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A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups

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Psoriasis is a chronic skin disorder with multifactorial aetiology. Genome-wide scans have provided unambiguous evidence for a major disease susceptibility locus on chromosome 6p21 (PSORS1). A minimal PSORS1 interval has been defined which encompasses three genes (*HLA-C*, *HCR* and *CDSN*) carrying psoriasis-associated SNPs. On the basis of this genetic evidence, we have undertaken an assessment of *CDSN* allele functional impact. A comparison of CDSN intragenic haplotypes showed that SNPs exclusive to disease-associated chromosomes are located in regions implicated in the stabilization of RNA transcripts. As CDSN is over-expressed in psoriatic lesions, we hypothesised that disease-associated intragenic SNPs may alter the rate of its mRNA decay. Here, we demonstrate that mRNAs transcribed from a CDSN risk haplotype present a 2-fold increase in stability, compared with those transcribed from a neutral haplotype (*t*-test $P = 0.004$). Site-directed mutagenesis revealed that a single synonymous SNP (CDSN*971T) accounts for the observed increase in RNA stability. CDSN*971T maps to a RNA stability motif and UV cross-linking analysis demonstrated that the SNP affects the transcript affinity for a 39 kDa RNA binding protein. Association analyses show that haplotypes bearing CDSN*971T confer psoriasis susceptibility in a wide range of ethnic groups. These results demonstrate the effect of synonymous variation upon allele specific gene expression, a finding of relevance to future studies of the pathogenesis of common and complex traits.

INTRODUCTION

Psoriasis is an inflammatory skin disorder characterized by disruption of keratinocyte terminal differentiation and premature desquamation of the stratum corneum (1). The disease is inherited as a multifactorial trait, with at least nine disease susceptibility loci (PSORS1-9) identified by independent genome-wide scans (2,3). Of these, the PSORS1 region on chromosome 6p21 may account for up to 50% of psoriasis familial clustering (2,3). Linkage disequilibrium (LD) based fine mapping of PSORS1 has highlighted a consensus minimal interval, spanning 200 kb of the major histocompatibility complex (MHC) class I region (4–7). Genetic analyses of the eight transcripts contained within the refined PSORS1 interval (reviewed in 8) have identified psoriasis-associated SNPs in the HLA-C (HLA-Cw6 allele), α-helix coiled coil rod homolog (*HCR*WWCC*) and corneodesmosin (*CDSN*TTC*) genes (9–12).

The *CDSN* gene encodes the only PSORS1 transcript to be specifically expressed in terminally differentiated keratinocytes (13,14). Its product, the adhesive protein corneodesmosin, localizes to the modified desmosomes that ensure the intercellular cohesion of keratinocytes (13,14). The serine and glycine rich terminal domains of corneodesmosin are essential for cell adhesion and are sequentially cleaved during the process of skin desquamation (13,14). Thus CDSN

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represents an attractive positional and biological candidate for psoriasis susceptibility.

Genetic analyses have identified a cluster of three intragenic CDSN haplotypes showing significant disease association in diverse ethnic groups (15–18). These findings have been validated in both case–control (15,16,18) and family-based datasets (17), yet their interpretation remains controversial, due, at least in part, to the confounding effect of LD conservation across the MHC (8). In this context, CDSN associations have often been ascribed to LD with HLA-C alleles (16,18), which have been more frequently analysed as markers of disease risk (9–12).

We have previously generated a dense SNP map (1 SNP per 3.5 kb) of the PSORS1 interval that we used as a framework to derive ancestral risk haplotypes segregating in populations of north-European descent (7,17). The analysis of 59 SNPs spanning the 200 kb PSORS1 minimal interval identified two distinct association peaks mapping to the HLA-C and CDSN genomic regions (7). Haplotype analysis revealed a cluster of rare risk chromosomes likely to have originated from recombination events which had removed HCR*WWCC, whilst preserving HLA-Cw6 and CDSN*TTC on either side (7,17). The occurrence of these putative recombinant risk chromosomes was also confirmed in a psoriatic population of Indian descent (7,17). Altogether, these observations argue against the hypothesis that association at the CDSN locus is due only to LD with HLA-Cw6 and suggests that both HLA-C and CDSN alleles may contribute to psoriasis susceptibility.

To further explore this hypothesis, we have undertaken an investigation of the functional impact of CDSN alleles. CDSN is an extremely polymorphic gene, with a SNP density approaching 1:100 bp (17,19). Coding polymorphisms cause both synonymous and non-synonymous substitutions, as well as deletions of single amino-acids (Table 1; SNP identifiers are reported in Supplementary Material, Table S1). SNPs have also been identified within the putative gene promoter, the single intron and the UTR regions (Table 1). However, the alignment of CDSN common haplotypes (i.e. those accounting for >2% of patient chromosomes) shows that only three SNPs (highlighted in bold in Table 1) are unique to over-transmitted/disease-associated chromosomes. Indeed, the alleles at SNPs 9, 971 and 1675 are shared by risk haplotypes 1.11, 1.21 and 1.41, whereas they are not observed on the under-transmitted/neutral chromosomes designated as 1.52, 2.11 and 2.21 (Table 1). Although none of the these SNPs introduces an amino acid change, two (SNPs 971 and 1675) map to gene regions (i.e. the coding sequence and the 3′-UTR) where the occurrence of SNPs has previously been associated with abnormal RNA stability (20,21). As corneodesmosin is up-regulated in the skin lesions of psoriatic patients (22,23), we hypothesised that CDSN risk alleles may contribute to the gene over-expression by altering the rate of mRNA decay. Using a combination of transfection-based RNA stability assays, site-directed mutagenesis and UV cross-linking, we were able to show that the synonymous SNP CDSN*971T confers altered transcript stability through a diminished affinity for a cytoplasmic RNA binding protein.

Table 1. Alignment of CDSN common haplotypes[a]

Status[b]	Haplo[c]	−690	−636	−461	−31	−22	**9**	75i	2450i	2729i	137	206	442	460	614	619	722	767	902	**971**	1118	1215	1236	1243	1331	1358	1593	1606	**1675**	1740	1748	Origin of population
													S/N	S/...		F/S						S/G	S/A	S/L			D/N					
Over-transmitted	**1.11**	c	g	c	a	c	c	g	g	t	c	c	g	gaa	a	t	t	g	c	t	g	a	t	c	g	t	g	...	g	c	c	UK (17), Gujarat (17), Sardinia (15)
Over-transmitted	**1.21**	c	g	c	a	c	c	g	g	t	c	c	g	gaa	a	t	t	g	c	t	g	g	t	c	g	t	g	...	g	c	c	UK (17), Thailand (18)
Disease-associated	**1.41**	c	g	c	a	c	c	g	a	t	c	c	g	gaa	a	c	t	g	t	t	g	a	t	c	g	t	g	...	g	c	c	Japan (16)
Neutral	1.52	t	g	t	a	c	t	g	g	t	c	c	g	gaa	a	t	t	a	c	c	g	a	g	c	g	t	g	...	t	c	c	
Under-transmitted	2.11	t	g	t	g	c	t	g	g	g	c	c	g	...	a	t	c	g	c	c	a	a	t	t	c	c	g	aag	t	t	t	
Neutral	2.21	c	a	c	a	t	t	a	g	g	t	t	a	gaa	g	t	c	g	c	c	a	a	t	t	c	t	a	aag	t	t	t	
		Promoter					5′-UTR	Intron			Coding region																	3′-UTR			

[a]CDSN intragenic haplotypes have been derived by sequencing of long-PCR products (19) and validated by segregation analysis of three-generation pedigrees (17).
[b]The terms 'over-transmitted' and 'disease-associated' refer to haplotypes showing disease association in family-based and case-control studies, respectively.
[c]Haplotype numbers are given according to the classification proposed by Guerrin *et al.* (19).

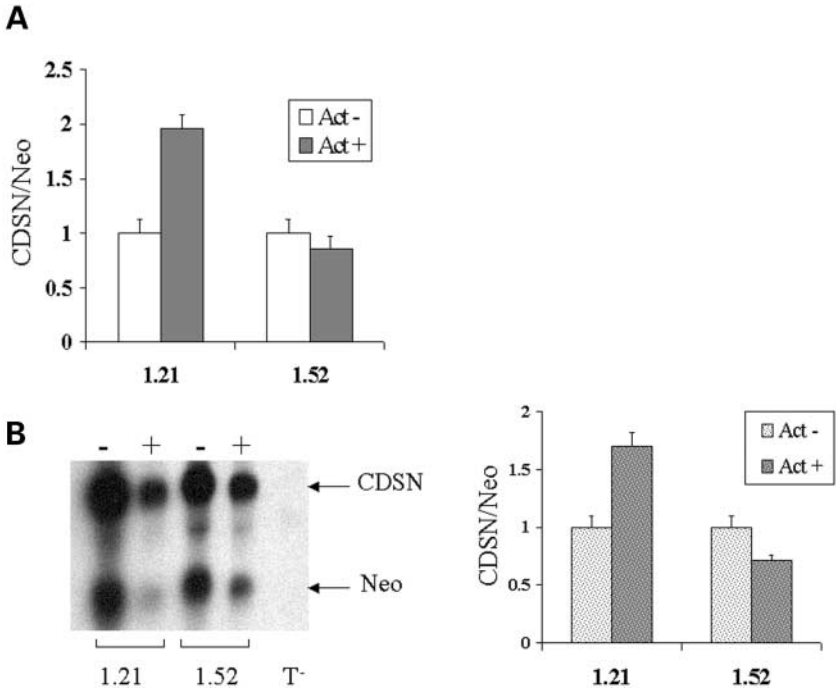

Figure 1. Risk haplotype 1.21 shows altered RNA stability. (**A**) Relative stability of 1.21 and 1.52 transcripts, as determined by semi-quantitative RT–PCR analysis of two independent transfection experiments. The basal CDSN/Neo value was set as 1 and the relative ratio after actinomycin treatment is shown. The rise in the CDSN/Neo ratio observed for construct 1.21 following actinomycin treatment demonstrates that the transcript is more stable than the Neo mRNA. (**B**) Northern blot analysis of two further transfection experiments. A representative blot is shown on the left, with − and + symbols, respectively, denoting RNAs extracted before and after treatment with Actinomycin D. T^- indicates RNA extracted from non-transfected cells. The mean CDSN/Neo values obtained following to densitometric analysis of the two blots are plotted on the right.

RESULTS

CDSN risk haplotype 1.21 shows altered RNA stability

In order to establish the impact of psoriasis-associated SNPs on CDSN transcript stability, we compared the decay rate of RNAs transcribed from haplotypes 1.21 (risk bearing) and 1.52 (neutral). To assess transcript stability, we transfected COS7 cell lines with the relevant cDNA clones and quantified CDSN mRNAs before and after incubation with an inhibitor of transcription (i.e. actinomycin D). Figure 1A shows the results that were obtained by semi-quantitative RT–PCR of CDSN transcripts, using the Neo RNA (which is also transcribed from the transfection vector) as an internal control. The analysis of two independent transfection experiments indicated that the relative stability of 1.21 transcripts was almost 2-fold greater than that of Neo mRNAs (1.95 × Neo), whereas the relative stability of 1.52 transcripts was only 0.86 × Neo (unpaired *t*-test two-tailed $P = 0.004$). These results were confirmed by northern blot analysis of RNAs from two further transfection experiments (Fig. 1B).

CDSN*971T is necessary and sufficient to confer altered mRNA stability

We next sought to identify the CDSN variant(s) accounting for the observed increase in 1.21 mRNA stability. Our constructs encompassed both the CDSN coding region and the 3′-UTR (nt 15–1986), so that the transfected 1.21 and 1.52 cDNAs were carrying divergent alleles at both nucleotides 971 and 1675 (nucleotide 9 maps to the 5′-UTR, hence it was not included in our constructs and could not have accounted for the altered RNA stability). To investigate the impact of these two SNPs, we used site-directed mutagenesis to modify the corresponding nucleotides in 1.21 cDNAs. Figure 2A shows the results that were obtained by semi-quantitative RT–PCR analysis of these modified transcripts. The relative stability of 1.21 mRNAs was not affected by the substitution of nucleotide 1675. In contrast, the mutagenesis of nucleotide 971 had a dramatic effect, reducing the relative mRNA stability by >50% (1.21 stability: 1.95 × Neo; 971mut stability: 0.68 × Neo; two-tailed $P = 0.0008$). These results were confirmed by northern blot analysis of RNAs from two further independent transfection experiments (Fig. 2B). The 1.21 haplotypes carrying both the 971 and the 1675 substitution, did not display any further decrease in RNA stability (data not shown).

In order to assess whether RNAs transcribed from haplotypes bearing CDSN*971T present an increase in their steady-state levels *in vivo*, we examined the endogenous CDSN mRNAs from the non-tumorigenic HaCaT keratinocyte cell line. Figure 3 shows the results of semi-quantitative PCR carried out on HaCaT genomic DNA and cDNA. The cell line is heterozygous for SNP 971 and both alleles are transcribed as shown by *Hae* III restriction analysis. Densitometric analysis of duplicated experiments revealed that haplotypes bearing 971T account for only 17% of CDSN genomic

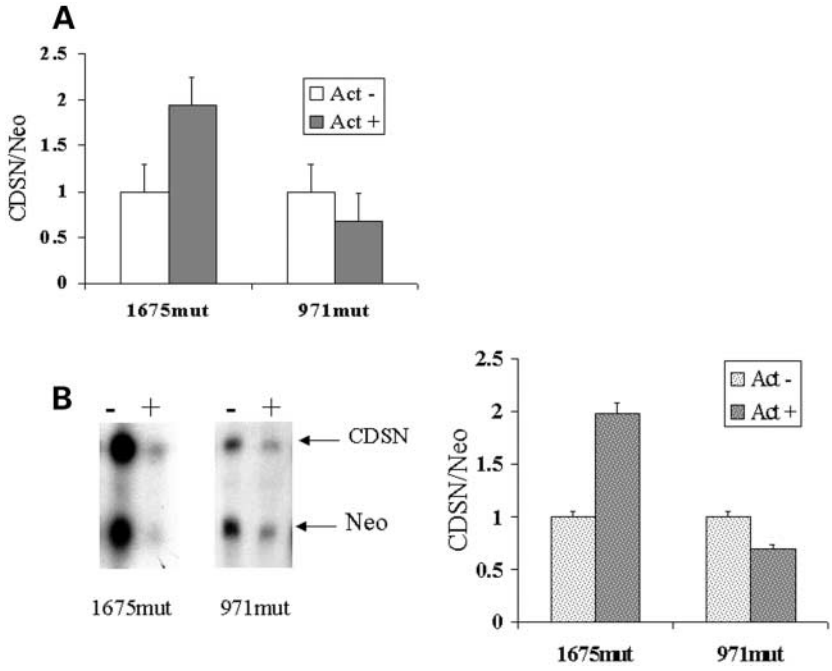

Figure 2. CDSN*971T accounts for the increase in RNA stability. (**A**) Relative stability of 1.21 transcripts bearing mutations of nucleotides 971 and 1675, as determined by semi-quantitative RT–PCR analysis of two independent transfection experiments. The basal CDSN/Neo value was set as 1 and the relative ratio after actinomycin treatment is shown. The rise in the CDSN/Neo ratio observed for construct 1675mut, following actinomycin treatment, demonstrates that the transcript is more stable than the Neo mRNA. (**B**) Northern blot analysis of two further transfection experiments. A representative blot is shown on the left, with − and + symbols, respectively, denoting RNAs extracted before and after treatment with actinomycin D. The mean CDSN/Neo values obtained following densitometric analysis of the two blots are plotted on the right.

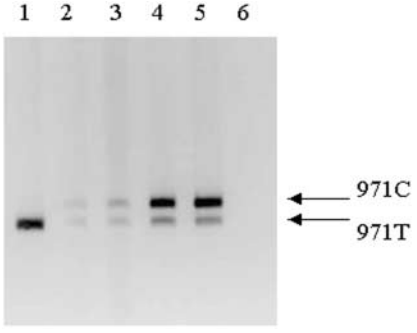

Figure 3. Semi-quantitative RT–PCR analysis of HaCaT endogenous CDSN transcripts. cDNAs bearing CDSN*971T were identified by *Hae* III restriction of RT–PCR products. 1, restriction control; 2 and 3, HaCaT cDNA; 4 and 5, HaCaT genomic DNA; 6, RT^-.

DNA, but represent 36% of the cDNA [the deviation from the 1:1 allele ratio in genomic DNA is due to the co-existence of several chromosome populations within the cell line (24)].

CDSN*971T decreases the affinity for a cytoplasmic RNA binding protein

To explore the mechanisms whereby CDSN*971T influences RNA decay, we compared the sequence surrounding the SNP with that of known stability determinants lying within coding regions (25). The alignment illustrated in Figure 4A shows that the sequence immediately upstream SNP971 closely matches the consensus for the mRNA stability/instability motifs that overlap the binding sites for cytoplasmic RNA binding proteins (25). To assess whether CDSN*971T affects the interaction with any such protein, we carried out UV cross-linking analysis to compare the efficiency with which cytoplasmic HaCaT proteins bind to RNA probes carrying different SNP971 alleles. Figure 4B shows that both CDSN alleles bind a cytoplasmic protein with an approximate molecular weight of 39kDa. Figure 4C illustrates an autoradiograph representative of two independent competition assays where increasing amounts of either unlabelled probe were added to the UV cross-linking reaction containing labelled 971T RNAs. These experiments showed that unlabelled RNAs carrying allele 971C compete with the radioactive probe more efficiently than those carrying allele 971T (Fig. 4C). The same results were obtained in two further competition assays where 971C RNA was used as the labelled probe (data not shown). Taken together, these observations suggest that allele 971T has a diminished affinity for the 39 kDa RNA-binding protein.

CDSN*971T displays highly significant disease association on diverse HLA-C backgrounds

As SNP971 does not introduce any amino acid substitution, it has not been investigated as extensively as CDSN non-conservative variants. To characterize the association at this locus, we have, therefore examined a dataset including 171 cases and 90 controls. χ^2 analysis confirmed that CDSN*971T is

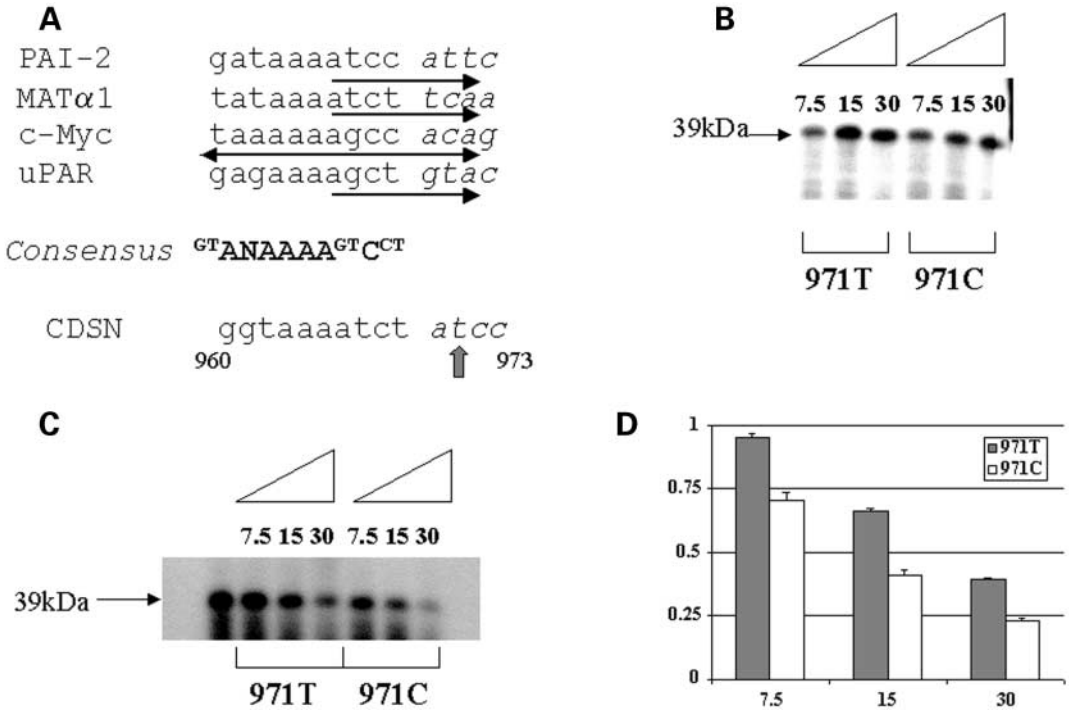

Figure 4. CDSN*971T affects the interaction with a RNA binding protein. (**A**) Alignment of CDSN nucleotides 960–973 with the sequences of previously characterized mRNA stability/instability motifs. Horizontal arrows indicate the localization of the cytoplasmic protein binding sites which overlap with the stability motifs. The grey vertical arrow indicates the position of nucleotide 971. (**B**) UV cross-linking analysis of RNA probes carrying either 971T or 971C alleles. The figures above the lane indicate amounts (μg) of cytoplasmic cell extract that were incubated with the probe. (**C**) UV cross-linking competition assay using unlabelled RNA probes carrying either 971C or 971T alleles. The figures above the lanes indicate the fold excess of unlabelled probe. (**D**) The results obtained following densitometric analysis of two independent experiment are plotted on the right, with the intensity of the complex in the first lane (no competitor added) set as 1.

Table 2. CDSN haplotypes bearing CDSN*971T are associated with psoriasis on the background of distinct MHC ancestral chromosomes[a]

Sample origin	Dataset	Associated CDSN allele (statistical significance)	Ancestral MHC haplotype (HLA-C allele)
UK (17)	171 Trios	1.11 ($P < 10^{-10}$); 1.21 ($P = 0.004$)	57.1 (Cw6); 13.1 (Cw6)
Gujarat (17)	30 Trios	1.11 ($P = 0.004$)	57.1 (Cw6)
Thailand (18)	139 Cases, 144 Controls	1.21 ($P = 9.2 \times 10^{-5}$)	13.1 (Cw6); 46.1 (Cw1)
Sardinia (15)	147 Cases, 120 Controls	1.11 ($P = 0.004$)	57.1 (Cw6); 58.1 (Cw7)
Japan (16)	101 Cases, 166 controls	1.41 ($P = 0.004$)	46.2 (Cw1)

[a]Numbers of MHC ancestral haplotypes are given according to the classification proposed by Degli-Esposti *et al.* (32).

significantly associated with psoriasis ($P = 5.3 \times 10^{-6}$), yielding an odd ratio of 2.52 (95% CI: $1.68 < \text{OR} < 3.78$). To investigate the relationship between CDSN*971T and HLA-Cw6 associations, we reviewed published studies of CDSN and HLA-C alleles. As shown in Table 2, CDSN*971T can be found on ancestral MHC chromosomes that carry non-Cw6 HLA-C alleles.

DISCUSSION

Associations between CDSN alleles and psoriasis susceptibility have been repeatedly described (reviewed in 8), however, their interpretation has been compounded by the high polymorphism content of the gene (i.e. different SNPs have often been examined by different groups) and the proximity to HLA-Cw6. We have previously generated genetic data indicating that CDSN association may not be secondary to LD with HLA-Cw6 (7,17). Here, we provide the first evidence for a functional impact of a psoriasis-associated CDSN allele. Using transfection based stability assays and site-directed mutagenesis, we were able to show that CDSN*971T confers abnormal RNA stability to CDSN transcripts. We also observed differential over-expression of haplotypes bearing CDSN*971T in the spontaneously immortalized HaCaT keratinocyte cell line. Despite having a destabilized karyotype that generates variant cell populations (24), HaCaT lines closely resemble normal keratinocytes in their growth and differentiation characteristics (24,26), so that they are widely used as model system for the study of this

cell type. Conversely, the amount of skin that is sampled in biopsy procedures would not allow an analysis of CDSN transcripts, given the low expression levels of the gene in normal keratinocytes (22,23).

The sequence immediately upstream of SNP971 closely matches the consensus for the stability/instability motifs, which overlap with the binding sites for cytoplasmic proteins involved in RNA metabolism (25). Our UV cross-linking analysis indicated that the CDSN*971T affects the rate of RNA decay by decreasing the affinity for a 39 kDa cytoplasmic protein. This effect is reminiscent of that obtained through the disruption of the PAI-2 mRNA instability motif, which results in a significant increase in transcript stability (25).

A recent study has shown that up to 50% of human genes with coding SNPs can present allelic variation in gene expression (27). In this context, the alteration of RNA instability motifs may well prove to be a more general phenomenon than previously thought.

We have previously generated evidence indicating that association at CDSN may not be accounted for by LD with HLA-Cw6. Our review of recent CDSN genetic studies provides further support for this observation, as it shows that HLA-C alleles other than Cw6 are found on psoriasis-associated chromosomes bearing CDSN*971T. Remarkably, CDSN haplotypes carrying CDSN*971T have been found to be associated with psoriasis in a very diverse range of populations (15–18,28). Indeed, a retrospective analysis of the published literature shows that negative association findings have only been obtained (12,29) when the number of SNP that were typed was not sufficient to differentiate risk haplotypes. Such a degree of consistency is uncommon in the study of complex traits and suggests a key role of CDSN variation in the pathogenesis of psoriasis.

Our investigation was limited to the gene coding region and 3′-UTR, so that we cannot exclude the possibility that regulatory SNPs other than CDSN*971T may also contribute to corneodesmosin up-regulation in patients' skin. Likewise, amino acid substitutions encoded by non-synonymous SNPs might also participate to the disease pathogenesis, once the protein has been translated. Thus, our results add to the type of genetic variation underlying common diseases and warrant further investigations of CDSN role in the molecular pathogenesis of psoriasis.

MATERIALS AND METHODS

Generation of constructs

The constructs used for transfection-based assays encompassed both the CDSN coding region and the 3′-UTR (nucleotide 15–1986). Primers *Eco*-CDf (GAATTCATGGGCTCG TCTCGGGCAC) and *Not*-CDr (GCGGCCGCGGCGTCAG AGGTGCTCTGAG) were used to amplify CDSN haplotypes 1.21 and 1.52 from the cDNA of an heterozygous individual (the skin biopsy used as a RNA source was obtained with patient's informed consent). The resulting 1.21 and 1.52 PCR products were then cloned into a pCI-Neo mammalian expression vector (Promega). Constructs with substitutions at nucleotides 971 and/or 1675 were generated by using the QuikChange site directed mutagenesis kit (Stratagene) to modify the sequence of 1.21 clones. Constructs used for *in vitro* transcription were generated by amplifying a 300 bp portion of the *CDSN* gene (nucleotide 831–1131; primers *Eco*-831F: GAATTCTGTAGCAATGGTGGCCTTCC and *Not*-831R: GCGGCCGCCACTGGCTGGAACGCAATGG) from the same heterozygous individual and by cloning the resulting PCR products in a pCI-Neo vector. The accuracy of all cloning and mutagenesis procedures was verified by direct sequencing of constructs.

RNA stability assay

Cell cultures and transfection experiments. COS7 cells were grown in Dulbecco's modified Eagle's medium (DMEM), (Gibco), supplemented with 10% fetal calf serum (FCS) (Gibco), 50 U/ml penicillin and 50 μg/ml streptomycin (Gibco). Cells were transfected with CDSN constructs, using the FuGENE6 Reagent (Roche) at a reagent to DNA ratio of 3:1. Forty-eight hours after transfection, 5 μg/ml actinomycin D (Sigma) was added to the medium, and cells were grown for further 24 h, before being harvested. All transfection experiments were carried out in quadruplicate.

Quantification of transcripts. The RNeasy mini kit (Qiagen) was used to extract total RNA from transfected cells, before and after incubation with actinomycin D. Semi-quantitative RT–PCR was used to analyse two of the four sets of paired RNAs that were obtained for each transfected construct. Following to DNase I (Invitrogen) treatment and reverse transcription, serial cDNA dilutions were amplified for 23 cycles using *Eco*-CDf (see earlier-mentioned sequence) and CD2p2 (GGTTTAGTATTCCGCGGTAAG) primers. *Neo*-F (CTGGGCACAACAGACAATCG) and *Neo*-R (AAGGCGA TAGAAGGCGATGC) primers were used to amplify the neomycin cDNA, which was utilized as an internal control. Following to electrophoresis on a 2% agarose gel, images were acquired on a Gene Genius Bio-Imaging System (Syngene) and PCR products were quantified using the Image J software (available at http://rsb.info.nih.gov/ij). The third and fourth sets of paired RNAs were quantified by northern blot analysis. Filters prepared according to standard protocols were sequentially probed with CDSN and Neo PCR products, respectively, obtained using the *Eco*-CDf/CD2p2 (1.21 and 1.52 alleles share the same sequence along the amplified genomic segment) and *Neo*-F/-*Neo*-R primer pairs. Following autoradiography on a Cyclone Phosphor Screen (Packard), images were acquired and analysed using a Cyclone Storage Phosphor System (Packard).

Analysis of HaCaT endogenous CDSN transcripts

HaCaT cells were grown in DMEM, supplemented with 10% FCS, 50 U/ml penicillin and 50 μg/ml streptomycin. Following to RNA extraction, 3 μg of total RNA was reverse transcribed and the 3′-UTR of CDSN was amplified using the CD2p9 (GCATGCCTTGGGCACAAACAAGC) and *Not*-CDr (see earlier-mentioned sequence) primers. RT–PCR product was digested with *Hae* III, in order to identify the G allele of SNP1675, which defines all haplotypes bearing CDSN*971T.

UV cross-linking analysis

In-vitro transcription. In order to generate DNA templates for *in vitro* transcription, the pCI-Neo vectors containing the 831–1131 region of alleles 1.21 and 1.51 were linearized by *Not* I restriction. Labelled probes and unlabelled RNA competitors were then obtained by using the Riboprobe *in vitro* Transcription System (Promega) to incorporate [α-^{32}P]rUTP (Amersham) and rUTP, respectively. Following phenol/chloroform purification, labelled probes and unlabelled competitors were, respectively, quantified by Cherenkov scintillation and ethidium bromide staining of agarose/formaldehyde gels.

Preparation of cytoplasmic cell extracts. HaCaT cells harvested at 90% confluence, washed in PBS and incubated for 5 min with 75 μl per 10^6 cells cytoplasm extraction buffer (10 mM HEPES pH 7.1; 3 mM MgCl; 14 mM KCl; 0.2% Nonidet P-40; 1 mM DTT; 0.5 mM PMSF; 1 × Roche Complete Mini Proteinase Inhibitor Cocktail). Following 1 min centrifugation at 1000 G, the supernatant was collected and the protein content was determined using the Biorad Protein Assay (Biorad).

UV-cross-linking. Cytoplasmic extracts were incubated with 5 μg/μl heparin for 10 min at room temperature, prior to the addition of 2 pmol (3×10^5 cpm) of labelled probe. For competition assays, 15–60 pmol of unlabelled probe (7.5–30-fold excess) were also added. After 30 min incubation at room temperature, samples were UV cross-linked for 15 min in a UVC 500 crosslinker (Amersham), digested with 200 μg/ml RNase A (Roche) for 20 min at 37°C, and separated on a 10% SDS–PAGE gel. Labelled RNA protein complexes were detected and quantified, using a Cyclone Phosphor Storage System.

Association analysis

One hundred and seventy one UK patients were sampled from a psoriasis family cohort that has been described elsewhere (7). Samples from 90 unrelated healthy controls were obtained from the DNA bank of the Tissue Typing lab, Guys Hospital, UK. Ethical approval and informed consent were obtained from all patients and controls included in this study. Samples were genotyped for the CDSN*971 SNP by hybridization of allele specific oligonucleotides (sequences of probes and PCR primers are reported in Supplementary Material Table S2), as described elsewhere (7). χ^2 values and odd ratio 95% confidence intervals were computed using the SISA software available at http://home.clara.net/sisa/twoby2.htm.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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