, homozygotes at MHC S (+1243) and carriage of Cw6 at HLA-C was tested for association with psoriasis and its subtypes. Table 4 shows the ORs and 95% CIs for these analyses. It can be seen that in the type 1a psoriatic group, the additional specification of genotype 2.2 at MHC S (+1243) when an individual already carries the HLA-Cw6 allele has no effect, since the OR does not increase. However, for the other two subtypes (1b and 2), the additional specification of genotype 2.2 at MHC S (+1243) did increase the risk for disease over HLA-Cw6 alone. This increased risk was also seen when psoriasis was considered as a whole disease entity.

Linkage disequilibrium analyses were performed on the control samples using the EH program (25). As expected, very
strong pairwise linkage disequilibria were detected. The pairwise results for HLA-Cw6 with MHC S (+619) and MHC S (+1243) showed that the disequilibria were 99 and 91% of their possible maxima, respectively. Between MHC S (+619) and MHC S (+1243), the disequilibrium was 96% of the maximum possible. Results were slightly curious due to the direction of these disequilibria. Both MHC S (+619) and HLA-Cw6 are in positive disequilibrium with MHC S (+1243), but in negative disequilibrium with each other. However, this is simply explained when we consider that the two alleles at MHC S (+1243) are almost equifrequent.

In this patient population, we tested a third published MHC S gene non-synonymous substitution (+1240) (26), but we did not use these data in the analysis as this locus was not polymorphic in our British Caucasian population (frequency of rare allele = 0% in 235 psoriatic patients and 150 healthy controls).

**DISCUSSION**

We have shown a strong, significant association between psoriasis and the rarer allele at position +1243 in the MHC S gene. This result was also seen in the three subtypes of psoriasis, as defined by age at onset. The strongest evidence for association was found in the earliest onset subtype (type 1a). Evidence decreases, although it achieved study-wide significance, for the type 1b psoriasis, and was nominally significant in the late onset individuals.

In a previous study, Ishihara et al. (26) genotyped and tested 63 Japanese psoriatic patients for association with MHC S (+1243) and eight other markers in the MHC S gene. Their results suggested no association with either allele at position +1243. Since psoriasis is associated with HLA-Cw6/Cw7 in Japan, it could be argued that MHC S does not infer susceptibility to psoriasis. On closer inspection, however, there are two good reasons why these contradictory results may have occurred. Firstly, there are approximately four times as many patients considered in the study presented here, and hence our analyses had substantially greater power. For example, given a locus with a rare allele frequency of 0.47 in the general population (as found here), our study has 94% power to detect a difference in allele frequencies of 0.15 or more between populations, whereas the 63 patients in the Japanese study generate a test with only 39% power. It is likely, therefore, that even if the association existed in the Japanese sample, it would be missed. Secondly, it has been observed in our study that the strongest associations were with those patients with the earliest age at onset of disease (type 1a), who are commonly those with familial clustering. In the study of Ishihara et al. (26), all but one individual had no family history, indicating that the vast majority of their sampled psoriatrics would be classified as type 2, the group with the weakest association found here. Therefore, the previous result should not be considered as contrary to the finding presented here. Other explanations which are compatible with an involvement of the S gene in psoriasis, and the previous and present data, include the possibility that +1243 is marking for another S gene locus which would be common in both Japanese and European populations (and not yet reported), or that the disease-causing mutation is different in the two populations, perhaps near the +1243 marker in our sample, and elsewhere in the Japanese population. Either way, more studies of the S gene, mutation screening and more comprehensive and powerful studies in other ethnic groups need to be carried out.

Another important issue is the proximity of the MHC S gene to HLA-C which has already been implicated in psoriasis and exhibits a stronger association than the one we see here for MHC S (+1243). At first glance, this indicates that perhaps there is no true association at MHC S (+1243), and that linkage disequilibrium to HLA-Cw6 is the driving force behind the association with MHC S (+1243). It is very difficult to partition associations in cases such as here, where the linkage disequilibrium is extremely strong. It is known that the power of a test is influenced not only by the allele frequencies at marker loci, but also by the relative frequencies of the marker and true disease alleles. It must be borne in mind, therefore, that the most significantly associated locus need not necessarily be the closest to the true disease locus. Jenisch et al. (15) recently suggested that HLA-Cw6 may not be the major gene involved in psoriasis in this region because the psoriasis-associated B-Cw6 haplotype frequencies vary within the same population and between different populations.

An intriguing result found here is the lack of association between MHC S (+619) and psoriasis. This marker is physically closer and has tighter linkage disequilibrium with HLA-Cw6 than MHC S (+1243). The simplest (and most likely) explanation is one of low power, since this marker has a much lower rare allele frequency (0.13) in the control population than MHC S (+1243). Hence, to see the same percentage difference in allele frequency, the absolute difference is much smaller. An increase of 0.18 in allele frequency for MHC S (+1243) is a 1.3-fold increase. The same increase for MHC S (+619) is 0.04, which this study had only 29% power to detect. There are also other possible explanations, including selection of the chromosome which carries allele 2 at the MHC S (+1243) site but not necessarily HLA-Cw6 and/or a specific allele at MHC S (+619).

**Table 3.** Type of association analysis performed and P-values found at HLA-Cw6, MHC S (+619) and MHC S (+1243)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Analysis performed</th>
<th>Total psoriasis</th>
<th>Type 1a</th>
<th>Type 1b</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cw6</td>
<td>Carriage</td>
<td>$8 \times 10^{-4}$</td>
<td>$2 \times 10^{-4}$</td>
<td>$1 \times 10^{-15}$</td>
<td>$1 \times 10^{-6}$</td>
</tr>
<tr>
<td>MHC S (+619)</td>
<td>Carriage</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MHC S (+1243)</td>
<td>Trend</td>
<td>$2 \times 10^{-9}$</td>
<td>$1 \times 10^{-9}$</td>
<td>$1 \times 10^{-3}$</td>
<td>0.0512</td>
</tr>
</tbody>
</table>

Proposed study-wide significance threshold = $6 \times 10^{-3}$.

*All analyses were performed on allele 2.

NS, normal $P > 0.05$. 

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The analysis of composite genotypes across HLA-C and MHC S (+1243) indicates that in type 1b, type 2 and psoriasis as a whole, adding an extra stipulation of genotype 2,2 at MHC S (+1243) increased the risk for disease (Table 4). In type 1a, the ORs are approximately the same with and without the +1243 polymorphic site, making it more difficult to determine a role for MHC S (+1243) within this subgroup. However, this form of composite genotype analysis may not be optimal for recognizing a true epistatic effect if one exists. It was shown here that the result for MHC S (+1243) is strongest when considered as a trend. For a trend test to be extended to the two-locus case, across both loci in all subtypes of psoriasis.

The evidence for involvement of the MHC S gene in psoriasis is strengthened by its biological involvement in desquamation. The predicted amino acid sequence of the MHC S gene product/corneodesmosin is serine–glycine-rich, and it has been proposed that such domains form loops favouring the interaction with other protein loops in the cornified cell envelope (e.g. keratin and loricrin) (24). S/corneodesmosin proteolysis in granular and cornified layers has been demonstrated and may be involved in a mechanism resulting in desquamation. Using monoclonal antibodies, Guerrin et al. (24) showed that the cleavage occurs in both N- and C-terminal domains of the protein. The MHC S (+1243) polymorphic site encodes a serine to leucine substitution [amino acid position 394 (26) or 410 (24)] in the C-terminal domain which was shown to be cleaved during the corneodesmosin maturation process. It is possible that the amino acid change may affect the proteolysis, which may be related to the impaired desquamation characteristic of psoriasis.

A previous study has reported an increase in expression of a 56 kDa epidermal keratin polypeptide in lesional psoriatic skin compared with normal skin (27). This observation may represent an up-regulation of the MHC S gene in lesional psoriatic skin. Therefore, differing genotypes at position +1243 could influence the level of expression and/or the stability of mRNA of the MHC S gene. Semi-quantitative RT–PCR should give an indication of the stable mRNA levels for patients with different genotypes at position +1243 compared with non-psoriatic individuals, and these experiments currently are being pursued. In addition, the transformation of desmosomes into corneodesmosomes is altered in psoriatic epidermis, and corneodesmosin may be involved in this dysfunction.

We have demonstrated a novel association between a marker at position +1243 in the MHC S gene and psoriasis. This association varies with psoriatic subtype based on AAO of disease in a similar way to that seen for HLA-Cw6, with early onset psoriasis showing the strongest association and highest OR. The association cannot be attributed wholly to the strong linkage disequilibrium in the region since composite genotype analysis of HLA-C and MHC S (+1243) indicates increased risk in most disease groups when the genotype at MHC S (+1243) is included, compared with the HLA-Cw6 genotype alone. In addition, there is some evidence for a biological role of the MHC S gene product in the pathogenesis of psoriasis.

Our results therefore indicate that a polymorphism in the MHC S gene (either at position +1243 or nearby) could be important in the development of the psoriatic phenotype. We currently are establishing DNA collections with nuclear families with the aim of confirming the present findings using TDT and sib-TDT (28) testing of the current hypothesis, and three-generation pedigree families to construct haplotypes across the HLA-C and MHC S region.

### MATERIALS AND METHODS

#### Patient and clinical assessment

A total of 235 Caucasian patients with chronic plaque psoriasis were recruited from the South Yorkshire region of the UK. From a series of consecutive blood donors from the Trent Blood Transfusion Service (Sheffield), we selected 374 samples which ethnically matched the disease population (Caucasian, North English) and could be used as healthy controls. Demographic details were comparable between psoriatics and controls (MF ratio = 0.96 versus 1.25, not significant; mean age ± SEM = 37.6 ± 1.03 years versus 39.7 ± 0.57 years, not significant). All of the patients entered into this project were diagnosed and managed by one consultant dermatologist (M.J.C.) in a specialist psoriasis clinic. The patients had been followed up by this consultant dermatologist for between 1 and 8 years. The clinical information

<table>
<thead>
<tr>
<th>Psoriasis type</th>
<th>HLA-Cw6$^a$</th>
<th>MHC S (+1243)$^b$</th>
<th>Composite$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>15.16 (9.49,24.22)</td>
<td>3.43 (2.21,5.32)</td>
<td>15.14 (9.06,25.31)</td>
</tr>
<tr>
<td>1b</td>
<td>7.27 (4.19,12.62)</td>
<td>2.42 (1.34,4.37)</td>
<td>9.06 (4.63,17.75)</td>
</tr>
<tr>
<td>2</td>
<td>3.45 (2.05,5.81)</td>
<td>1.69 (0.92,3.11)</td>
<td>4.43 (2.11,9.30)</td>
</tr>
<tr>
<td>Total</td>
<td>7.75 (5.68,10.56)</td>
<td>2.66 (1.86,3.82)</td>
<td>9.38 (5.99,14.70)</td>
</tr>
</tbody>
</table>

Odds ratios (95% CIs) for each locus separately and in composite are shown.

$^a$Carriage of Cw6.

$^b$Homozygosity for allele 2.

$^c$‘Composite’ indicates carriage of Cw6 at HLA-C and homozygosity for allele 2 at MHC S (+1243).
was updated into the clinical database from the patient notes during this period to ensure the accuracy of diagnosis and epidemiological subtypes. The diagnosis of chronic plaque psoriasis was based on the presence of characteristic skin lesions (29) with one of scalp, nail or joint involvement (30). We subdivided the psoriatic patients into three groups on the basis of the two epidemiological subgroups reported by Henseler and Christoplers (31) and three subgroups according to Swanbeck et al. (21). We used the nomenclature 1a for the group age at onset 0–20 years and 1b for age at onset of 21–39 years because these two groups reported by Swanbeck et al. (21) appeared to be subgroups of the type 1 psoriasis as previously described (31). Type 2 psoriasis had an age at onset of 40 years or more as reported by both of these groups. These subgroups reasonably explain the AAO and presence of family history distribution for the collected samples (data not shown).

### Polymorphism analysis

**MHC S** gene. Genomic DNA was extracted from whole blood according to standard protocols and stored at 100 ng/µl. Three reported exonic polymorphisms were analysed in the **MHC S** gene: a C→T substitution at nucleotide position +619, a G→T base change at +1240 and a C→T transition at +1243. Primers SS/S6 were used to amplify the polymorphic loci (+619), whereas both polymorphic loci (+1240) and (+1243) were amplified using primers S15/S16, as previously described (26). PCRs were prepared in bulk and aliquoted to 25 µl volumes comprising 50 mM KCl, 20 mM Tris–HCl, 1.5 mM MgCl2, 200 µM each dNTP, 1.2 µM each primer, 1 U of Taq polymerase (Gibco BRL, Paisley, UK) and 200 ng of genomic DNA. Thermocycling conditions were 2 min at 95°C, 28 cycles of 1 min at 95°C, 1 min at either 62°C (+619) or 58°C (+1240)/(+1243) and 15 s at 72°C. Restriction digests were performed in 20 µl reactions containing 10 µl of PCR product and 2.5 U of either *MnlI* (+619), *MspI* (+1240) or *HphI* (+1243) and appropriate manufacturer’s buffer (New England Biolabs, Hitchin, UK) at 37°C overnight. Allelic discrimination was performed by electrophoresis using either 15% polyacrylamide (+619) or 2% agarose (+1240)/(+1243). *HphI* produced 123 + 89 bp for allele 1, while it did not cut allele 2 (212 bp). *MspI* digestion generated 126 + 60 bp (allele 1) fragments. *MnlI* cleaved the PCR product (261 bp) into 66 + 12 bp (allele 2) or uncut 78 bp (allele 1), in addition to the common 126 and 57 bp fragments. As per standard nomenclature, the more common allele in the control population was designated allele 1, and the rarer allele 2.

**HLA-C** gene. Genomic DNA was amplified by PCR in 25 µl reactions, comprising 50 mM KCl, 20 mM Tris–HCl (pH 8.4), 2 mM MgCl2, 200 µM of each dNTP, 0.625 U of *Taq* polymerase, 0.125 µl of W1 (Gibco BRL), 750 nM of each primer (forward, 5′-TTG AGG ATT CTC CAC TCC CCT GAG-3′; reverse, 5′-CTG TGC CTC GCG CTT GTA CTT-3′) and 200 ng of DNA. Reactions were thermocycled thus: 95°C for 2 min (one cycle), 95°C for 1 min, 60°C for 1 min and 72°C for 30 s (30 cycles), 72°C for 5 min and 15°C hold. A 10 µl aliquot of PCR product was digested in a 20 µl reaction containing 10 U of *SmaI*, 50 mM KCl, 10 mM Tris–HCl, 7 mM MgCl2 and 1 mM dithiothreitol (DTT) and incubated at room temperature for 4 h (32). Alleles were determined by 9% polyacrylamide gel electrophoresis. The 618 bp PCR product was cleaved to fragments of 348 and 270 bp in non-Cw6 individuals, whereas carriage resulted in fragments of 348, 270, 196 and 74 bp in heterozygous individuals and 348, 196 and 74 bp in the homozygous state.

### Statistical analysis

Disease and control populations were compared non-parametrically. To investigate the possibility of a dose effect, ORs for the heterozygotes and homozygotes were calculated separately by comparing their risk with that for individuals homozygous for the alternative allele. If a dose effect was evident, a χ² test for trend was carried out, weighted by the number of putative disease susceptibility alleles in each genotype group. Otherwise, a χ² analysis for carriage of the relevant allele was performed. These analyses were carried out on the overall data set, and also for the three subtypes defined by age at onset (see Patient and clinical assessment).

Pairwise linkage disequilibria were calculated across the three loci using the EH program (25). Possible interactions between loci were investigated using composite genotype analysis.

Several analyses were performed here, many of which were highly correlated since the markers of interest were close and in strong linkage disequilibrium with each other. Due to the difficulty in determining a reasonable correction for multiple testing, and the loss of interpretability of P-values if they were corrected, we chose to present raw P-values throughout. However, the multiple testing must be taken into consideration. We suggest that a reasonable critical value to assess the significance across this study is 0.006 (= 0.05/9), which accounts for three distinct AAO groups tested at three loci, although this is probably still a conservative threshold.

**ABBREVIATIONS**

AAO, age at onset; CI, confidence interval; HLA, human leukocyte antigen; MHC, major histocompatibility complex; OR, odds ratio.

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### REFERENCES