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ORIGINAL ARTICLE

# HLA-C, CSTA and DS12346 susceptibility alleles confer over 100-fold increased risk of developing psoriasis: evidence of gene interaction

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**Psoriasis is an inflammatory skin disorder that exhibits multifactorial mode of inheritance. In addition to the well-known susceptibility locus *PSORS1* many other loci have been shown to be implicated in the genetic predisposition for disease. However, interactions between loci have not been thoroughly explored. Thus, we measured the effect of potential interaction between human leukocyte antigen (*HLA)-C*, *CSTA* and D1S236 at *PSORS1*, *PSORS4* and *PSORS5*, respectively, in the development of psoriasis. Analysis of 130 Caucasian psoriatic families showed that the risk to an HLA-*Cw6* +ve individual who carries two copies of the risk allele at both the *CSTA* and D1S2346 is 105 times the risk to an HLA-*Cw6* +ve individual who does not carry any risk alleles at the *CSTA* or D1S2346. This is the first demonstration of an interaction between risk alleles in three susceptibility loci suggesting possible functional interaction between genes in these loci, which might explain the complexity of the pathogenesis of psoriasis.**
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## INTRODUCTION

Psoriasis is a chronic inflammatory disease affecting ~4% of the Caucasian population.[1] Twin and family-based studies have suggested that psoriasis is a complex genetic trait associated with multiple factors.[2] Despite extensive efforts focused on identifying psoriasis susceptibility loci using genome-wide studies, only the *PSORS1* locus, located within the major histocompatibility complex (*MHC*) on chromosome 6p21.3, has been consistently replicated.[3] The *PSORS1* locus is known to be by far the strongest susceptibility locus and although 19 loci on 15 different chromosomes have been implicated in total, a meta-analysis of six genome-wide studies revealed evidence for linkage to only two regions.[4] Because of the difficulties in replicating and identifying actual susceptibility genes within these identified regions, gene–gene interactions have not been extensively explored.

Although epistatic interactions have been documented in other common complex diseases such as Alzheimer's and Crohn's disease,[5,6] it was almost a decade ago that for the first time an interaction between the *PSORS4* locus and human leukocyte antigen (HLA)-*Cw6* at the *PSORS1* locus was identified[7] and it has been suggested that the effect of the *PSORS1* locus should be conditioned on in future studies of non-major histocompatibility complex susceptibility loci.[4] We have previously shown that the *CSTA* '*TCC*' haplotype located at *PSORS5* is only associated with psoriasis in individuals harbouring the risk allele HLA-*Cw6*.[8] In this study, we investigated the interaction between three susceptibility loci, PSORS1, PSORS4 and PSORS5 in 130 Caucasian psoriatic families and identified a highly significant close interaction between them and development of psoriasis.

## MATERIALS AND METHODS

### Psoriasis families

Families with psoriasis were recruited from the Sheffield and Edinburgh regions of the United Kingdom, after obtaining their informed consent. This was approved by the Sheffield Local Ethics Committee and conformed to the guidelines set forth in the Helsinki protocol. The total number of families involved in this study was 130 Caucasian families from Scotland and North England. All of the families recruited from Sheffield and Edinburgh were Caucasian. All of the psoriasis patients and their family members used in this study were diagnosed and managed by a single consultant dermatologist (MJC) in a specialist psoriasis clinic. Overall, the psoriasis patient diagnosis was: 121 chronic plaque; 60 chronic plaque and guttate; 10 guttate; one pustular; one chronic plaque and pustular; and one chronic plaque, guttate and pustular. The mean age at onset for all the patients was 16.26 years (age range: 0–52) and 145 of the 194 cases (74.74%) reported a positive family history of psoriasis.

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### Genotyping and data analysis

Genomic DNA was extracted from whole blood, obtained from all family members included in this study, using standard protocols (Amersham Biosciences, Little Chalfont, UK) and stored in 96-well microtiter plates, with each well containing 500 µl of DNA (100 ng µl$^{-1}$). For the restriction fragment length polymorphism genotyping of the *PSORS1* (HLA-*Cw6*) locus, genomic DNA was amplified by PCR using the conditions and primers as described previously.[9] PCRs were conducted using fluorescently labelled primers (HEX and FAM dyes) for the microsatellite marker genotyping at *PSORS4*. Conditions, primers and details for the individual microsatellite markers are listed in Table 1. Alleles at each microsatellite marker were numbered according to their frequency with the most frequent being labelled allele 1. The genotyping methodology for *PSORS5* locus has been described previously.[8] HLA-*Cw6* (*PSORS1*) and *CSTA* (*PSORS5*) genotypes were analysed in 130 and 126 psoriatic families, respectively, using the Transmit[10] and FBAT programs.[11] Both programs allow the user to test for association/linkage between disease phenotype and genetic markers and can handle both bi-allelic and multi-allelic data while performing the classical TDT and sib-TDT. The genotyping at seven microsatellite markers on chromosome 1q21 (*PSORS4*) in 130 psoriatic families were also analysed using the Transmit and FBAT programs. Alleles for each individual microsatellite marker were coded by frequency with alleles of $<5\%$ frequency combined together.

### Statistical analysis

Conditional logistic regression (CLR) was used to model and quantify the association between genetic polymorphisms and disease risk. In a family-based CLR analysis one would expect to use unaffected siblings as matched controls. In the absence of such sibling controls, we used the method of Cordell *et al.*[12,13] to generate pseudo controls for the affected offspring conditional on the parental genotypes. The genotypes of the pseudo controls represent the untransmitted parental alleles. Several families in our study had more than one affected offspring. In the presence of linkage, nuclear families with more than one affected sib cannot be assumed to be independent. We can allow for this non-independence of observations by choosing a robust variance estimator. To determine the mode of inheritance for each locus (which dictates the coding of the genotypes in the regression analysis), we selected the mode of inheritance that provided the largest log-likelihood. For the D1S2346 locus, we considered to be the risk allele and grouped all the others together. The mode of inheritance for the D1S2346 locus was then determined based on this two-allele configuration. We previously showed that the *CSTA* −190T/+162C/+344C (*CSTA TCC*) allele is only associated with psoriasis in those individuals carrying the HLA-*Cw6* risk allele.[8] As a result of this finding, we stratified our data by presence of the HLA-*Cw6* risk allele and performed the analyses in the two strata (presence/absence of risk allele) separately. Within each stratum that we tested, using CLR, for association between the putative risk conferring alleles at the D1S2346 locus and psoriasis. Where an association was seen with the alleles at the D1S2346 locus, we again used CLR to quantify the relationship between the risk of psoriasis and carriage of the risk alleles at the *CSTA* and D1S2346 loci by inclusion of both terms in the logistic model. We also determined whether there was any evidence of a super-multiplicative (synergistic) effect by inclusion of an interaction term in the model. The *P*-values for the regression models are calculated using the likelihood ratio test for nested models. This provides a test of the 'improvement' in the fit of a model following inclusion of further terms (loci) in the logistic model rather than tests of individual terms (loci) in the model (Wald's tests). Both the generation of sibling pseudo controls and the CLR analysis were both undertaken using STATA 10 (StataCorp LP, College Station, TX, USA).

## RESULTS

A total of 130 psoriatic families were genotyped for HLA-*Cw6*. HLA-*Cw6* allele frequencies in these 130 families were found to be 73.2% HLA-*Cw6* −ve and 26.8% HLA-*Cw6* +ve. Our analysis shows the over-transmission of the HLA-*Cw6* +ve allele to diseased individuals with a Z score of 3.575 ($P$=0.00035) (Table 2). At the *PSORS4* locus, seven microsatellite markers were genotyped in all 130 families, whereby the strongest evidence for linkage is at marker D1S2346 (Z score 5.8, $P<0.000001$). Of the other six markers, only D1S498 produced evidence for linkage (a Z score$\geqslant$3) with a Z score of 3 ($P$=0.0027). When the relationship between the *PSORS1* and *PSORS4* loci was investigated, six of the seven microsatellite markers showed an increased over-transmission when stratified by HLA-*Cw6* (Table 2). The evidence for linkage increased with the presence of the HLA-*Cw6* +ve allele except for marker D1S2346, which decreased slightly although still showed evidence for over-transmission with the +ve HLA-*Cw6* allele. We showed previously that *CSTA* '*TCC*' at *PSORS5* is strongly associated with psoriasis (Z score 3.8, $P$=0.00013) (ref. 9). The single strongest association from these three SNPs was found with *CSTA* c162T>C (Z score 3.6, $P$=0.00025). When the relationship between *PSORS1* and *PSORS5* was investigated, the 'TCC' was over-transmitted with HLA-*Cw6* +ve families (Z score 3.81, $P$=0.0001). When the *CSTA* c162T>C was analysed with HLA-*Cw6*, the +162C allele was over-transmitted with HLA-*Cw6* +ve allele (Z score 3.49, $P$=0.00054). For the investigation into a possible three-loci interaction, D1S2346 and *CSTA* c162T>C markers were chosen as susceptibility alleles for *PSORS4* and *PSORS5*, respectively.

As we have noticed previously,[9] *CSTA* significantly increases the risk only in HLA-*Cw6* +ve individuals. This is not the case for D1S2346,

**Table 1 Primers and PCR conditions for microsatellite markers genotyped at the PSORS1 locus**

| *Marker name* | *Marker location (Mb)* | *Genbank accession* | *Marker type* | *Estimated fragment size (bp)* | *PCR conditions* | *PCR primers (5′–3′)* |
|---|---|---|---|---|---|---|
| D1S498 | 148.07 | Z24441 | $(CA)_n$ | 183–205 | 56 °C, 1.5 mM $MgCl_2$ | F—TTGCTGAAGGGACATAGTG<br>R—TGCTGGGTTATATCCAATATC |
| D1S2343 | 148.71 | Z51597 | $(CA)_n$ | 218–268 | 59 °C, 1.5 mM $MgCl_2$ | F—GGGTGGATCACTTAAGCCT<br>R—CTAGCATATTCGTCCTGAACTAA |
| D1S2399 | 148.89 | G05569 | $(N)_n$ | 338–339 | 56 °C, 1.5 mM $MgCl_2$ | F—ATGCAGATGTGGTGTGCATT<br>R—AAGTTTTTATGCAGAGGTCACTCC |
| D1S1664 | 149.35 | G09753 | $(TAGA)_n$ | 149–150 | 50 °C, 1.5 mM $MgCl_2$ | F—GGTCTGAGAAAGACGGTGAG<br>R—ACATCGCAGCTAAGTGTTCC |
| D1S2346 | 149.98 | Z51162 | $(CA)_n$ | 89–115 | 59 °C, 1.5 mM $MgCl_2$ | F—TATCTTGCCCTGCACC<br>R—AAGTGGGTCTCCCCAG |
| D1S2858 | 150.86 | Z51536 | $(CA)_n$ | 125–129 | 56 °C, 1.5 mM $MgCl_2$ | F—GGGTGGATCACTTAAGCCT<br>R—CTAGCATATTCGTCCTGAACTAA |
| D1S305 | 151.09 | Z17260 | $(CA)_n$ | 156–176 | 56 °C, 1.5 mM $MgCl_2$ | F—CCAGNCTCGGTATGTTTTTACTA<br>R—CTGAAACCTCTGTCCAAGCC |

**Table 2 TDT results for HLA-*Cw6* at PSORS1 and seven microsatellite markers at PSORS4 in 130 psoriatic families**

| | *Marker* | *No of Obs* | *No of Exp* | *Var (O−E)* | *Z Score* | P-*value* |
|---|---|---|---|---|---|---|
| *PSORS1 locus* | | | | | | |
| HLA-*Cw6* −ve | | 184 | 205.17 | 35.27 | −3.575 | 0.00035 |
| HLA-*Cw6* +ve | | 100 | 78.83 | 35.27 | 3.575 | 0.00035 |
| *PSORS4 locus* | | | | | | |
| D1S498 | Allele 1 | 109 | 105.95 | 27.35 | 0.32 | 0.75 |
| | Allele 2 | 72 | 58.66 | 20.27 | 3.00 | 0.0027 |
| | Allele 3 | 19 | 24.77 | 8.69 | −1.72 | 0.086 |
| | Allele 4 | 16 | 24.25 | 11.9 | −2.31 | 0.021 |
| | Allele 5 | 21 | 19.27 | 7.91 | 0.48 | 0.63 |
| | Allele 6 | 49 | 53.1 | 19.53 | −0.81 | 0.42 |
| | | | | | Global $\chi^2$=18.79 (5df) | Global *P*-value=0.0021 |
| D1S2343 | Allele 1 | 86 | 82.12 | 26.80 | 0.096 | 0.92 |
| | Allele 2 | 74 | 83.5 | 25.53 | −0.22 | 0.83 |
| | Allele 3 | 71 | 70.13 | 18.91 | −0.7 | 0.49 |
| | Allele 4 | 26 | 21.88 | 7.88 | 0.93 | 0.35 |
| | Allele 5 | 29 | 28.36 | 9.93 | 0.29 | 0.77 |
| | | | | | Global $\chi^2$=(4df) | Global *P*-value=0.2997 |
| D1S2399 | Allele 1 | 254 | 245.21 | 19.19 | 1.71 | 0.087 |
| | Allele 2 | 14 | 17.12 | 7.97 | −0.87 | 0.38 |
| | Allele 3 | 13 | 15.11 | 6.97 | −0.76 | 0.45 |
| | Allele 4 | 5 | 8.55 | 4.24 | −1.5 | 0.13 |
| | | | | | Global $\chi^2$=4.8409 (3df) | Global *P*-value=0.18382 |
| D1S1664 | Allele 1 | 124 | 116.79 | 32.72 | 1.15 | 0.25 |
| | Allele 2 | 58 | 68.65 | 24.99 | −1.8 | 0.072 |
| | Allele 3 | 55 | 58.34 | 20.98 | −0.79 | 0.43 |
| | Allele 4 | 18 | 13.74 | 6.74 | 1.4 | 0.16 |
| | Allele 5 | 17 | 14.32 | 5.93 | 1.28 | 0.2 |
| | Allele 6 | 14 | 14.16 | 4.44 | −0.24 | 0.81 |
| | | | | | Global $\chi^2$=13.258 (5df) | Global *P*-value=0.02108 |
| D1S2346 | Allele 1 | 153 | 124.23 | 22.58 | 5.8 | <0.000001 |
| | Allele 2 | 64 | 75.16 | 13.54 | −2.77 | 0.0055 |
| | Allele 3 | 53 | 56.39 | 13.97 | −0.54 | 0.59 |
| | Allele 4 | 6 | 18.64 | 8.73 | −4.23 | 0.000024 |
| | Allele 5 | 10 | 11.58 | 4.74 | −1.15 | 0.25 |
| | | | | | Global $\chi^2$=29.802 (4df) | Global *P*-value=$5.37\times10^{-6}$ |
| D1S2858 | Allele 1 | 145 | 150.8 | 31.024 | −1.04 | 0.3 |
| | Allele 2 | 97 | 88.87 | 26.59 | −0.18 | 0.86 |
| | Allele 3 | 34 | 35.25 | 7.43 | 1.42 | 0.15 |
| | Allele 4 | 10 | 11.08 | 3.97 | −0.5 | 0.62 |
| | | | | | Global $\chi^2$=3.999 (3df) | Global *P*-value=0.26157 |
| D1S305 | Allele 1 | 79 | 87.52 | 25.48 | −1.61 | 0.11 |
| | Allele 2 | 72 | 60.45 | 19.01 | 2.43 | 0.015 |
| | Allele 3 | 43 | 37.52 | 16.16 | 1.25 | 0.21 |
| | Allele 4 | 40 | 36.86 | 16.17 | 0.9 | 0.37 |
| | Allele 5 | 22 | 23.21 | 10.45 | −0.31 | 0.76 |
| | Allele 6 | 15 | 16.67 | 5.71 | −0.63 | 0.53 |
| | Allele 7 | 15 | 23.77 | 10.19 | −2.65 | 0.0082 |
| | | | | | Global $\chi^2$=21.485 (6df) | Global *P*-value=0.00152 |

which significantly increase the risk of psoriasis in both HLA-*Cw6* +ve and HLA-*Cw6* −ve individuals (Table 3). There does appear to be a difference though in the magnitude of the OR in HLA-*Cw6* +ve versus HLA-*Cw6* −ve individuals. In a logistic regression model, significance at two loci (when both are included in the model) implies a multiplicative risk across the two loci. Hence, we have multiplicative risk within and between the loci. This means that for HLA-*Cw6* +ve individuals, each copy of the risk allele at the CSTA and D1S2346 loci increases the odds of disease multiplicatively by 1.9 and 5.39, respectively. Therefore, this suggests that the risk to an HLA-*Cw6* +ve individual who carries two copies of the risk allele at both the *CSTA* and D1S236 loci is $1.9\times1.9\times5.39\times5.39=$**105** times the risk to an HLA-*Cw6* +ve individual who does not carry any risk alleles at the *CSTA* or D1S2346 loci.

## DISCUSSION

It has long been accepted that psoriasis has a genetic basis, albeit a complex one that involves the interaction of multiple genes and

**Table 3 Conditional logistic regression analysis of the *CSTA* +162 and D1S2346 risk allele combinations in the family data stratified by the absence/presence of the HLA-Cw6 risk allele**

| *Terms in the model* | *OR* | P-*value* | *95% CI* |
|---|---|---|---|
| *(a) HLA positive* | | | |
| CSTA | 2.15 | $1\times10^{-4}$ [a] | (1.38, 3.37) |
| D1S2346 | 6.18 | $1\times10^{-7}$ [a] | (2.74, 13.94) |
| D1S2346+CSTA | 5.39 (D1S2346) | 0.0028 | (2.37, 12.28) |
| | 1.90 (CSTA) | | (1.15, 3.16) |
| D1S2346+CSTA+ | 5.29 (D1S2346) | 0.96 | (1.69, 16.57) |
| D1S2346*CSTA | 1.86 (CSTA) | | (0.70, 4.98) |
| | 1.02 (synergistic interaction) | | (0.48, 2.16) |
| *(b) HLA negative* | | | |
| CSTA | 1.19 | 0.517[a] | (0.69, 2.02) |
| D1S2346 | 3.17 | 0.008[a] | (1.10, 9.13) |
| D1S2346+CSTA | 3.61 (D1S2346) | 0.53 | (1.20, 10.90) |
| | 1.42 (CSTA) | | (0.80, 2.53) |
| D1S2346+CSTA+ | 2.23 (D1S2346) | 0.55 | (0.49, 10.08) |
| D1S2346*CSTA | 1.00 (CSTA) | | (0.38, 2.61) |
| | 1.69 (synergistic interaction) | | (0.64, 4.49) |

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio.
[a]Determined by comparison with the null model containing no covariates.

environmental factors.[2] In this study, we investigated possible epistatic interactions between *PSORS1*, *PSORS4* and *PSORS5* loci and psoriasis. In selecting markers for genotyping, we chose the well-known HLA-*Cw6* allele for the *PSORS1* locus and *CSTA* c162T>C for *PSORS5*, a locus on chromosome 3q21, previously shown to be associated with psoriasis.[9,14] In addition, refinement work for the *PSORS4* locus has narrowed down the region to the proximity of the D1S2346 microsatellite marker.[15] We have therefore genotyped seven microsatellite markers on chromosome 1q21, four of which (D1S498, D1S1664, D1S305 and D1S2346) have been previously reported to show linkage to psoriasis.[8,15] Three additional markers (D1S2343, D1S2399 and D1S2858) were chosen to fit between the other markers to reduce the inter-marker spacing. Our data shows that the strongest evidence for linkage is at marker D1S2346 (Z score 5.8, $P<0.000001$). This marker is located distal to the epidermal differentiation complex, a 2.0 Mb region that includes at least 45 genes that become activated in terminal stages of keratinocyte differentiation involved in the development, maturation and cross-linking of the epidermis[16] and ~0.5 Mb away from the recently identified *LCE3C_LCE3B-del* locus associated with psoriasis.[17] Interestingly, this locus is in a distinct linkage-disequilibrium block compared with the other genes of the epidermal differentiation complex reported for association (blocks of low linkage-disequilibrium for rs4112788, a tag SNP for *LCE3C_LCE3B-del*),[17] suggesting a second susceptibility locus on chromosome 1q21 region. Although there seems to be a redundancy in genes with a role in epidermal barrier function contributing to disease susceptibility, recent studies highlight also the synergistic role of the immune system in disease pathogenesis by identifying several new susceptibility loci for psoriasis[18–21] and suggesting a close interaction between epidermal barrier impairment and both innate and adaptive immune system dysfunction for disease development.[22]

In the present study, data analysis point towards a very close interaction between the three susceptibility loci *PSORS1*, *PSORS4* and *PSORS5*, suggesting a striking 105-fold risk for an HLA-*Cw6* +ve individual who carries two copies of the risk allele at both the *CSTA* and D1S2346 loci. Such increased risk could translate functional interaction between proteins encoded by genes in these loci. This is in keeping with the recent study by the Genetic Analysis of Psoriasis Consortium and the welcome trust Case Control Consortium showing an interaction between HLA-C and endoplasmic reticulum aminopeptidase 1 (ERAP1), encoding an amino peptidase that regulates the quantity of peptides bounds to class I molecule such as HLA-C.[22] Interestingly, cystatins have been shown to modulate lysosomal cathepsin activities in antigen presentation.[23] In fact, lysosomal cysteine proteases regulate antigen presentation by both major histocompatibility complex class II and class I within the endosomal/lysosomal compartment.[24] In addition, cathepsin activity is decreased in human dendritic cells in response to HIV-1 infection, which enhance HIV-1 survival. It also decreased HIV-1 antigen processing and presentation to T cells.[25] Cathepsins are also essential for the progression of CD8+ T-cell-mediated autoimmune diabetes.[26] The regulation of cathepsin activity by cystatins seems to be important in antigen presentation and T-cell response conforming the fundamental role of immune system in the pathogenesis of psoriasis.

Our study provides strong evidence of an interaction between three susceptibility loci and incites to explore the possibility of functional interaction between recently identified susceptibility alleles.[18–21]

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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