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Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus

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PSORS1, near HLA-C, is the major genetic determinant of psoriasis. We present genetic and structural evidence suggesting a major role for the HCR gene at the PSORS1 locus. Genotyping of 419 families from six populations revealed that coding single-nucleotide polymorphisms of HCR formed a conserved allele HCR*WWCC that associated highly significantly with psoriasis and with the HLA-Cw6 allele in all populations. Because of strong linkage disequilibrium between HLA-Cw6 and HCR*WWCC, the two genes could not be genetically distinguished by this sample size. However, the variant HCR allele was predicted to differ in secondary structure from the wild-type protein. HCR protein expression in lesional psoriatic skin differed considerably from that observed in normal skin. These results provide strong evidence for the HCR*WWCC allele as a major genetic determinant for psoriasis, probably by a mechanism impacting on keratinocyte proliferation.

INTRODUCTION

Psoriasis vulgaris is a chronic disfiguring inflammatory skin disorder, prevalent in many populations at frequencies ranging from 1 to 3%. Clinically, psoriasis is characterized by red, indurated, scaly plaques either localized or widespread in extent, which typically relapse and remit over many years. These features mirror the characteristic pathological changes, namely keratinocyte hyperproliferation and loss of differentiation, dilatation and proliferation of dermal blood vessels and accumulation in the skin of neutrophils and T lymphocytes. However, the molecular basis of the pathogenesis of psoriasis remains unclear (1).

Increased concordance rates in twins together with familial clustering support a significant genetic component in the disease aetiology (2). However, segregation analysis of multi-generational families shows no clear pattern of inheritance and, cumulatively, suggests that psoriasis represents an example of a common, but genetically complex, disease. In non-Mendelian disorders, attributing causality to a single gene, even within a defined susceptibility interval, may be problematic. The

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Table 1. Clinical characteristics of the psoriasis patients in different study groups

	Family study						Case-control study	
	UK	Finland	Sweden	Spain	Italy	India	Japan	India
Number of families	175	91	64	52	48	27	–	–
Number of affecteds	175	116	64	130	48	27	82	61
Male:female ratio of affecteds	71:104	69:47	33:31	30:67	26:22	21:6	57:25	22:34
Age of affecteds at entry to the study								
Median	32	40	34	39	22	32	49	45
Range	9–54	5–77	18–54	7–83	4–41	15–54	9–79	13–74
Age of affecteds at psoriasis onset								
Median	14	20	18	17	12	23	36	28
Range	2–35	1–72	3–40	2–75	4–31	5mo–35[a]	1–65	2–65
Number of affecteds with age of onset								
≤40 years	173	101	64	102	29	25	24	49
>40 years	0	10	–	6	–	0	15	10
unknown	2	5	–	22	19	2	43	3
Number of probands with affected first degree relative	161 (92%)	39 (34%)	7 (11%)	130 (100%)	34 (71%)	3 (11%)	n/a	n/a
Number of affecteds with plaque psoriasis	175 (100%)	91 (78%)	64 (100%)	108 (83%)	47 (98%)	27 (100%)	43 (52%)	61 (100%)

[a]Five months to 35 years old.

contribution of any given locus to a disease phenotype may vary in different populations. Even high density association-based studies may be limited by the unknown degree to which a disease locus may harbour a number of causal alleles, and mutation in a single gene may be neither necessary nor sufficient for disease development. Hence, the correlation between identified genotype and observed phenotype is unlikely to be complete. Despite these potential analytical difficulties, psoriasis is unusual amongst the non-Mendelian common diseases studied so far, with strong association to HLA-C and highly reproducible evidence for a susceptibility locus (PSORS1) within the major histocompatibility locus (MHC). This was initially identified by case–control association studies for HLA protein polymorphisms, specifically the HLA-Cw6 antigen (3–6) and confirmed through robust non-parametric linkage analysis of family material (7–10). These data suggest that PSORS1 confers significant risk for disease, estimated to account for between 35 and 50% of the familial clustering observed in psoriasis. Hence, any gene near HLA-C at chromosome 6p21.3 may be considered a positional candidate for psoriasis susceptibility. The psoriasis susceptibility region has recently been refined to an ~300 kb interval around HLA-C, yet with high probability distal to it (11–13). Altogether, five genes are characterized in this completely sequenced region: HLA-C, OTF3 (OMIM 164177), TCF19 (SC1; OMIM 600912), HCR (OMIM 605310) and corneodesmosin (CDSN; OMIM 602593), and the presence of three more genes (SEEK1, SPR1 and STG1) has been predicted (Fig. 1). Of these, CDSN marks the distal end of the candidate region (14,15). To determine the genes most likely to represent PSORS1, we set out to compare all the candidate psoriasis susceptibility alleles, identified in a diverse range of populations, using standardized genotyping methods. We studied a total of 419 families recruited from the UK, Finland, Sweden, Spain, Italy and India, and an additional 143 cases with population-matched controls from Japan and India (Table 1).

RESULTS

Search for new polymorphisms in genes within the susceptibility region

To avoid selection bias and in order to search for previously undetected single-nucleotide polymorphisms (SNPs) with possibly significant association, we resequenced the OTF3, TCF19, HCR and CDSN genes, which map within the refined psoriasis susceptibility region, and also >90% of the region encompassing the three predicted genes (Fig. 1). Resequencing was performed on eight selected patients heterozygous for the putative high-risk psoriasis susceptibility haplotype (11), but no previously unreported contributory polymorphisms were detected (none approaching the frequency of HLA-Cw6) so that variants associating strongly with psoriasis occur only in the HCR and CDSN genes as reported previously. In addition, we verified two further exons in the HCR gene, adding 135 bp 5′ of the gene and 26 amino acids to the beginning of the protein (Fig. 1; GenBank accession no. AY029160). These additional exons did not contain new polymorphic sites in psoriasis patients.

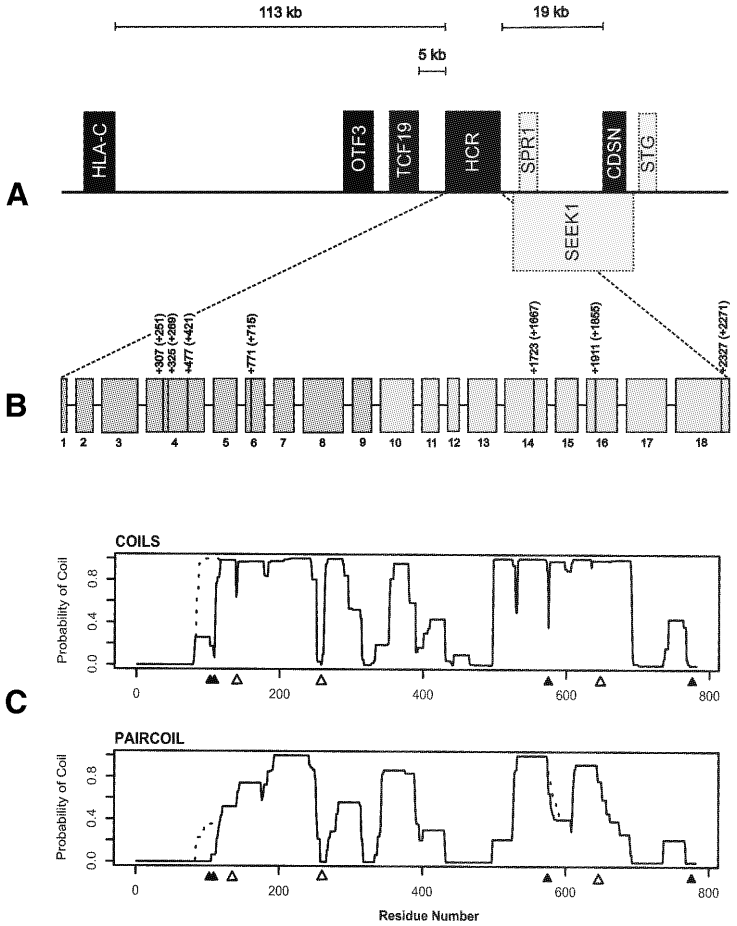

Figure 1. (**A**) Physical map of the MHC region near HLA-C showing the verified genes (black boxes) and predicted genes (open boxes). (**B**) The genomic structure of the HCR gene, showing two new exons (1 and 2, exon numbering below the boxes) and positions of SNPs addressed in this study (above the boxes). For each SNP, the position is numbered according to GenBank accession no. AY029160; for reference, numbering used in a previous association study (15) is given in parentheses. (**C**) Secondary structure predictions using two different programs, COILS and PAIRCOIL are shown. The structural prediction for the wild-type allele is shown as a dotted line, and for the psoriasis susceptibility allele as a solid line (note that the predictions overlap for most of the protein length). Distinct differences in structural prediction are induced by the two most N-terminal changes from Arg→Trp (C→T at nucleotides 307 and 325) and by the Gly→Cys change (G→T at nucleotide 1723), marked by black arrowheads. SNPs showing less transmission disequilibrium were not predicted to cause structural alterations (open arrowheads).

SNP association analysis

We selected three genes for further genetic analysis, each previously associated with psoriasis and verified by our resequencing to possibly carry susceptibility alleles, namely HLA-C (3–6), CDSN (16,17) and HCR (15). For HLA-C, we genotyped three SNPs defining the Cw6 allele, for CDSN two SNPs defining the CDSN*5 allele, and for HCR seven SNPs potentially associated with psoriasis (Table 2). Of the HCR SNPs, four (HCR-307, 325, 1723, 2327) changed an amino acid. Three of these substitutions (Arg→Trp at HCR-307, Arg→Trp at HCR-325 and Gly→Cys at HCR-1723) were predicted to change the secondary structure of the protein (Fig. 1). Of note, the two first amino acid changes caused a dramatic decrease in the probability of coiled-coil structure.

We first considered SNP associations and transmission disequilibrium in each study population (Table 2). When families were pooled together (908 patient chromosomes, 772 control chromosomes), the strongest overall effects [odds ratio (OR) > 2] and most significant associations ($P < 10^{-10}$) were observed for HLA-Cw6, HCR-307*T, HCR-325*T and HCR-1723*T (Table 2).

A specific HCR susceptibility allele with predicted altered structure

We next examined haplotype associations (Table 3). The HPM algorithm was used to extract the most strongly associated haplotypes. We calculated two index numbers to quantify the effects (the proportion of chromosomes found in patients of all chromosomes with a given haplotype and OR), and performed two statistical tests [2 × 2 association test and transmission disequilibrium test (TDT)]. The ORs were similar for HLA-Cw6 and the different associating HCR SNP combinations [2.9 and 2.5–3.2, respectively, confidence intervals (CIs) overlapping in the range 1.9–4.4], whereas the OR for CDSN*5 was distinctly lower (2.0, CI 1.6–2.5).

Of special interest is the specific coding HCR four-SNP haplotype (Table 3). This SNP haplotype, redefined as a susceptibility allele HCR*WWCC (corresponding to SNPs at

Table 2. Allele associations and transmission disequilibrium for HLA-Cw*6 and HCR and CDSN SNPs

Allele	Allele association analysis (% patient chromosomes/% control chromosomes)									Transmission disequilibrium test		
	British families $n = 164$	Finnish families $n = 91$	Swedish families $n = 62$	Italian families $n = 47$	Indian families $n = 29$	Spanish families $n = 26$	All families $n = 419$			All families $n = 419$		
								OR	$P\chi^2$	Transmitted	Untransmitted	$P\chi^2$
HLA-Cw6	38/13	18/7	27/10	19/ND	14/11	23/10	26/11	2.9 (2.1–3.9)	2×10^{-11}	113	56	1.2×10^{-5}
HCR-307*T	49/27	28/12	34/22	34/16	28/21	21/21	36/21	2.1 (1.7–2.7)	1×10^{-10}	220	123	1.6×10^{-7}
HCR-325*T	54/27	27/10	33/20	34/21	32/24	24/8	38/19	2.6 (2.0–3.3)	$4.x10^{-14}$	234	112	5.4×10^{-11}
HCR-477*C	88/86	90/88	96/89	82/88	95/89	65/84	88/87	1.1 (0.8–1.5)	NS	100	88	NS
HCR-771*C	86/73	83/80	82/76	93/89	78/79	98/85	86/77	1.8 (1.4–2.4)	3×10^{-5}	144	100	0.0049
HCR-1723*T	51/27	28/11	33/19	36/7	31/23	24/17	37/19	2.4 (1.9–3.1)	3×10^{-13}	246	119	3.0×10^{-11}
HCR-1911*G	84/74	81/76	76/66	88/86	72/63	91/69	82/73	1.7 (1.4–2.2)	1×10^{-5}	153	105	0.0028
HCR-2327*G	55/32	42/27	38/30	47/53	35/34	39/18	46/32	1.8 (1.5–2.3)	1×10^{-7}	251	154	1.4×10^{-6}
CD-619*T	89/83	92/88	89/90	92/88	100/96	ND/ND	91/87	1.5 (1.1–2.1)	0.01	90	58	0.0085
CD-1243*C	67/48	61/52	65/49	72/63	74/55	72/81	67/52	1.9 (1.5–2.3)	1×10^{-8}	216	122	3.2×10^{-7}

ND, not typed; NS, not significant. For OR, 95% CIs are given in parentheses.

nucleotides 307, 325, 1723 and 2327, and involving amino acids 103, 109, 575 and 776, respectively), was found in 35% of patient chromosomes and 18% of the control chromosomes (Table 3). HLA-Cw6 and HCR*WWCC were in strong linkage disequilibrium ($D' = 0.67$, $r^2 = 0.34$ in all subjects; $D' = 0.73$, $r^2 = 0.46$ in patients), whereas linkage disequilibrium was weaker between HCR*WWCC and CDSN*5 ($D' = 0.37$, $r^2 = 0.067$ in all subjects; $D' = 0.41$, $r^2 = 0.08$ in patients), suggesting a relative recombination hot-spot between HCR and CDSN.

To differentiate the genetic effect of HLA-Cw6 and HCR*WWCC, we performed conditional TDTs and tested nested models. None of the results offered significant support for either of the two genes over the other (data not shown), consistent with strong linkage disequilibrium but leaving also open the possibility of locus interaction. Thus, genetically HLA-Cw6 and HCR*WWCC remain equally good candidates.

To study whether the same alleles also associate with psoriasis in Japanese and an independently ascertained cohort of Indian patients, we considered cases and controls. The HLA-Cw*6 allele and HCR*WWCC occurred in Japanese patients as well as controls less frequently than in Europeans, but the OR was high and the association still significant (8/83 in patients versus 1/70 in controls, $P = 0.04$). The HCR susceptibility allele was thus conserved and associated with HLA-Cw6 as indicated by their presence in the same individuals in all populations studied from both Europe and Asia.

Three of the four amino acid substitutions specifying the high-risk HCR*WWCC allele were predicted to induce secondary structural alterations of the HCR protein (Fig. 1). Essentially, the first α-helical loop of the putative psoriasis susceptibility allele was predicted to start approximately 30 amino acids toward the C-terminus compared to the major allele, suggesting a mechanism for a putative biochemical effect.

Expression of HCR in normal and psoriatic skin

Because the genetic and protein prediction evidence suggested the possible involvement of HCR in psoriasis susceptibility, we raised polyclonal antibodies to determine HCR protein distribution in normal and psoriatic lesional skin.

In healthy and non-lesional skin, HCR protein was confined uniformly to basal keratinocytes, whereas in psoriatic skin this pattern was disturbed (Fig. 2). In lesional psoriasis, expression was enhanced within nuclei and cytoplasm of basal keratinocytes at the tips of dermal papillae, whereas basal keratinocytes at the rete ridges were mostly negative. This altered pattern was not seen in other inflammatory (eczema, lichen planus) or acanthotic (pityriasis rubra pilaris) disorders of the skin that resemble psoriasis (data not shown). For comparison, we used an antibody against Ki-67 (a cell proliferation marker) on parallel sections of normal and psoriatic skin. Proliferating cells were most abundant at rete ridges in psoriatic skin. Thus, HCR and Ki-67 showed inverse patterns of staining

Secondary structure predictions for the HCR protein suggested that it contains several segments of coiled-coil structure (Fig. 1). It was predicted to be either a nuclear or a cytoplasmic protein, and a dual localization in keratinocytes was supported by our immunohistochemical results (Fig. 2). Taken together, the nuclear localization and inverse relationship to a proliferation marker suggest a role for HCR in regulating keratinocyte differentiation or proliferation.

DISCUSSION

Our genetic analysis indicated that a specific combination of SNPs of the HCR gene forms an allele that confers susceptibility to psoriasis at a level similar to HLA-Cw6, thus making HCR a prime candidate for PSORS1. In each population studied, either association tests or TDT, or both, supported association of the specific allele HCR*WWCC with psoriasis and, when combined, the effect surpassed that observed for CDSN and at least paralleled that observed for HLA-Cw6. Other genes in this interval are unlikely to show stronger effects based on our sequencing results.

The genotyping data indicate strong linkage disequilibrium between the three genes in all populations. Essentially, HLA-Cw6

Table 3. HLA-Cw*6, HCR*WWCC and CDSN*5 haplotype association and TDT results among the affected compared to control chromosomes in 419 families

Haplotype									Haplotype analysis					TDT analysis		
HLA-Cw*	HCR							CD*	Patient chromosomes	Control chromosomes	Proportion of chromosomes in patients	$P\chi^2$	OR (95% CI)	Transmitted	Untransmitted	$P\chi^2$
	+307	+325	+477	+771	+1723	+1911	+2327									
6	–	-	–	–	–	–	–	–	193/729 (26%)	60/536 (11%)	0.76	2×10^{-11}	2.9 (2.1–3.9)	157	48	4×10^{-13}
–	T	T	C	C	T	G	G	–	184/487 (38%)	61/351 (17%)	0.75	1×10^{-10}	2.9 (2.1–4.0)	144	55	9×10^{-12}
–	-	T	C	C	T	G	–	–	216/565 (38%)	67/412 (16%)	0.76	8×10^{-14}	3.2 (2.3–4.4)	164	60	3×10^{-14}
–	T	T	–	–	T	–	G	–	218/620 (35%)	88/494 (18%)	0.71	1×10^{-10}	2.5 (1.9–3.3)	178	82	3×10^{-11}
–	–	–	–	–	–	–	–	5	355/656 (54%)	202/545 (37%)	0.64	4×10^{-9}	2.0 (1.6–2.5)	275	180	1×10^{-7}
6	T	T	C	C	T	G	G	–	93/414 (22%)	19/269 (7.1%)	0.83	1×10^{-7}	3.8 (2.3–6.4)	75	15	6×10^{-11}
–	T	T	C	C	T	G	G	5	112/383 (29%)	21/265 (8.0%)	0.84	4×10^{-11}	4.8 (2.9–7.9)	84	19	3×10^{-11}
6	T	T	C	C	T	G	G	5	69/322 (21%)	13/201 (6.5%)	0.84	5×10^{-6}	3.9 (2.1–7.3)	54	11	5×10^{-8}

Note that the denominator for chromosome numbers vary because ambiguous allelic data were discarded and excluded from constructing haplotypes (e.g. offspring and both parents heterozygous, resulting in ambiguity of transmitted alleles). –, denotes any allele.

resides mostly in chromosomes carrying HCR*WWCC, whereas more ancestral recombinations between HLA-Cw6 or HCR*WWCC on one hand, and CDSN*5 on the other, are evident from an inspection of the haplotypes or linkage disequilibrium measures between the three genes. Such a block of little recombination is compatible with recent measurements in European populations (18) and preservation of the same association in the Japanese, though at lower frequencies, argues for an old European founder effect for the psoriasis susceptibility mutation within the HLA-C–HCR block. However, these results also suggest that further dissection of the genetic association within this block may be impractical because very large sample sizes with very low genotyping error rates would be required to identify very rare historical recombinations between the genes.

The putative psoriasis risk allele HCR*WWCC is more common among controls than HLA-Cw6, suggesting a low penetrance. Even though such a finding is unexpected, it is in full accordance with the common disease–common variant hypothesis as well as previous examples of susceptibility genes in complex diseases, such as Alzheimer's, type 2 diabetes, susceptibility to deep venous thrombosis and Crohn disease (reviewed in 19).

However, dissonant interpretations of the role of HCR SNPs in psoriasis have recently been published and need further scrutiny. One analysis is based on a small sample of Swedish families (20) and the other on a large set of previously studied families of mixed ancestry from the USA (21). O'Brien *et al.* (20) conclude that HCR is unlikely to have an independent genetic effect on psoriasis, but their genetic data lack power to differentiate between HLA-C and HCR, given their small sample size ($n = 48$) and the strong linkage disequilibrium, and indeed reveal that the same HCR SNPs described earlier (15) associate strongly with psoriasis in their material.

Chia *et al.* (21) suggest rejecting HCR as a susceptibility gene based on two arguments. In their first argument, they rely on previous analysis by Nair *et al.* (13) that divides the susceptibility region into two parts, RH1 and RH2 [figure 2 from Nair *et al.* (13)]. This division, however, is based on two markers (M6S111 and M6S169) and may be artefactual, because historical marker mutations remain a likely explanation for the interrupted haplotypes and further to artefactual biphasic TDT peaks [figure 3 from Nair *et al.* (13)]. This alternative interpretation of the data of Nair *et al.* (13) is supported further by two notions. First, a genetic mechanism allowing double recombinations around the two markers while simultaneously preserving the cosegregation of two separate but almost adjacent chromosome segments (in this case, RH1 and RH2) is unusual. Secondly, HCR localizes within the RH2 block (13) but, as shown by Chia *et al.* (21), forms a continuous haplotype with HLA-Cw6, in accordance with our present data. These observations suggest that the RH1 and RH2 blocks in fact form a continuous haplotype block with two unstable markers near its centre. The second argument of Chia *et al.* (21), to disregard HCR, relies on a small number of HLA-Cw6 and HCR*WW negative risk haplotypes, leading these authors to conclude that a role in susceptibility is unlikely. However, the haplotypes are poorly documented as susceptibility haplotypes, based only on a meeting abstract and a borderline *P*-value, and the HCR gene has not been sequenced to identify possible novel coding variants. Finally, none of these studies (13,20,21) considers the combination of SNPs that we here found to represent the specific susceptibility allele HCR*WWCC.

Our working hypothesis portrays HCR as a regulator of keratinocyte proliferation or differentiation. An allelic variant of HCR (named HCR*WWCC) has a tendency to adopt a functionally different conformation with the potential to affect the growth or antigenic properties of keratinocytes, at least when

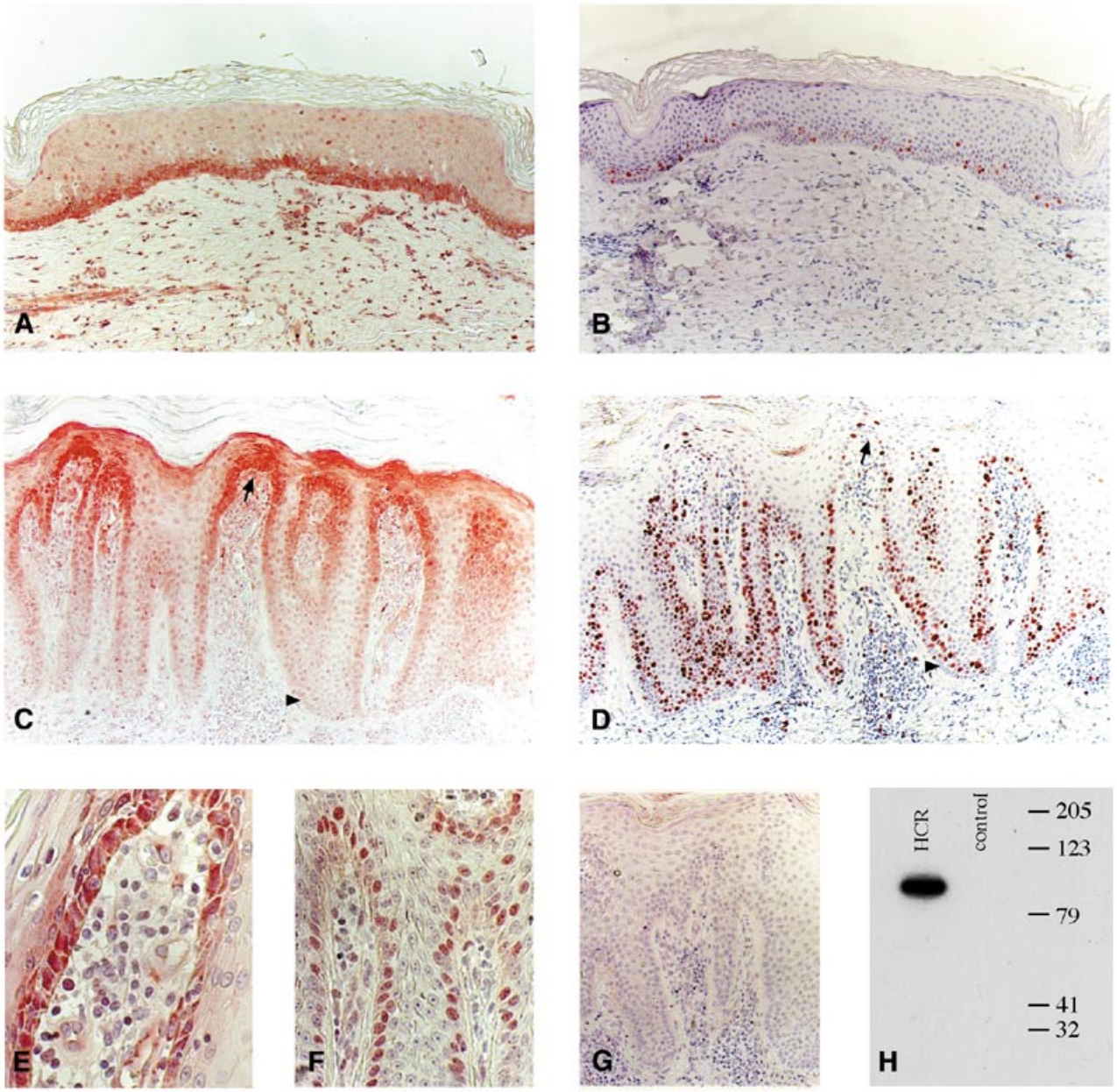

Figure 2. (**A**) Immunohistochemical staining using HCR antibodies on normal skin sections revealed strong and uniform staining of basal keratinocytes. (**C**) In psoriatic skin, basal keratinocytes at the tips of dermal papillae (arrow) showed strong staining for HCR, whereas basal keratinocytes at the rete ridges (arrowhead) were mostly negative. For comparison, we used an antibody against Ki-67 (a cell proliferation marker) on parallel sections of normal (**B**) and psoriatic skin (**D**). Proliferating cells were most abundant at rete ridges in psoriatic skin [arrowheads in (C) and (D)]. High magnification suggested that the HCR protein occurred in the cytoplasm (**E**) or also in the nucleus (**F**) in different cells and areas of the skin. Preimmune serum did not stain any cells in skin sections (**G**), and the specificity of the antibody was further supported by western blot analysis of cells transfected with an HCR coding construct (HCR) or with a non-coding construct (control) (**H**).

triggered by certain external factors. Such a subtle effect is compatible with the low penetrance and expressivity of the susceptibility allele, both at the population level, during the lifetime of an individual patient, and regionally in the skin. The change in the secondary structure of the HCR susceptibility form compared to the wild-type form may affect its biochemical properties (and alter its putative role in cell proliferation or differentiation) or antigenic properties (and thus contribute to the immunological response characteristic of psoriasis). Obviously, the structural prediction must be viewed cautiously and assessed experimentally. Finally, we showed an altered pattern of HCR protein expression as compared to healthy skin or clinically mimicking disorders. We have not observed differences in HCR expression that would depend on a patient's genotype.

Our data support the concept that HCR may constitute an essential element of PSORS1 and HCR*WWCC is likely to represent the disease susceptibility allele. Historically, the association of HLA-Cw6 with psoriasis was detected long

before the HCR*WWCC association, but that fact does not make one a more likely candidate than the other when both genes are in fact included in the same DNA block. Indeed, it may be impractical by genetic linkage or association to further dissect the role of the different genes within the region spanning 120 kb because of the strong linkage disequilibrium and large sample size needed. Future studies investigating PSORS1 should concentrate on increasing our understanding of the cellular roles of HCR and its neighbouring genes.

MATERIALS AND METHODS

Study populations and samples

Study populations consisted both of nuclear families and case–control sample sets from populations with different ethnic origin. Most of the families were trios with an affected offspring. 175 trios were recruited in Great Britain, 91 in Finland (in 21 trios there was also one affected parent), 64 in Sweden, 48 in Italy (in 32 trios there was also one affected parent). Gujarati Indian samples consisted of 27 trios and a separate case (n = 61)–control (n = 73) population. Spanish families (n = 52) were large multiplex pedigrees. The Japanese sample set consisted of 83 psoriasis patients and 70 population-based controls. Clinical characteristics of the patients are shown in Table 1. Predominantly, the patients in all surveys had early-onset, plaque psoriasis with familial background.

SNP genotyping

The collection of samples for genetic study was by informed consent and approved by local ethical review boards according to applicable regulations in each country. SNP genotyping was done in two centres. The Finnish, Italian, Spanish and Swedish samples were genotyped for HCR and CDSN*1243 using altered restriction site recognizing enzymes. In PCR amplification the following fluorescently labelled primer pairs were used: TCGACAGCTGCAAGAGCTGCCG and CCCCTCTTCCA-AGTTCTTCC (HCR*307); ATCTTCAGGTCCAGCAGGC-TGAGGTGATCGT and TTTCTACCCCTGCATTCACC (HCR*325); CACCTGCACTAACCTGTCTTTG and tacttgg-caattTTTCTACCCCTGCATTCACC (HCR*477); CTTCTTT-CCGCAGCTGTCCT and TCCCTAAGTCTGCACACAGAT (HCR*771); gatcacgccagtgTCCTTTTAGGGGAGGCAGAG and AAGGCCCTATCCACCCTG (HCR*1723); GATGGTT-CAGGGATGAGGAA and CCTGCAGACGCCTGAGTT (HCR*1911); TGGTGCTCATCTGCTGTCTT and agctccag-ctgcCTTTCCCTCCAACTGTCAGC (HCR*2327); CATTG-CGTTCCAGCCAGTGG and aggctcgccggttcaccAACTGGA-GCTGCTGCTGAAGGA (CDSN*1243). In non-labelled primers, 8–13 bp tail (shown in lower case letters in the primer sequences above) was added. The tail contained the SNP specific restriction site acting as an internal control for digestion. Since HCR*251 did not alter any restriction site, a mutation was incorporated into the primer sequence (shown underlined above). For electrophoresis, the digestion products were pooled into two panels and run on ABI 377 sequencer. Allele calling was done using Genotyper program (PE Biosystems).

PCR assays were carried out in 10 µl volumes containing 25 ng of genomic DNA, 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl, 0.1% Triton X-100), 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM primer mix, 1% DMSO and 0.3 U of DNA polymerase (DyNAzyme II, Finnzymes, Espoo, Finland). Digestion reactions were performed overnight in 10 µl reactions containing 5 µl of PCR product and 0.125–1 U of either *Bst*UI (HCR*307), *Ava*II (HCR*325), *Tsp*509I (HCR*477), *Bsm*FI (HCR*771), *Msl*I (HCR*1723), *Hha*I (HCR*1911), *Mwo*I (HCR*2327) or *Hph*I (CDSN*1243) and the appropriate manufacturer's buffer (New England Biolabs, Beverly, MA).

Genotyping for CDSN*619 and HLA-Cw*6 was also done using altered restriction sites as described previously. Briefly, CDSN*619 allele was genotyped using restriction enzyme *Mnl*I (22). HLA-Cw6 allele was genotyped using restriction enzymes *Msp*A1I and *Dde*I, which together detected the SNPs +102, +142 and +213 (base number in coding sequence) of exon 2 of the HLA-C gene (23). The digestion products for both CDSN*619 and HLA-Cw6 were electrophoresed on agarose gel and photographed under UV illumination. British, Indian and Japanese samples were genotyped using allele-specific hybridization assay (24) and HLA-Cw6 allele was genotyped using SSP–PCR (25).

To validate genotyping results between the two centres, we blindly genotyped 14 control DNAs in both centres. For all HCR and CDSN SNPs the results were identical. HLA-Cw6 genotyping was done in 100 Finnish patients using both methods described above. The results showed congruency of 98% between the methods. All the markers were in Hardy–Weinberg equilibrium. The average proportion of missing genotypes was 6.6% (range 4.9–11.5% for different markers). Mendel errors in family data were detected in 10/1428 genotypes (0.007%).

Haplotyping

Haplotyping was done within each trio and from each trio four independent chromosomes were obtained. If the child was affected, the transmitted chromosomes were considered as disease associated and untransmitted as controls. If one of the parents was affected, his/her chromosomes were considered as disease associated and the spouse's chromosomes as controls. If both the parent and the child were affected, only the untransmitted chromosome of an unaffected parent was considered as control, the other three as disease associated. In case of ambiguities (missing genotypes, identical heterozygotic genotypes in all of the family members or Mendel errors), the alleles were discarded. Before haplotyping, large Spanish pedigrees were divided into trios using a computer program developed in-house. The program randomly identified one trio per pedigree in which at least one of the members was affected, and genotyping information was available for all of the members. These trios were then combined with the rest of the family data. After haplotyping, the number of zeroed alleles was, on average, 17% (range 9–25%) per marker, the vast majority due to identical heterozygotes in the family.

Associated haplotype identification was done by using the Haplotype Pattern Mining (HPM) algorithm (26). The input data consisted of chromosomes tagged with the disease status (affected or control). The algorithm finds all the haplotype patterns that are significantly disease associated, using a χ^2 test. TDT analyses were done manually (for haplotypes; Table 3) or using GENEHUNTER 2.0 (27). For association tests, P-values

were calculated using the χ^2 test, or Fisher's exact test when the number of expected observations was less than five.

Antibody production

Antisera against HCR protein were raised in rabbits by immunization with a synthetic 18mer peptide ERDVSSDR-QEPGRRGRSW (amino acids 62–79). Peptide synthesis and antibody production were performed using materials purchased from Sigma Genosys (Cambridge, UK) and specific antibodies were affinity purified with peptide bound to an epoxy-activated Sepharose column (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions.To ascertain the specifity of the antibody, we carried out SDS–PAGE and western blotting according to standard procedures. HCR cDNA was cloned into the pCMV5 vector and transiently expressed in COS-1 cells. Affinity-purified HCR antibody (1 μg/ml) was used as the primary and peroxidase-conjugated anti-rabbit IgG as the secondary antibody, detected with enhanced chemiluminiscence (Boehringer Mannheim, Mannheim, Gemany).

Immunohistochemistry

Skin specimens were obtained from the centre of untreated psoriatic lesions ($n = 16$) and from healthy control skin ($n = 7$). Immunostaining was performed on formalin-fixed paraffin embedded specimens using the avidin–biotin–peroxidase complex technique for HCR and Ki67 antibodies [Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA) and StreptABComplex/HRP Duet Mouse/Rabbit kit (Dako A/S, Glostrup, DK), respectively]. Affinity-purified HCR antibody was used at 4 μg/ml and anti-Ki67 (A047; Dako A/S) was diluted 1:200. Paraffin sections were pretreated with trypsin (10 mg/ml) (HCR) or antigen retrieval (Ki67). Ki67 immunohistochemistry was performed on sections serial to those used for HCR. Both diaminobenzidine and 3-amino-9-ethylcarbazole were used as chromogenic substrates. The tissues were counterstained with haematoxylin. Controls were performed with preimmune serum.

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