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Protective Effect of Human Endogenous Retrovirus K dUTPase Variants on Psoriasis Susceptibility

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Abstract

Previous genetic and functional studies have implicated the human endogenous retrovirus K (HERV-K) dUTPase located within the PSORS1 locus in the MHC region as a candidate psoriasis gene. Here, we describe a variant discovery and case-control association study of HERV-K dUTPase variants in 708 psoriasis cases and 349 healthy controls. Five common HERV-K dUTPase variants were found to be highly associated with psoriasis, with the strongest association occurring at the missense SNP rs3134774 (K158R, $p=3.28 \times 10^{-15}$, OR=2.36 [1.91-2.92]). After adjusting the association of the HERV-K dUTPase variants for the potential confounding effects of HLA alleles associated with psoriasis, the HERV-K SNPs rs9264082 and rs3134774 remained

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significantly associated. Haplotype analysis revealed that HERV-K haplotypes containing the non-risk alleles for rs3134774 and rs9264082 significantly reduced the risk of psoriasis. Functional testing showed higher antibody responses against recombinant HERV-K dUTPase in psoriasis patients compared to controls ($p<0.05$), as well as higher T-cell responses against a single HERV-K dUTPase peptide ($p<0.05$). Our data support an independent role for the HERV-K dUTPase on psoriasis susceptibility, and suggest the need for additional studies to clarify the role of this dUTPase in the pathogenesis of psoriasis.

Introduction

Psoriasis is an inflammatory, immune-mediated disorder of the skin, joints, and nails that affects approximately 3% of the U.S. population (Kurd and Gelfand, 2009). Significant morbidity is associated with psoriasis due to its chronic, debilitating nature and its established link to other systemic inflammatory diseases (Kim *et al*., 2010). Although the risk of developing psoriasis is known to be influenced by multiple genetic loci, the strongest genetic effect appears to reside within the MHC region on chromosome 6, a locus known as PSORS1. Historical efforts to fine map the causative variant(s) within PSORS1 were made difficult due to the extensive linkage disequilibrium within the region. Several studies localized PSORS1 to an approximately 300 kb region between HLA-B and HCG22, with varying boundaries identified between studies (Fan *et al*., 2008; Lench *et al*., 2005; Martinez-Borra *et al*., 2005; Nair *et al*., 2006; Oka *et al*., 1999; Orru *et al*., 2005; Veal *et al*., 2002). From this region, the HLA-C*06:02 allele has been identified as the variant most strongly associated with psoriasis (Nair *et al*., 2006). Other HLA alleles that have been associated with psoriasis or psoriatic arthritis are *HLA-C*12, B*27, B*38, B*39, B*40*, and *B*57* (Feng *et al*., 2009; Helms *et al*., 2005; Rahman *et al*., 2011). Whether additional non-HLA variants within PSORS1 may contribute to psoriasis susceptibility is still unclear.

We were interested in exploring whether the PSORS1 human endogenous retrovirus K (HERV-K) dUTPase, located 32 kb telomeric of HLA-C (Figure 1), might be independently associated with psoriasis. A previous analysis of several hundred psoriasis families identified a strong psoriasis risk haplotype, termed RH1, which localized to a 60 kb region telomeric to HLA-C (Nair *et al*., 2000) and which harbors the HERV-K dUTPase. A subsequent analysis of the same dataset using a haplotype sharing statistic demonstrated that the HERV-K dUTPase may be a candidate susceptibility gene for psoriasis (Foerster *et al*., 2005). Most recently, HERV-K dUTPase protein has been shown to stimulate the production of psoriasis-associated Th1/Th17 cytokines in immune cells through interaction with Toll-like receptor 2 (Ariza and Williams, 2011).

Human endogenous retroviruses (HERVs) are retroviruses that have historically infected human populations and have permanently integrated into the human genome via the infection of germline cells. These retroviral sequences are thereafter vertically transmitted from generation to generation. Although most HERV sequences have become defunct through the accumulation of mutations, HERV sequences possessing an intact open reading frame can lead to the transcription and translation of viral proteins. The HERV-K family of HERVs represents the historically most recent addition to the human genome and many

HERV-K proviruses contain intact open reading frames (Turner *et al*., 2001). The HERV-K dUTPase at PSORS1 occurs within an open-reading frame and mRNA transcripts of the dUTPase are detectable in the skin and peripheral blood mononuclear cells of both psoriasis patients and healthy controls (Foerster *et al*., 2005).

HERVs, which constitute a large portion (~8%) of the human genome (Lander *et al*., 2001), have been implicated in the development of psoriasis and several other autoimmune diseases, though no definitive relationship has been demonstrated. Retroviral-like particles have been detected in the urine and skin of psoriasis patients (Dalen *et al*., 1983; Iversen, 1983) and over-expression of HERV-E envelope glycoprotein (Bessis *et al*., 2004) and HERV-W RNA transcripts (Moles *et al*., 2005) were found in psoriatic skin lesions compared to control skin. HERVs have also been associated with autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, alopecia areata, scleroderma, and multiple sclerosis (Banki *et al*., 1992; La Placa *et al*., 2004; Moyes *et al*., 2005; Rebora, 2005; Reynier *et al*., 2009; Urnovitz and Murphy, 1996). The proposed mechanisms by which HERVs may trigger autoimmune diseases include HERV encoding of autoantigens, molecular mimicry between HERV and other self antigens, HERV superantigens, the insertion of HERV sequences near immune genes affecting gene regulation, and the activation of innate immunity through pattern recognition receptors.

Considering the genomic localization of the HERV-K dUTPase adjacent to HLA-C within PSORS1 and the functional evidence of the ability of the dUTPase to stimulate production of inflammatory cytokines in immune cells, we here used a case-control approach to investigate the hypothesis that the HERV-K dUTPase may be associated with psoriasis independent of known HLA-C and HLA-B associations. We also examined whether psoriasis patients generate B-cell and T-cell immune responses against HERV-K dUTPase recombinant protein and peptides, respectively.

Results

HERV-K dUTPase Variants at PSORS1 are Strongly Associated with Psoriasis

We sequenced a 431 bp genomic fragment of the PSORS1 HERV-K dUTPase in 708 Caucasian psoriasis cases and 349 Caucasian controls. Previous sequencing of a larger 4.2 kb region around the dUTPase showed that the 431 bp fragment contained the variants associated with high-risk and low-risk psoriasis haplotypes (Foerster *et al*., 2005). The 431 bp fragment encompasses the C-terminal portion of the dUTPase protein starting at consensus amino acid 108 and includes dUTPase domains 3, 4, and 5. We identified a total of 14 DNA variants, 9 of which were novel and rare (MAF<5%) (Table 1). We then conducted association testing of the identified HERV variants with psoriasis using logistic regression, adjusting the results for potential population stratification using a panel of 87 European ancestry informative markers. We found that all five common (MAF>5%) HERV-K dUTPase polymorphisms were highly associated with psoriasis ($p < 10^{-4}$, exceeding the Bonferroni threshold of 0.05/14=0.0036, Table 1). The HERV-K SNP most highly associated with psoriasis was rs3134774, an A/G polymorphism corresponding to the non-synonymous change K158R ($p=3.28 \times 10^{-15}$, OR=2.36). rs3134774 was in nearly perfect linkage disequilibrium (LD) with the non-coding SNP rs3134775 ($r^2=0.99$ in our dataset)

and in high LD with the non-coding SNP rs3132531 (r^2=0.83) (Figure 2). The synonymous HERV-K SNP rs9264082, which was in low LD with rs3134774 (r^2=0.16), was associated with psoriasis protection ($p=1.68 \times 10^{-9}$, OR=0.50), as was the non-synonymous SNP rs114780460 ($p=2.98 \times 10^{-5}$, OR=0.59). Although none of the nine rare HERV-K variants (MAF<5%) demonstrated statistical association with psoriasis, as would be expected from power limitations, we did note that rare variant NOVEL8 encoding the non-synonymous change D166G was seen in 4 psoriasis cases and none of the controls (Table 1).

We also imputed the HLA-C*06:02 genotypes of all cases and controls using a set of 4 tag SNPs and the accuracy of the imputation was empirically validated (see Materials and Methods). None of the HERV-K SNPs were as strongly associated with psoriasis as HLA-C*06:02 ($p=1.39 \times 10^{-22}$, OR=4.45, Table 1). Indeed, stepwise conditioning of the HLA-C*06:02 association signal on the top HERV-K SNP rs3134774 followed by rs9264082 (the next most significant HERV SNP after all HERV SNPs were conditioned on rs3134774) resulted in a residual association signal for C*06:02 of $p=2.17 \times 10^{-13}$, OR=3.51. Since HLA-C*06:02 demonstrates the strongest overall association signal with psoriasis and its association cannot be fully explained by the effects of the HERV SNPs, we conclude that HLA-C*06:02 is still the most significant risk factor for psoriasis at PSORS1.

HERV-K dUTPase SNPs are Associated with Psoriasis Protection Independently of Known HLA Alleles

To evaluate whether the HERV associations with psoriasis were independent of HLA alleles previously reported to be associated with psoriasis or psoriatic arthritis (*HLA-C*06, C*12, B*27, B*38, B*39, B*40,* and *B*57*) (Feng *et al*., 2009; Helms *et al*., 2005; Rahman *et al*., 2011), we first examined the linkage disequilibrium pattern between the HERV SNPs and these HLA alleles in a subset of our psoriasis cases (n=256) for which direct HLA typing data were available. We found that C*06 and B*57 showed moderate LD with the HERV SNPs (max r^2=0.46 and 0.19, respectively) and thus among HLA alleles had the greatest likelihood of influencing HERV associations, whereas the other HLA alleles showed significantly lower LD (all r^2<0.09) and were not likely to influence HERV associations, despite some having high D' values (see Table S1 for full explanation). Therefore, in addition to our imputed *HLA-C*06:02* data, we also determined *HLA-B*57:01* in all cases and controls using the tag SNP rs2395029, which is in very high LD (r^2=1.0) with *HLA-B*57:01* in Caucasians of European descent (de Bakker *et al*., 2006). We conditioned the association signals of the five significant HERV SNPs on *HLA-C*06:02* and *HLA-B*57:01* using logistic regression (Table 1, rightmost columns). We found that several HERV-K SNPs showed independent associations with psoriasis. The strongest independent effect was from rs9264082 ($p=2.50 \times 10^{-4}$, OR=0.64) followed by rs3134774/rs3134775 (p=0.0020, OR=1.46 and p=0.0037, OR=1.43, respectively). All three of these HERV SNPs remained significant (q<0.05) after adjusting for multiple testing using the false discovery rate method (Benjamini and Hochberg, 1995). A trend toward independence was seen for rs3132531 (p=0.026, OR=1.33, q=0.092) and rs114780460 (p=0.051, OR=0.78, q=0.143).

To further elucidate the relationship of the HERV-K dUTPase SNPs with each other, we estimated the frequencies of haplotypes containing rs114780460, rs3134774, and rs9264082

and performed haplotype association testing, adjusting for the effects of *C*06:02* and *HLA-B*57:01* (Table 2). We did not include rs3134775 or rs3132531 in the haplotype analysis due to their high LD with rs3134774 (r^2=0.99 and r^2=0.83, respectively). We identified four haplotypes, H1 to H4, which accounted for greater than 99.5% of the total number of haplotypes in both cases and controls. We found that three haplotypes—H2, H3, and H4—were independently associated with psoriasis protection. All three haplotypes contained the non-risk allele A of rs3134774, whereas the presence of the protective allele G of rs9264082 in H3 and H4 further reduced psoriasis risk compared to H2. Our haplotype data demonstrate that haplotypes containing protective HERV alleles (allele A of rs3134774, allele G of rs9264082) can independently reduce the risk for psoriasis.

Evaluation of B cell immunity to HERV-K dUTPase protein

To determine whether HERV-K dUTPase protein elicits humoral responses, we tested sera from 23 psoriasis patients and 16 healthy donors for responses to recombinant wild-type HERV-K dUTPase. As shown in Figure 3, psoriasis subjects had a greater humoral response against HERV-K dUTPase compared to the healthy blood donors (p=0.0334, Mann-Whitney U test). No response was observed against vehicle control or against human dUTPase control protein even at the lowest dilution used (data not shown). Our data suggest that certain epitopes in the HERV-K dUTPase protein may be eliciting an immunological response in psoriasis patients. Future studies examining the specificity of the antibody response against the high-risk and low-risk dUTPase proteins as defined by our genetic analysis are warranted.

Evaluation of T cell immunity to HERV-K dUTPase peptides

To evaluate whether HERV-K dUTPase peptides might elicit T cell responses, we tested the peripheral blood mononuclear cells of 13 psoriasis patients and 11 healthy controls for ELISPOT interferon-gamma responses to peptides from the PSORS1 HERV-K dUTPase. Peptides were designed as overlapping 13-mer sequences spanning the region from amino acid 129 of the dUTPase (just beyond conserved domain 3) to the carboxy terminus of the dUTPase (Figure S1). Peptides were designed to contain either the wild-type dUTPase sequence or various combinations of the amino acid substitutions corresponding to rs114780460, rs3134774, or variant NOVEL8 identified in our study. Within the psoriasis samples, no overall differences were observed when comparing PBMC reactivity against the psoriasis risk-associated peptides versus the psoriasis protection-associated peptides, with only one psoriasis patient reacting against the risk-associated peptides (Table S2). However, interestingly, we identified a HERV-K dUTPase peptide (PGECIAQLLIVPY) which elicited reactivity in 5/13 (38.5%) of psoriasis patients versus 0/11 (0%) of controls (p=0.0473, Mann-Whitney U test) (Table S2). 2/5 responses were of moderate strength, with SFU/10^6 >200. This sequence appears to be specific for the PSORS1 dUTPase as it differs by 4 amino acids from the wild-type HERV-K dUTPase sequence (PGDRIAQLLLLPY, differences underlined) and protein BLAST alignment of PGECIAQLLIVPY against the human protein database did not reveal any other identical matches. This sequence also contains a number of potential epitopes based upon HLA allele motif scanning, including peptides restricted through HLA-C*06:02. Although requiring further investigation, this

suggests the possibility that immune reactivity against HERV-K dUTPase peptides might be involved in the pathogenesis of psoriasis.

Discussion

In this study, we took a candidate gene approach to evaluate whether a human endogenous retroviral K (HERV-K) dUTPase sequence in the MHC region is associated with psoriasis. Sequencing the dUTPase in 708 cases and 349 controls, we found that HERV-K dUTPase variants at PSORS1 are strongly associated with psoriasis but that this association was not as strong as the association of psoriasis with HLA-C*06:02. To determine whether dUTPase variants were associated with psoriasis independent of psoriasis-associated HLA alleles, we determined the LD between the HERV variants and these HLA alleles and identified HLA-C*06:02 and HLA-B*57:01 as the most likely potential confounders. We then performed association testing of HERV variants conditional on HLA-C*06:02 and HLA-B*57:01 and found that two variants within the dUTPase, rs9264082 and rs3134774, remained independently associated. The minor allele A of the missense SNP rs114780460 was also protective against psoriasis (OR=0.78), but its effect was not quite independent (p=0.051).

rs9264082 encodes the synonymous change L161L, whereas rs114780460 and rs3134774 encode missense SNPs corresponding to G155R and K158R substitutions, respectively. Our haplotype analysis shows that the haplotype 155G/158R, previously termed “high-risk” (Foerster *et al*., 2005), is the most common haplotype in psoriasis patients (Table 2). Foerster et al.’s “low-risk” haplotype 155R/158K corresponds to protective haplotype H3 in our analysis; however, the haplotype 155G/158K which was not reported by Foerster et al. also corresponds to protective haplotypes H2 and H4 (Table 2). Ariza and Williams studied whether the 155G/158R substitutions and the 155R/158K substitutions (the latter associated with psoriasis protection) influenced the ability of recombinant HERV-K dUTPase protein to stimulate cytokine responses when added to human primary cells (Ariza and Williams, 2011). In agreement with the genetic prediction from our data, recombinant protein containing 155G/158R showed a significantly increased ability to stimulate Th1 and Th17 cytokines compared to protection-associated 155R/158K recombinant protein in human primary dendritic cells. The authors also tested the effects of the single substitutions 158R and 158K which did not show the expected trend; however, in these two cases 158R and 158K were paired with 155K, a situation which is never observed at the PSORS1 HERV-K dUTPase in which amino acid position 155 can only harbor a G (Glycine) or R (Arginine). Indeed, it is important to note that the recombinant protein experiments conducted by Ariza and Williams employed as their baseline protein the wild-type HERV-K dUTPase sequence, which is present in many copies throughout the human genome but which differs in sequence slightly from the PSORS1 HERV-K dUTPase at the N-terminus and C-terminus. Therefore, interpretation of the functional effects of the G155R and K158R polymorphisms require additional studies which examine these changes within the context of the PSORS1 dUTPase specifically.

We tested whether psoriasis patients mounted humoral responses against the HERV-K dUTPase protein. Using an ELISA assay, we found higher antibody responses against wild-type HERV-K dUTPase in psoriasis patients compared to healthy controls. The response

was specific for HERV-K dUTPase, as human dUTPase did not elicit reactivity. Future work is needed to define the relevant reactive epitopes and to determine whether there is a differential antibody response to dUTPase protein containing lysine at position 158, corresponding to the protective genetic variant identified here. We also tested whether HERV-K dUTPase peptides could elicit immunologic responses from PBMCs of psoriasis patients and healthy controls. We found T cell responsivity to several of the peptides, with peptide P04 being recognized in 5/13 psoriasis patients, including 2 at moderate strength. This 13 amino acid sequence has several potential HLA anchor motifs, including that of HLA-C*06:02, amongst others. That our screen did not detect a differential response against the psoriasis-associated genetic variants does not rule out a potential functional role for these variants. For efficiency, we tested 13-mer peptides staggered by 5 amino acids, but this does not cover all possible peptide sequences. We also did not examine the possibility that skin residents immune cells might react to these peptides. In addition, it is possible that these genetic variants might be important for the secondary or tertiary structure of the dUTPase protein which could affect interaction with innate pattern receptors such as TLR-2, a mechanism which is not addressed with peptide experiments.

Apart from modulating immune response, endogenous retroviral dUTPase may harbor alternative psoriasis-relevant properties. Related dUTPases have been found to interact with peroxisome proliferator-activated receptor (PPAR) isoforms (Chu *et al*., 1996) and human dUTPase specifically with PPAR delta (Rual *et al*., 2005), previously implicated in psoriasis (Romanowska *et al*., 2010; Westergaard *et al*., 2001). In addition, human dUTPase was recently shown to act as an unconventional high-affinity DNA binding protein that interacts with specific target sequences independently of enzymatic function (Hu *et al*., 2009). It is quite possible that such functions are retained in truncated homologous HERV-K dUTPase fragments.

In conclusion, using a large case-control cohort, we have identified variants within the HERV-K dUTPase gene at PSORS1 which have an effect on psoriasis susceptibility independent of HLA loci known to be associated with psoriasis. Our functional studies preliminarily suggest that certain HERV-K dUTPase epitopes and peptides may elicit immunologic responses more frequently in psoriasis patients than in healthy controls. Given the extensive linkage disequilibrium within the MHC region, replication of our genetic findings in additional cohorts is warranted and further mapping studies are needed to evaluate the possibility that the independent HERV variants identified here are markers of other causal variants in the region. Nevertheless, this study advances the evidence that the HERV-K dUTPase at PSORS1 may be involved in the pathogenesis of psoriasis and suggests the importance of additional genetic and immunologic work to clarify the role of this dUTPase in psoriasis.

Material and Methods

Samples

DNA samples from 708 Caucasian psoriasis cases and 359 Caucasian healthy controls were collected from the University of California, San Francisco and Washington University, St. Louis. Psoriasis cases were 50.4% female and had a mean age of onset 24.6 yrs (SD 15.5).

Psoriasis serum and PBMC samples were collected from the University of California, San Francisco, and healthy control serum and PBMCs from volunteer donors at the Blood Centers of the Pacific. All subjects provided informed written informed consent for study participation under the approval of local institutional review boards. This study adheres to the principles of the Declaration of Helsinki.

HERV-K Sequencing and Identification of Variants

PCR was used to amplify a 431 bp segment of the HERV-K dUTPase at PSORS1 for sequencing. SNPs within this amplicon have previously been shown to be associated with high risk and low risk psoriasis haplotypes (Foerster *et al*., 2005). The forward primer used was: 5'-AAGTTTAAAAGGCATACAAATACATACAGGG-3'. The reverse primer used was: 5'-GGACCTTCACAATGCAAAATATAATGG-3'. These primers are specific for the PSORS1 HERV-K dUTPase as confirmed by BLAT analysis. The PCR conditions were: 95 °C for 2 min; 35 cycles of (92 °C for 10 sec, 58 °C for 20 sec, 72 °C for 1 min); 4 °C. PCR products were sequenced at a commercial facility (Quintara Biosciences, Berkeley, CA) using an ABI 3730xl. Polymorphisms were identified through a visual examination of the DNA sequencing chromatograms (Sequencher 4.0, Gene Codes Corporation, Ann Arbor, MI). All detected variants were successfully sequenced in greater than 95% of samples and no variants had a Hardy-Weinberg equilibrium p-value < 0.01. Amino acid numbering for the dUTPase protein was done in concordance with the previously published report by Ariza and Williams (Ariza and Williams, 2011).

HLA-C*06:02 and HLA-B*57:01 Genotype Imputation and Validation

The HLA-C*06:02 genotypes for cases and controls were imputed using a modification of de Bakker et al. (de Bakker *et al*., 2006) based on a combination of four SNPs that were in linkage disequilibrium with HLA-C*06:02. These four SNPs were genotyped using a combination of TaqMan assays (rs2894207, rs887466) and Illumina GoldenGate assays (rs1062470, rs10484554). The program SNPAnalyzer (Yoo *et al*., 2005) was used to generate haplotypes from these SNPs. HLA-C*06:02 corresponds to the haplotype CGTT for SNPs rs2894207, rs887466, rs1062470, and rs10484554, respectively. HLA-B*5701 was determined by genotyping all samples on the Illumina Goldengate platform for rs2395029, a tag SNP in high LD (r^2=1.0) with HLA-B*57:01 (de Bakker *et al*., 2006). The validity of the imputation for HLA-C*06:02 was confirmed by comparing the imputed genotypes with actual genotypes that were known for a subset (n=233) of samples based on sequence-specific oligonucleotide probe (SSOP) HLA typing (Helms *et al*., 2005). Overall, the imputation accuracy rate for HLA-C*06:02 was 98.7%.

Ancestry Informative Markers

To adjust for potential population stratification between cases and controls, 98 European ancestry informative markers (AIMs), which comprised a subset of the top 100 European ancestry informative markers reported by Price et al. (Price *et al*., 2008), were genotyped in both cases and controls using the Illumina GoldenGate platform. After removing AIMs with less than 95% genotyping in all samples, Illumina GenTrain quality score < 0.5, and Hardy-

Weinberg equilibrium p-value < 0.0001, 87 high-quality AIMs remained which were used in the subsequent analysis.

Association Testing and Statistical Analysis

Additive logistic regression models in PLINK (Purcell *et al*., 2007) were used for the association testing. To account for potential population stratification or admixture in these samples, principal component analyses was performed using EIGENSTRAT (Price *et al*., 2006). Four principal components derived from 87 ancestry informative markers were included as covariates in the logistic regression models. Calculation and visualization of linkage disequilibrium between HERV variants and HLA alleles was performed in Haploview (Barrett *et al*., 2005). False discovery rate (FDR) q-values were calculated in R. Haplotype frequency estimation and haplotype association testing were performed using THESIAS, which utilizes the stochastic E-M method (Tregouet and Garelle, 2007).

HERV-K dUTPase ELISA Assays

96 microtiter well plates (Nunc-Immuno Plate MaxiSorp Surface) were coated overnight at 4°C with the recombinant HERV-K dUTPase or human dUTPase protein at 2.5 μg/ml in PBS. Purification of recombinant dUTPase was performed as previously described (Ariza and Williams, 2011). Plates were then washed 3 times with 200 μL of PBS/0.05%-Tween 20 and blocked with 100 μL of blocking buffer (PBS/2.5%-BSA) at room temperature (RT). The samples were diluted in blocking buffer and incubated for 2 h at RT in duplicates. Plates were then washed 3 times with 200 μL of PBS/0.05%-Tween 20. An anti-human-IgG HRP-conjugated secondary antibody was diluted at 1:1000 in blocking buffer and incubated at RT for 1 hour. Plates were then washed 6 times with 200μL of PBS/0.05%-Tween 20 and incubated for 10 minutes with 100 μL of TMB (Invitrogen). Addition of 50 μL H_2SO_4 2 M was used to stop the reaction. The plates were read at 450 nm and 690 nm for the background on a plate reader. The background from the 690 nm uncoated wells and PBS-BSA (negative controls) were subtracted from the mean absorbance of the coated wells. A high responder serum was used to normalize the results. Statistical analysis was performed using a non parametric Mann and Whitney test using Prism 5.0a from GraphPad Software. A positive result was defined as a serum which led to a signal twice over the background OD.

HERV-K dUTPase peptide ELISPOT Assays

Peripheral blood mononuclear cells (PBMC) were isolated from EDTA-anticoagulated blood, and cryopreserved in liquid nitrogen until being thawed overnight before the ELISPOT assay. ELISPOT IFN-γ assays were carried out with individual 13-mer peptides (Figure S1). Peptides were used at a final concentration of 10 μg/mL. Briefly, 96-well flat-bottomed nitrocellulose plates (Multiscreen, Millipore, Bedford, MA) were quickly humidified with 1x phosphate buffered saline (PBS) and then coated with anti-IFN-γ monoclonal antibody (Mabtech, Nacka, Sweden) in 1x PBS for a 1 h incubation at room temperature. Plates were washed four times with 1x PBS and then incubated at 37°C in 5% CO2 for 1h with RPMI 1640 supplemented with penicillin, streptomycin, and 10% fetal bovine serum (R10). One hundred thousand viable thawed PBMC in R10 were added to the

wells. Phytohemagglutinin (PHA) and a peptide pool from cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF) were used as positive controls, and media only as a negative control. Plates were incubated for 16 to 18 h at 37°C in 5% CO2, after which the cells were discarded. After washing the plates twice with 1x PBS and twice with 1x PBST (1xPBS containing 0.1% Tween 20), biotinylated anti-IFN-γ monoclonal antibody (Mabtech) in 1xPBSTB (1x PBST containing 2% bovine serum albumin) was added. Plates were incubated for further 30min at room temperature. Following two additional washes with 1x PBST, streptavidin-alkaline phosphatase conjugate (Jackson ImmunoResearch, West Grove, PA) in 1x PBSTB was added for 30 min to 1 h at room temperature. Plates were then soaked in 1x PBST for 30 min to 1 h. Developer solution (Vector Laboratories, Inc., Burlingame, CA) was added for about 15 min. Plates were rinsed with tap water and dried and then spots were counted using a AID reader (AID, Obenhochen, Germany). HERV-K-specific responses were reported as the number of spot-forming units (SFU)/ 1.0×10^6 PBMC after subtraction of twice the background IFN-γ secretion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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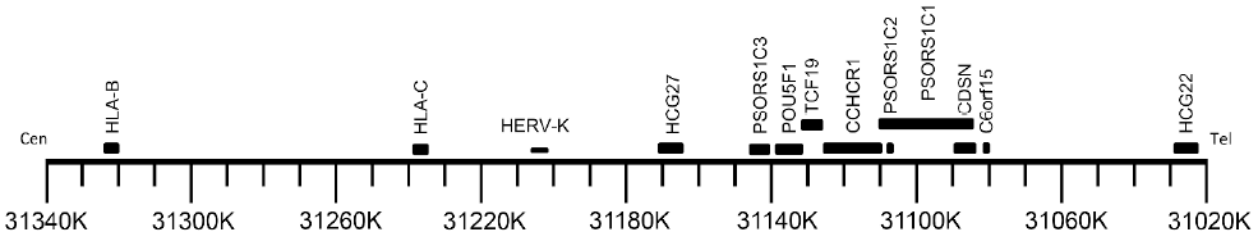

Figure 1. Location of human endogenous retrovirus K (HERV-K) sequence adjacent to HLA-C within the MHC region on chromosome 6

The PSORS1 susceptibility locus containing one or more psoriasis risk variants has been mapped in various studies to the region between HLA-B and HCG22. Cen: centromeric; Tel: telomeric. Scale at bottom is human reference sequence assembly GRCh37/hg19.

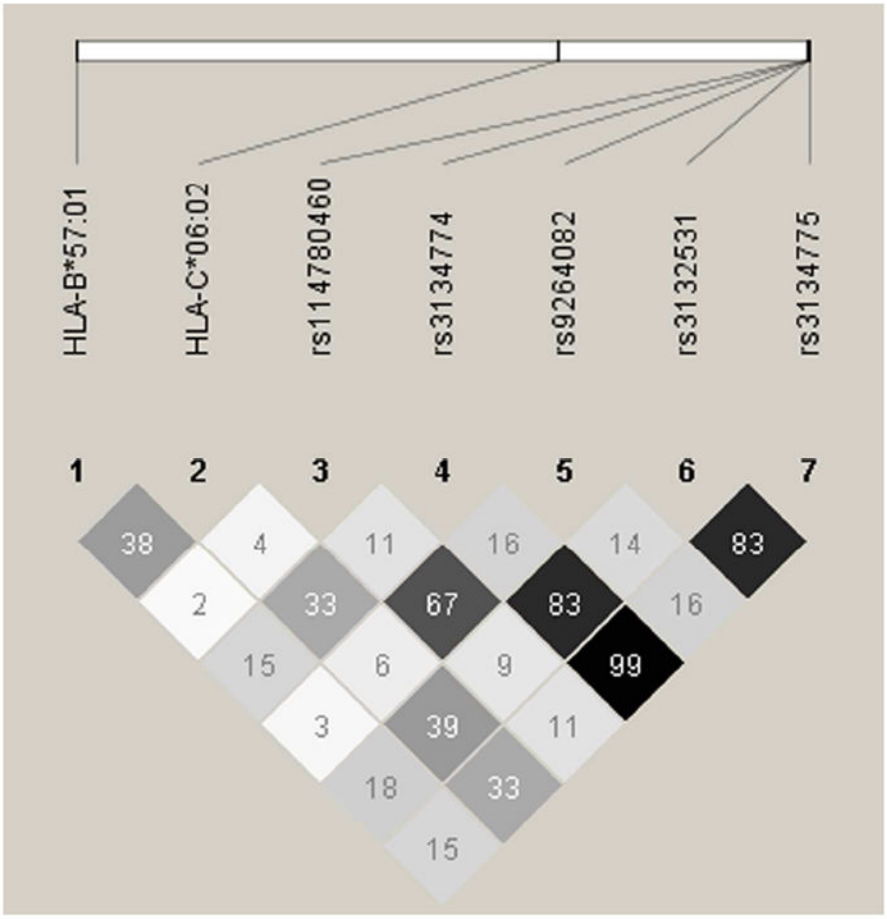

Figure 2. Linkage disequilibrium plot for HLA-C*06:02, HLA-B*57:01, and HERV-K dUTPase SNPs

r^2 values between pairs of SNPs are shown.

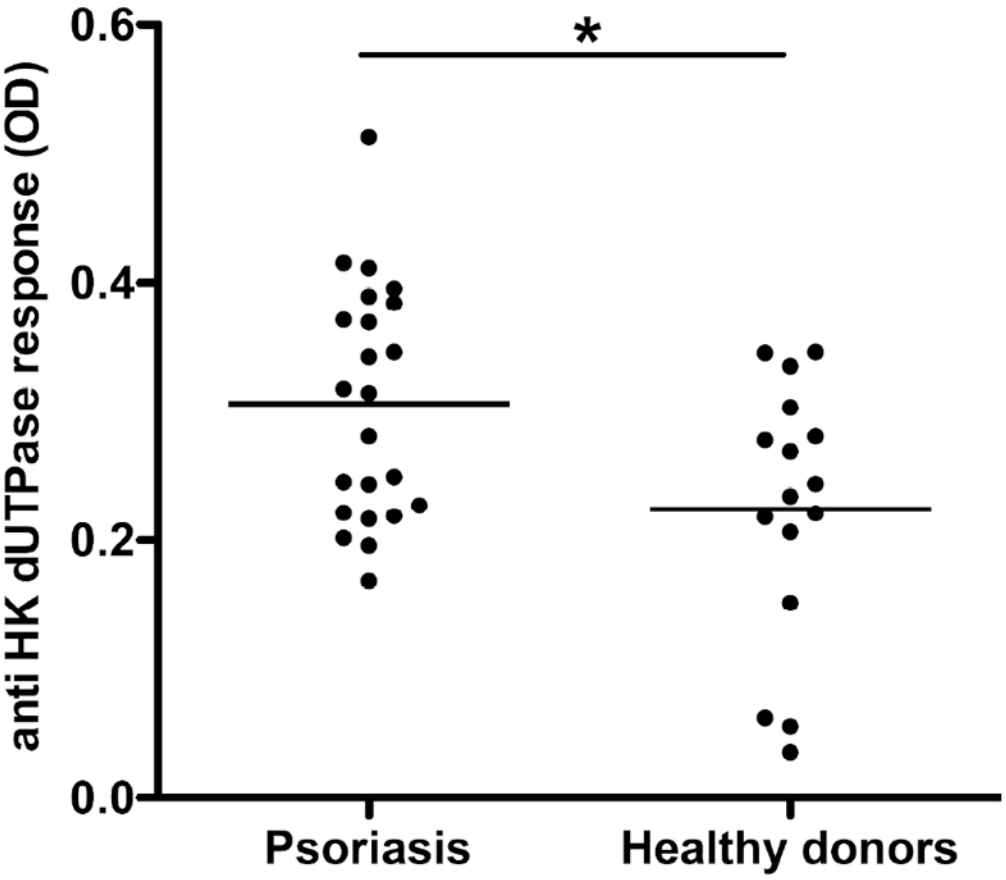

Figure 3. IgG antibody responses against recombinant HERV-K dUTPase

The response against HERV-K dUTPase was screened using 23 psoriasis patient and 16 healthy donor serum samples at a 1:800 dilution. The human dUTPase was used as a control. Psoriasis subjects had a greater humoral response against HERV-K dUTPase compared to the healthy blood donors. No response against the human dUTPase was detected even at the lowest dilution used (data not shown). * $p<0.05$, Mann-Whitney U test.

Table 1

Association Testing of PSORS1 HERV-K dUTPase Variants with Psoriasis

P-values and odds ratios (ORs) for association with psoriasis are shown before and after adjustment for HLA-C*06:02 and HLA-B*57:01 (rightmost columns). False discovery rate q-values for the conditional tests are shown. MAF, minor allele frequency.

SNP / Variant	Chr 6 Position (hg19)	Major/Minor Alleles	Amino Acid Change	MAF Cases	MAF Controls	P-value	OR [95% CI]	$P\text{-value}_{adj}$	$OR\ [95\%\ CI]_{adj}$	FDR q-val
NOVEL1	31201267	A/T	-	0.0007	0.0000	0.9993	-	-	-	1
NOVEL2	31201299	T/G	-	0.0014	0.0000	0.999	-	-	-	1
rs3134775	31201357	T/G	-	0.4595	0.2855	**9.97E-15**	2.31 [1.87-2.86]	0.0037	1.43 [1.22-1.81]	**0.0173**
NOVEL3	31201357	T/C	-	0.0000	0.0014	0.9993	-	-	-	1
NOVEL4	31201376	G/A	-	0.0404	0.0390	0.8532	-	-	-	0.4000
NOVEL5	31201378	C/A	-	0.0021	0.0042	0.4581	-	-	-	0.9454
NOVEL6	31201378	C/T	-	0.0014	0.0000	0.9991	-	-	-	1
NOVEL7	31201438	G/A	-	0.0000	0.0111	0.998	-	-	-	1
rs3132531	31201472	G/A	-	0.4224	0.2535	**3.48E-14**	2.31 [1.86-2.86]	0.0263	1.33 [1.03-1.71]	0.0921
NOVEL8	31201499	A/G	D[166]G	0.0028	0.0000	0.9987	-	-	-	1
rs9264082	31201514	A/G	L[161]L	0.1622	0.2779	**1.68E-09**	0.50 [0.40-0.63]	2.50E-04	0.64 [0.51-0.81]	**0.0035**
rs3134774	31201524	A/G	K[158]R	0.4596	0.2827	**3.28E-15**	2.36 [1.91-2.92]	0.0020	1.46 [1.15-1.85]	**0.0140**
rs114780460	31201534	G/A	G[155]R	0.1199	0.1927	**2.98E-05**	0.59 [0.46-0.76]	0.0511	0.78 [0.60-1.00]	0.1431
NOVEL9	31201541	G/A	P[152]P	0.0000	0.0056	0.9986	-	-	-	1
HLA-C*06:02	-	-	-	0.2722	0.0879	**1.39E-22**	4.45 [3.30-6.00]	-	-	-
HLA-B*57:01	-	-	-	0.1412	0.0385	**3.28E-11**	4.06 [2.68-6.15]	-	-	-

Table 2

Association of 3-Marker HERV-K Haplotypes with Psoriasis

For missense SNPs rs114780460 and rs3134774, the corresponding amino acids at HERV-K dUTPase protein positions 155 and 158 are shown. Haplotype associations were adjusted for the effects of C*06:02 and B*57:01. Haplotypic odds ratios are expressed in reference to the most frequent haplotype. [G]=Glycine; [K]=Lysine; [R]=Arginine.

Haplotype	rs114780460 [AA 155]	rs3134774 [AA 158]	rs9264082	Case Freq	Control Freq	P value	OR [95% CI]
H1	G [G]	G [R]	A	0.4583	0.2848	-	1.00 [reference]
H2	G [G]	A [K]	A	0.3743	0.4322	0.0370	0.77 [0.60-0.99]
H3	A [R]	A [K]	G	0.1229	0.1955	9.43E-04	0.59 [0.43-0.81]
H4	G [G]	A [K]	G	0.0422	0.0838	2.80E-05	0.40 [0.26-0.61]