Acta Derm Venereol 2005; 85: 2–8

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INVESTIGATIVE REPORT

HLA-Cw*0602 Associates More Strongly to Psoriasis in the Swedish Population than Variants of the Novel 6p21.3 Gene *PSORS1C3*

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The *PSORS1* locus in the major histocompatibility complex region on chromosome 6p21.3 contains a major predisposing factor for psoriasis for which several candidate genes have been tested. The analyses are complicated by strong linkage disequilibrium in the region and the complex genetic background of psoriasis. In the search for an alternative to *HLA-C* we have identified a novel gene, *PSORS1C3*, and characterized it with regard to psoriasis. *PSORS1C3* is located approximately 7 kb centromeric to *POU5F1*. A putative protein of 58 amino acids was predicted and expression was detected in both normal and psoriasis skin. Sequencing of the coding region revealed a total of 11 single nucleotide polymorphisms. When comparing the frequencies of *PSORS1C3* variants in a case-control material in the Swedish population, three single nucleotide polymorphisms displayed significant association with psoriasis. This association appeared to be HLA-Cw*0602-dependent due to linkage disequilibrium, thus *HLA-C* remains the strongest associating factor in the region. ***Key words: multifactorial; genetic predisposition; linkage disequilibrium; major histocompatibility complex; single nucleotide polymorphisms.***

(Accepted July 23, 2004.)

Acta Derm Venereol 2005; 85: 2–8.

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Psoriasis is a chronic skin disease affecting approximately 2% of the Caucasian population. The characteristic features seen in cutaneous psoriatic lesions are abnormal proliferation and differentiation of keratinocytes and infiltration of inflammatory cells (1, 2). Psoriasis is a clinically heterogeneous disease of which chronic plaque psoriasis is the main phenotype (1, 3). Other variants include guttate psoriasis, often preceded by streptococcal throat infection, and psoriasis arthritis (4, 5). In addition, it has been suggested that psoriasis ought to be separated into two clinical subtypes (type I and II) based on age of onset and family history, where type I is described as familial and with an age of onset below 40 (6).

The genetic contribution to psoriasis is well described and several psoriasis susceptibility loci have been identified, PSORS1 to PSORS9 (http://www.ncbi.nlm.nih.gov/omim, http://www.gene.ucl.ac.uk/nomenclature). However, the individual role of these loci in the architecture of predisposition to the disease still remains unclear. Psoriasis is considered a disease with a complex genetic background (7), thus the identification of susceptibility genes is prone to a number of complicating factors. Diseases displaying a non-Mendelian inheritance pattern are the consequence of subtle effects of common alleles modulated by genetic background and environmental triggers/modifiers, the individual contributions of which are not easily deciphered (8). In addition, heterogeneity can be a further complicating factor in studying the genetic cause of a genetic disease. One would expect that even the strongest genetic factor in psoriasis would be absent in some affected individuals, would be present in some unaffected individuals, would display complex/unpredictable inheritance patterns in psoriasis families and would be difficult to implicate in actual disease involvement even upon identification.

The PSORS1 region on chromosome 6p21.3 has been extensively investigated in the search for a psoriasis gene. The MHC (major histocompatibility complex) locus has demonstrated strong association with psoriasis in various ethnic populations and is therefore believed to contain a major predisposing factor in the development of psoriasis (7). The HLA-Cw*0602 allele of the *HLA-C* gene shows the strongest association to date. However, it is still disputed whether this allele is the predisposing psoriasis gene or simply a marker in strong linkage disequilibrium with the true disease gene (9–12). Arguments used to support the latter hypothesis are (i) the high level of linkage disequilibrium demonstrated across the MHC region, (ii) the existence of non-Cw*0602 risk haplotypes and (iii) the low incidence of disease among HLA-Cw*0602 carriers (7).

The search for an alternative to *HLA-C* in the PSORS1 region has led to the characterization of the genes *STG*, corneodesmosin (*CDSN*), *PSORS1C1* (*SEEK1*), *PSORS1C2* (*SPR1*), *HCR*, *TCF19* and *POU5F1* with regard to psoriasis susceptibility.

 ISSN 0001-5555
DOI: 10.1080/00015550410023527

However, except in some isolated studies none of these candidates have associated more strongly than HLA-Cw*0602 across diverse populations (13–20). Therefore we have continued the screening for new candidates in the area between *HLA-C* and *POU5F1*. Here, we present the cloning of a novel gene, psoriasis susceptibility 1 candidate 3 (*PSORS1C3*) (Fig. 1), and its association to psoriasis in the Swedish population. *PSORS1C3* is located approximately 7 kb centromeric to *POU5F1* and by virtue of its proximity to *HLA-C* is a potential candidate in psoriasis susceptibility.

MATERIALS AND METHODS

Patients and controls

In these analyses a total of 218 patients with psoriasis vulgaris were studied; 194 were recruited from the Stockholm area and 24 from the southwest of Sweden. Patients were enrolled at the Karolinska University Hospital and through the Swedish Psoriasis Association. Diagnostic ascertainment was performed through direct clinical examination. The patient group consisted of 108 women and 110 men and their age of disease onset ranged from 1 to 72 years with a mean of 20 and a median of 18. More than 70% had an age of onset below 25 years of age and 85% had a family history of psoriasis; thus the majority of the patients fall into the so-called type 1 psoriasis phenotype. As a control group 127 population-matched individuals were used (70 women and 57 men) with the criterion of no personal or family history of psoriasis.

Bioinformatics and expressed sequence tag characterization

Expressed sequences tags (ESTs) of interest (AI872271, AW015492 and AI796482) were obtained (UK MRC HGMP Resource Center, Cambridge) and purified using Qiagen Maxiprep columns (Qiagen, Valencia, CA, USA). The ESTs were sequenced using vector-specific primers and the DYEnamic™ sequencing chemistry (Amersham Pharmacia Biotech, Uppsala, Sweden) in a 6-μl reaction in a 96-well Robocycler™ thermocycler according to the recommendations of the supplier. The products were separated on LongRanger polyacrylamide (BMA Bioproducts, Rockland, ME, USA) 96-well gels on ABI 377 sequencers (Applied Biosystems, Foster, CA, USA). Also publicly available chromatograms were imported by file transfer protocol from www.wustl.genome.edu and assembled using the Pregap4 and Gap4 programs of the Staden package (21). EST and cDNA derived-sequences from the region under investigation were compared against genomic sequences using the Dotter program (22) to identify areas of interest. The RepeatMasker program (repeatmasker.genome.washington.edu) was used to block repetitive sequences in both sequence groups. When analysing *PSORS1C3* for putative function we performed homology searches using the BLAST family of programs (www.ncbi.nlm.nih.gov); Scanprosite (www.expasy.ch), Pfam (pfam.wustl.edu), Blocks (www.blocks.fhcrc.org) and Smart (smart.embl-heidelberg.de) to search for known protein families and domains; and Psort (psort.nibb.ac.jp) in the prediction of protein sorting signals and localization sites.

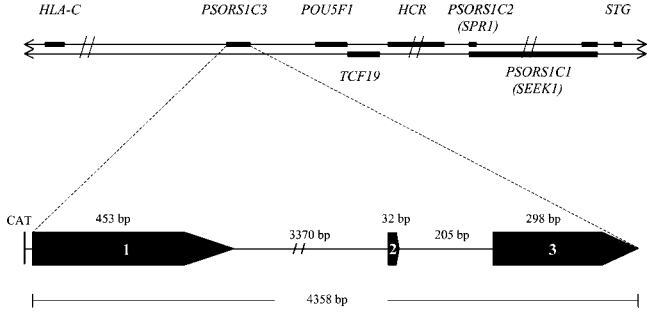

Fig. 1. The position and structure of *PSORS1C3* are described in relation to known genes in the PSORS1 locus on chromosome 6p21.3. *PSORS1C3* is 4358 bp long and is located between *HLA-C* and *POU5F1* in a centromeric to telomeric direction. A section showing the organization of the exons is enlarged and sizes of introns and exons are given. Figure is not to scale.

RT-PCR and rapid amplification of cDNA ends

Total RNA was extracted from tonsils and psoriasis lesional and unaffected skin as follows. Four-mm punch skin biopsies down to and including the dermis were obtained and snap-frozen from both lesional and non-lesional skin of individuals with stable untreated plaque psoriasis. Whole tonsils were removed and snap-frozen from a psoriasis patient who experienced recurrent streptococcal infections. The skin biopsies were sectioned in a cryostat and immediately immersed in RNA*later* according to the recommendations of the manufacturer (Qiagen) and homogenized using syringes. Tonsils were also homogenized in RNA*later*. Total RNA was extracted using the RNeasy Kit (Qiagen) according to the manufacturer's protocol. Normal skin total RNA was obtained commercially (Clontech, Palo Alto, CA, USA). A first strand cDNA synthesis kit was used in the first step of the RT-PCR using random priming hexamers (Amersham Biosciences). A 335-bp cDNA fragment stretching all three exons was then PCR amplified using *PSORS1C3* specific primers in exon 1 and 3 for RT-PCR (Table I). 3′ and 5′ rapid amplification of cDNA ends (RACE) was performed on *PSORS1C3* using the SMART RACE kit (Clontech) and gene-specific primers. Additional primers C3RP.U1-C3RP.U5 were tested for RT-PCR at the 5′ end of the sequence. The resulting products were sequenced as described above using the same primers as in the amplification step.

Northern blot analysis

As a probe, a sequence stretching the three exons was amplified using the same primers as for RT-PCR and excised from low melting point agarose. The *PSORS1C3* probe was labelled using random priming as described previously (23) and hybridized to commercial Human MTN™ Blot I and II with poly(A)$^+$ RNA from multiple human tissues according to the manufacturer's instructions (Clontech). Exposure was performed overnight and signals were visualized using a BAS 1500 phospho-imager (Fujifilm, Edison, NJ, USA).

Polymorphism analysis

In order to elucidate the exon-intron structure the obtained cDNA sequence (acc. no. AY484516) was plotted against the genomic sequence (acc. no. AP000509). For the detection and analysis of polymorphisms each of the three *PSORS1C3* exons were amplified in three separate PCR products in 87 patients and 50 controls (Table I). Any novel polymorphism or ambiguous sequence was re-sequenced and re-analysed. Amplification was achieved under standard conditions using

Table I. *Primers used in rapid amplification of cDNA ends, RT-PCR of the psoriasis susceptibility 1 candidate 3 (PSORS1C3) cDNA and amplification and sequencing of PSORS1C3 exons and individual single nucleotide polymorphisms (SNPs)*

Primer/method	Forward	Reverse	Size (bp)
RACE and RT-PCR			
C3.RT-PCR	5′-GCTATGACTTCATTACTCTTTCCCAG-3′	5′-ACTTGCTCTCGACCCGGTCTA-3′	335
C3RP.U1	5′-ATATAGGATGAGAGGCAGGTTGTTC-3′		
C3RP.U2	5′-TCAGTTTTGGATGTGTCAGATTTAAG-3′		
C3RP.U3	5′-CCAGATGACCAACAGGTAGGTAGT-3′		
C3RP.U4	5′-GATGAAGAGATTTGGGAGTCATTACT-3′		
C3RP.U5	5′-TAAGACTCCTGTTGGAAAATAACCC-3′		
raceC3.r1		5′-GGGACCAATAGAGACTTGCTCTCGACCCGGTCTAG-3′	
raceC3.r2[a]		5′-AGGCCTGTCAATCTCAAATAGAGGAGTC-3′	
Amplification and sequencing			
C3.exon 1	5′-TTTGGATGTGTCAGATTTAAGGCC-3′	5′-AATAACGAATGCAGCTGCACAT-3′	665
C3.exon 2	5′-CTCACCCACTCCTTTTGCTCTC-3′	5′-ACTTGCTCTCGACCCGGTCTA-3′	337
C3.exon 3	5′-GATTGACAGGCCTCGGAAGTC-3′	5′-CAGGCTTTCCTTCCTTCTCACT-3′	491
Pyrosequencing			
pC3.exon 1a[b]	5′-CAATGAGCCTAGATGAGGCC-3′	5′-(Biotin)GCACAGAGAGTGGGTTGCTGAGG-3′	211
pC3.exon 1b[b]	5′-ACGAAGTCTCAGGCTAC-3′	5′-(Biotin)AATAACGAATGCAGCTGCACAT-3′	283
pC3.exon 3	5′-GATTGACAGGCCTCGGAAGTC-3′	5′-(Biotin)CAAGACCCTTCACAGAG-3′	261
SNP 3868542	5′-TGAGCCTAGATGAGGCCCTGCAG-3′		
SNP 15721489	5′-TAGTCACACAGCCTCACC-3′		
SNP 15721491	5′-ACAGGCACCTTGTGCAGTG-3′		
SNP 3871247	5′-CAGGTAAGACTCCTGTTGG-3′		
SNP 15721492	5′-ACGAAGTCTCAGGCTAC-3′		
SNP 3130506	5′-GCCCAGCACCTTGTCACCATCC-3′		
SNP 3871246	5′-GGGGAGCATCATGGCAC-3′		
SNP 15721493, 15721494 and 887467	5′-ATCTCCCAGTGAGGGGAAG-3′		
SNP 2269711	5′-TCTCCCCACCCAGCCATGCCAGC-3′		

RACE, rapid amplification of cDNA ends.
[a]raceC3.r2 primer is exon2/exon3-boundary specific.
[b]Indicates two non-overlapping sequences of exon 1.

Taq polymerase (Promega, Madison, WI, USA) with 200 ng DNA as template, in a 50-μl reaction in a 96-well Robocycler™ thermocycler (Stratagene, La Jolla, CA, USA). Amplified products were purified using Microcon™ PCR 96-well filter plates (Millipore, Bedford, MA, USA) and sequenced as described above using the same primers utilized in the PCR amplification. Resulting sequences were compared against the genomic sequence of *PSORS1C3* (acc. no. AP000509) using the Pregap4 and Gap4 programs of the Staden package (21). Polymorphisms were detected using the TRACE_DIFF program of the same package. Any novel polymorphisms were submitted to the NCBI SNP database (dbSNP) and subsequent database numbers were obtained. All SNPs in this paper are referred to by their corresponding dbSNP number.

Genotyping of identified SNPs in an extended material was done through pyrosequencing in a 96-well format, according to the recommendations of the supplier (Pyrosequencing, Uppsala, Sweden). Briefly, short PCR fragments of exons 1 and 3 of *PSORS1C3* were amplified under standard conditions in three separate PCR products (Table I) with all reverse oligonucleotides biotinylated at the 5′ end. Fragments were captured using streptavidin-coated magnetic beads (Dynal Biotech, Sweden), denatured, annealed to their corresponding sequencing oligonucleotides (Table I) and sequenced on a PSQ96 Pyrosequencer using the reaction mixtures provided by the supplier (Pyrosequencing). The resulting luminous signals were converted to genotypes using the PSQ96 software version 1.2AQ. The HLA-Cw*0602 status was determined by phototyping as described previously (24).

Statistical analysis

The test for Hardy-Weinberg equilibrium was performed using GenePop (http://wbiomed.curtin.edu.au/genepop/). Odds ratios (OR) and 95% confidence intervals (CI) were calculated for the observed frequencies of *PSORS1C3* alleles between patients and controls. The significance of this distribution was determined by Fisher's exact test and corrected for multiple testing by the Bonferroni method (25). In order to test for confounding factors, the data were stratified according to presence or absence of HLA-Cw*0602 as well as for each of the associating SNPs and then tested for homogeneity of the odds ratios (26). Haplotype estimation was done with HPlus (http://qge.fhcrc.org/software.php) (27).

RESULTS

Characteristics of the PSORS1C3 gene

BLAST was used to compare the genomic sequence of the region located between *HLA-C* and *POU5F1* (acc. no. AC004195) to ESTs, proteins and protein domains.

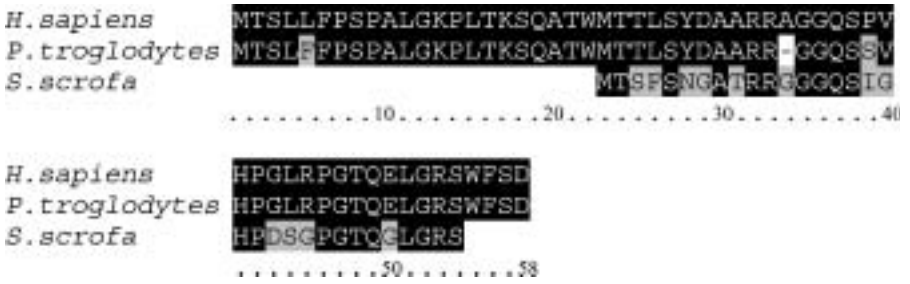

Fig. 2. A putative protein of *PSORS1C3* is conserved. A similar sequence of *PSORS1C3* was identified in the HLA-region in *Pan troglodytes* (chimpanzee) and the SLA-region in *Sus scrofa* (pig). These were translated and compared with the human putative protein sequence of 58 amino acids using ClustalW.

ESTs from the region were characterized by sequencing the entire clone inserts using vector-specific primers or by importing the pre-existing chromatograms (www.wustl.genome.edu). A contig was created using RT-PCR and primer walking to complete any gaps in the sequence, while 5′ and 3′ RACE were used to determine the true extent of the transcripts (Table I). This resulted in the identification of a novel gene: *PSORS1C3* (psoriasis susceptibility 1 candidate 3) (www.gene.ucl.ac.uk/nomenclature) consisting of three exons in total with a centromeric to telomeric orientation (Fig. 1) and a possible CAT-box located 53 bp upstream of the start of exon 1. The sequence transcript found was 600 bp long and the longest deduced open reading frame (ORF) was 58 amino acids (AY484516). The putative protein shows no similarity to any known proteins or domains but its sequence is conserved between human, chimpanzee (*Pan troglodytes*) and pig (*Sus scrofa*) (Fig. 2).

PSORS1C3 expression

To test *PSORS1C3* expression, RT-PCR was performed on total RNA from normal skin, tonsil and both lesional and non-lesional psoriasis skin. An RNA transcript of expected size was identified in all samples (Fig. 3a). It should be noted that the epidermal to dermal ratio varies greatly between lesional and unaffected skin, and that RT-PCR is not a quantitative assay. Thus the intensity of the RT-PCR bands should be interpreted with extreme caution. Expression was further analysed by Northern blotting in multiple tissues. A *PSORS1C3* probe stretching all three exons repeatedly detected a 1-kb band in testis and pancreas and a 2-kb band in skeletal muscle (Fig. 3b). The difference in size between the identified transcripts on the Northern blots may result from alternative splicing/polyadenylation sites. Thus we performed 3′ and 5′ RT-PCR to investigate the possibility of additional *PSORS1C3* sequence/isoforms. However, neither additional sequence nor isoforms were detected. Nevertheless, one cannot fully rule out the possibility that other splice variants exist, as isoform expression can be both organ- and time-specific (28).

Polymorphisms in the PSORS1C3 gene associated with psoriasis

To investigate the presence of polymorphisms in *PSORS1C3* and to test these for association to psoriasis, all three exons were completely sequenced in both directions for 87 patients and 50 controls. An additional 131 patients and 77 controls were genotyped using pyrosequencing. A total of 11 SNPs in *PSORS1C3* were identified; 7 located in exon 1 and the remaining 4 in

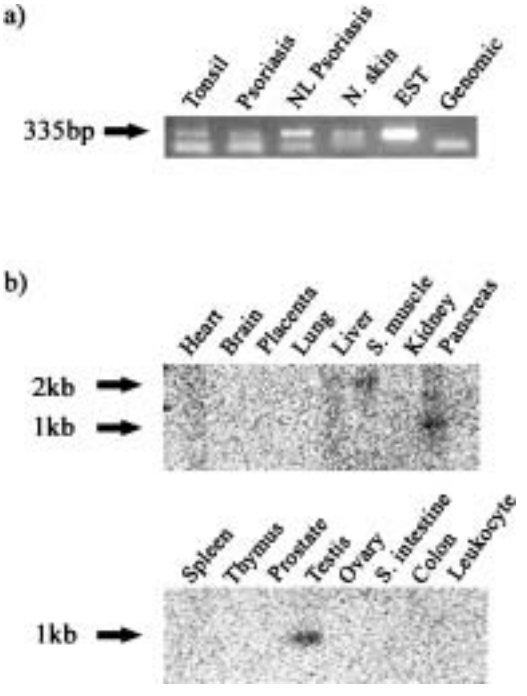

Fig. 3. PSORS1C3 is expressed in multiple tissues. (a) RT-PCR was performed using total RNA from normal skin, non-lesional and psoriasis skin and tonsil. The expressed sequence tag (EST) was used as a positive control and genomic DNA as negative control. An expected product of 335 bp was amplified in all samples. (b) Northern blots containing multiple tissues, as indicated, were probed with a sequence of 335 bp containing all three exons of *PSORS1C3*. The sizes of detected bands are shown.

Table II. *Allele frequencies of psoriasis susceptibility 1 candidate 3 (PSORS1C3) single nucleotide polymorphisms (SNPs) and their association with psoriasis*

Location	Polymorphism	SNP number[a]	Positive alleles: patients	Positive alleles: controls	OR (95% CI)	Fisher's *p* value	Corrected *p* value[b]
Exon 1	A→G	3868542	40% (173/436)	48% (121/254)	0.72 (0.53–0.99)	0.0461	NS
	C→T	15721489	19% (81/436)	31% (78/254)	0.51 (0.36–0.74)	0.0004	0.0032
	T→C	3871247	63% (273/436)	49% (125/254)	1.73 (1.26–2.36)	0.0008	0.0064
	T→C	3130506	26% (113/436)	32% (81/254)	0.75 (0.53–1.05)	NS	
	G→A	3871246	65% (283/436)	52% (131/254)	1.74 (1.27–2.38)	0.0007	0.0056
Exon 3	G→A	15721494	7% (29/436)	7% (18/254)	0.93 (0.51–1.72)	NS	
	A→T	2269711	29% (128/436)	37% (95/254)	0.70 (0.50–0.96)	0.0348	NS
HLA-Cw*0602	Positive		59% (128/218)	12% (15/127)	10.62 (5.81–19.40)	$8.16*10^{-19}$	$6.53*10^{-18}$

[a]According to NCBI SNP database.
[b]Fisher's *p* value corrected for multiple testing.

exon 3 (Table II). Three of these SNPs – 15721491 (T/A) in exon 1 and 15721492 (C/T) and 15721493 (G/C) in exon 3 – were rare variants (<1%) in patient and control populations and therefore were not analysed further in this study. Four exon 1 insertion/deletion SNPs reported in the NCBI SNP database were not detected upon the direct sequencing of the study population. One SNP, 887467 (G/C) in exon 3, was not in Hardy-Weinberg equilibrium and therefore was not analysed further. After correction for multiple testing, analyses of the remaining seven SNPs showed that three SNPs located in exon 1 associated significantly with psoriasis. SNP 15721489 showed the highest association (OR=0.51, CI 0.36–0.74, $P_{corr}=0.0032$) (Table II).

Testing for confounding

The HLA-Cw*0602 allele was detected in 59% of the patients and 12% of the controls, which gave the strongest and the most significant association with psoriasis in this study (OR=10.62, CI 5.81–19.40, $P_{corr}=6.53*10^{-18}$). In order to determine whether the association of *PSORS1C3* SNPs was dependent on HLA-Cw*0602 status, and vice versa, the data were stratified according to the presence or absence of each detected variant (Table III). When stratifying for all the various SNPs, HLA-Cw*0602 continued to show strong association with psoriasis but when stratifying for HLA-Cw*0602 status none of the *PSORS1C3* SNPs displayed association. The homogeneity test showed that the ORs obtained from the stratified tables differed significantly. Thus the observed associations between psoriasis and various SNPs in this novel gene are probably due to linkage disequilibrium with HLA-Cw*0602. As a supplementary test an estimation of intragenic *PSORS1C3* haplotypes was performed using HPlus (data not shown). However, none of the inferred haplotypes differed significantly in frequency between patients and controls.

DISCUSSION

The genetic component in the development of psoriasis is strong and multiple studies indicate that a major genetic determinant is located in the PSORS1 region at chromosome 6p21.3. The ongoing characterization of the area telomeric to *HLA-C* has not revealed any credible alternative to HLA-Cw*0602, a well-known marker of the disease at this locus. *PSORS1C3* was identified while screening the PSORS1 region for novel

Table III. *Stratification according to presence or absence of HLA-Cw*0602 and single nucleotide polymorphisms (SNP) variants*

	HLA-Cw*0602 association to psoriasis stratified by SNP status							SNP association to psoriasis stratified by HLA-Cw*0602 status						
		Cw6+		Cw6−					SNP+		SNP−			
SNP	Status	Pat	Contr	Pat	Contr	Cw6 OR	Cw6 *p* value	Status	Pat	Contr	Pat	Contr	SNP OR	SNP *p* value
15721489	SNP+	33	6	42	60	7.85	$3.4*10^{-6}$	Cw6+	33	6	95	9	0.52	NS
	SNP−	95	9	48	52	11.44	$4.5*10^{-12}$	Cw6−	42	60	48	52	0.76	NS
3871247	SNP+	112	14	68	79	9.30	$2.6*10^{-14}$	Cw6+	112	14	16	1	0.50	NS
	SNP−	16	1	22	33	24.00	$6.4*10^{-5}$	Cw6−	68	79	22	33	1.29	NS
3871246	SNP+	120	14	63	80	10.88	$2.0*10^{-16}$	Cw6+	120	14	8	1	1.07	NS
	SNP−	8	1	27	32	9.48	0.028	Cw6−	63	80	27	32	0.93	NS

Only associating SNPs are shown. Pat, patient; Contr, control.

transcription units and as it was located between *POU5F1* and *HLA-C* it was ideally positioned as a potential psoriasis susceptibility candidate gene. The facts that (i) the three exons of this gene showed proper gene-donor/acceptor sites, (ii) the exons were conserved in chimpanzee and pig, and (iii) positive transcript signals were identified by RT-PCR and Northern blot in multiple tissues, were taken as strong indications that this was an active transcriptional unit. The longest predicted ORF is 58 amino acids long; however, the sequence shows no homology to any known proteins or domains. *PSORS1C3* is located in the MHC, a region known to be involved in the immune system, which could suggest an immune function for this gene. Proteins involved in immunity can show rapid evolution and as a result show little similarity to evolutionary related proteins, e.g. LL-37 (29). Recent genomic studies indicate that the role of functional RNAs is more widespread and involved than previously thought (30–32). Taking into account the short size of the predicted *PSORS1C3* peptide and the lack of identifiable functional motifs one could speculate that the main functional role of *PSORS1C3* lies at the RNA level rather than the protein level. Future directions in the investigation of this gene's functions could include antibody production and Western blotting to test for translation and tissue-specific expression, cell localization and protein–RNA interaction screens to study RNA functionality.

It was anticipated that *PSORS1C3* SNPs would show some degree of association to psoriasis considering both the strong linkage disequilibrium that exists in the PSORS1 region (10) and the gene's proximity to *HLA-C*. Thus a more relevant question is whether these SNPs associate more strongly than, and/or independently of HLA-Cw*0602. *PSORS1C3* was indeed associated with psoriasis but the level of association was not as strong as for HLA-Cw*0602 in the present study. Extensive linkage disequilibrium is a major confounding factor for association studies in this region (10). Therefore, we stratified our data both for and against the presence of HLA-Cw*0602 and vice versa. None of the *PSORS1C3* SNPs showed HLA-Cw*0602-independent association to psoriasis. In contrast, HLA-Cw*0602 always associated to psoriasis regardless of *PSORS1C3* SNP status. Based on the analysis of the exonic variants, one could speculate that the association of *PSORS1C3* to psoriasis is wholly dependent on HLA-Cw*0602 status and therefore does not indicate causality. In summary, HLA-Cw*0602 remains the strongest psoriasis-associated allele in this region and is in linkage disequilibrium with several *PSORS1C3* SNPs.

A number of arguments have been used to question HLA-Cw*0602 as the causative factor in the PSORS1 region. These include: low incidence of psoriasis in HLA-Cw*0602 individuals, $>30\%$ of psoriasis individuals are HLA-Cw*0602-negative, the existence of HLA-Cw*0602-negative families, HLA-Cw*0602-positive extended haplotypes associating more strongly to psoriasis than HLA-Cw*0602 itself and alternative candidates that approach the same level of association as HLA-Cw*0602 in a number of studies (7, 9, 10, 33). However, as mentioned above, even the strongest genetic factor in psoriasis will probably be present only in a subset of the disease population and may be common in the general population; psoriasis is not a Mendelian disease. One cannot rule out HLA-Cw*0602 simply due to non-Mendelian behaviour. All psoriasis susceptibility candidates have to be compared with the strongest associating factor/variation at the same locus in order to determine candidature. Furthermore, linkage disequilibrium will raise the incidence of alleles in genes neighbouring the true psoriasis susceptibility gene. For example, the psoriasis-associated allele HCR*WWCC is in strong linkage disequilibrium with the HLA-Cw*0602 allele and it is thus difficult to distinguish them genetically without very large sample sizes (17). Nevertheless, HLA-Cw*0602 consistently associates more strongly to psoriasis than the associated HCR*WWCC allele (16–18). Thus, upon comparison with all other candidate variations, HLA-Cw*0602 has consistently been shown to be the strongest associating variation in psoriasis. Furthermore, HLA-Cw*0602 has recently been shown to have a dosage effect on the severity of psoriasis (12). The role of *HLA-C* in T cell biology and the fact that T cells are involved in the initiation and maintenance of psoriasis plaques further supports HLA-Cw*0602 involvement in disease pathogenesis (34). Thus, considering the functional role of *HLA-C* in the immune system, it seems prudent to investigate the effects of variations upon its functionality.

In conclusion the identification and characterization of this novel gene contribute further information to the PSORS1 region. Using the one locus-one gene model our study does not support the involvement of *PSORS1C3* in the pathogenesis of psoriasis. However, if one were to use a model allowing for interactions between multiple genes at this locus one could not conclusively exclude any candidate by association studies alone, including *PSORS1C3*. Irrespective of the model applied to PSORS1, interactions with other loci need to be investigated. In particular, investigation of the effects of HLA-Cw*0602 status on the allelic distribution at those loci containing known HLA-C binding partners may provide further clues to psoriasis biology by identifying epistatic interactions.

ACKNOWLEDGEMENTS

We thank Anna-Lena Kastman for her valuable technical assistance and Tomas Rosenmüller for his contributions. This study was supported by funds from the Swedish Psoriasis

Association, Karolinska Institutet, the Swedish Research Council, the Swedish Heart and Lung Foundation, the Welander-Finsen Foundation and HRM Silvia's Jubilee Foundation.

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