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The association of *ERAP1* and *ERAP2* single nucleotide polymorphisms and their haplotypes with psoriasis vulgaris is dependent on the presence or absence of the *HLA-C*06:02* allele and age at disease onset

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ABSTRACT

The aim of this case-control study was to elucidate the role of some single nucleotide polymorphisms (SNPs) in the *ERAP1* (rs27524, rs27044, rs30187, rs2287987 and rs26653) and *ERAP2* (rs2248374) genes in predicting the risk for psoriasis vulgaris in the Polish population. *ERAP1*, *ERAP2* and *HLA-C*06:02* typing was done using the TaqMan SNP genotyping assays. We confirmed a strong association of the *HLA-C*06:02* allele with early-onset psoriasis. In *ERAP1*, rs30187T increased the risk of psoriasis in *HLA-C*06:02*-positive patients, most strongly in late onset psoriasis, whereas it was protective when the *HLA-C*06:02* allele was absent. We also found a protective effect of the *ERAP2* rs2248374A allele and rs2248374AA genotype only in *HLA-C*06:02* carriers, especially in the subgroup of patients with juvenile psoriasis. Analysis of combined haplotypes for *ERAP1* and *ERAP2* also revealed differences when the patients and controls were stratified by *HLA-C*06:02*. An *ERAP1* haplotype known to possess high enzymatic activity was associated with psoriasis if *HLA-C*06:02* was present and a functional *ERAP2* allele was absent. In the absence of *HLA-C*06:02*, an *ERAP1* haplotype of low activity was conducive to psoriasis if a functional *ERAP2* allele was present, but the same *ERAP1* haplotype was protective if the *ERAP2* allele was defective.

1. Introduction

Endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 trim peptides to the optimal length for binding to human leukocyte antigen class I (HLA-I) molecules, which present them to CD8+ T lymphocytes [1]. These two aminopeptidases may function not only as monomers but also as ERAP1-ERAP2 heterodimers, which differ from monomers in activity and specificity [2,3]. Several single nucleotide polymorphisms (SNPs) of the *ERAP1* and *ERAP2* genes have already been described, and some of them result in amino acid changes in aminopeptidase molecules, changing their specificity and/or activity against substrate peptides [4,5]. In addition, about 25% of individuals in all human populations tested are homozygous for an *ERAP2* variant which results in the lack of an ERAP2 protein [6]. Therefore, the ability of the immune system to induce and execute the cytotoxic response to a given antigen, or to ignore it, depends not only on polymorphism of HLA molecules but also on that of ERAP1 and ERAP2. Not surprisingly, *ERAP1* and *ERAP2* genes have been found to be associated with several human diseases [7–10]. Some immunogenic peptides require ERAP1 and/or ERAP2 trimming to be bound by HLA-I molecules, while others may be eliminated by overtrimming, and still others are independent of ERAP activity [3,11].

Psoriasis vulgaris (PsV), an inflammatory condition and one of the most frequent skin diseases, affects about 2% of Caucasians [12]. Both genetic and environmental factors play a role in keratinocyte hyperproliferation and altered differentiation, and in infiltration of inflammatory cells to the epidermis and dermis [12–14]. The disease is immune-mediated, and is strongly associated with the HLA class I molecule *HLA-C*06:02* in most human populations [12]. It seems reasonable also to predict an association of psoriasis with *ERAP1* and *ERAP2* polymorphisms, which may affect the presentation of psoriatogenic peptide(s) by the HLA-C*06:02 molecule to CD8+ T cells. Indeed, this has been shown for *ERAP1* in several human populations both in genome-wide association studies (GWAS) [15,16] and in the candidate

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gene approaches [17] including Poles [18]. Here, we extend our investigation by adding four other *ERAP1* SNPs and a SNP in *ERAP2*, tested on higher numbers of cases and controls and controlled by *HLA-C*06:02* typing.

Psoriasis is commonly classified into two subtypes according to age of onset. Type I (early-onset) psoriasis appears before the age of 40 years, and affects 70% of all psoriatics. Type II psoriasis (late-onset) develops after the age of 40 years [19,20]. Type I psoriasis displays a strong family history, and a strong association with *HLA-C*06:02*. In contrast, type II psoriasis is rarely familial, and its association with *HLA-C*06:02* is weak or absent, depending on the population [20–22]. In this study we stratified our patients according to age at disease onset into three subtypes to compare the association of our SNPs in very early onset psoriasis (0–20 years) to middle early onset (21–40 years) and late onset (over 40 years).

2. Materials and methods

2.1. Study population

Four hundred and sixty-one unrelated Polish patients diagnosed with psoriasis vulgaris were enrolled in the study. Among these, 352 patients were recruited in the Department and Clinic of Dermatology, Venereology and Allergology of Wrocław Medical University, and 109 patients were from the Department of Dermatology, Venereology and Allergology of Medical University in Gdańsk. The diagnosis of psoriasis was made according to the well-established clinical criteria [23]. For all patients we had information on gender, age at disease onset and age at time of blood sampling. The clinical severity of the disease was assessed according to PASI (Psoriasis Area and Severity Index) [12,24] on 324 of the patients. All cases were divided into three subgroups based on age of onset: (I) – up to 20 years (very early onset psoriasis, vEOP), (II) – between 21 and 40 years (middle early onset psoriasis, mEOP) and (III) - above 40 years (late onset psoriasis, LOP). Detailed characteristics of the patients are shown in Table 1. Regarding the median PASI score, the studied individuals fulfilled the criteria for moderate-to-severe psoriasis (Table 1). Controls were 454 unrelated Polish blood donors with no history of PsV or other dermatoses. The study was approved by bioethical committees of participating medical universities, and all patients and controls gave written informed consent.

2.2. DNA extraction

Genomic DNA was extracted as described earlier [25] or using Invisorb Spin Blood Midi kit (Stratec Molecular, Berlin, Germany) from 3 ml of frozen venous blood according to the manufacturer's instructions.

Table 1
Characteristics of patients and controls.

	Age at onset				
	vEOP ≤20 yr N = 152	mEOP 21–40 yr N = 174	LOP > 40 yr N = 135	All N = 461	Controls (N = 454)
Sex, women (%)	65 (42.8%)	47 (27.0%)	78 (57.8%)	190 (41.2%)	189 (41.6%)
Mean age at onset	13.9	30.2	54.0	31.8	–
Median for PASI score	13.05	12.40	10.30	12.05	–
Mean age at the moment of blood sampling	34.4	47.9	62.1	47.6	33.1

vEOP – very early onset psoriasis, mEOP – middle early onset psoriasis, LOP – late onset psoriasis.

2.3. ERAP1 and ERAP2 SNPs typing

The polymorphisms were chosen based on previously reported associations with susceptibility to psoriasis [15,16,26] and ankylosing spondylitis (AS) [27,28]. Five SNPs in *ERAP1* (rs26653, rs2287987, rs30187, rs27044, rs27524) and one in *ERAP2* (rs2248374) were genotyped using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, USA) according to manufacturer's instruction. Assay IDs are provided in Supplementary Table 1. 7300 Real Time PCR System and SDS software ver. 1.4 (Applied Biosystems, Foster City, USA) were used to conduct reactions and allelic discrimination, respectively.

2.4. Determination of HLA-C*06:02 genotypes

The *HLA-C*06:02* genotypes for all individuals were imputed by using the method described by Lai et al. [29] with minor modifications in assay manufacturer and haplotype estimation software. For genotyping of four selected SNPs (rs1062470, rs887466, rs2894207 and rs10484554) from the *PSORS1* region (6p21.3) we used TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, USA), (Supplementary Table 1). The reaction conditions and Real Time PCR System were the same as for *ERAP1/2* typing. FAMHAP software ver. 19 was used to generate four point haplotypes for each individual [30].

2.5. Statistical analysis

Associations between genetic markers and disease status on the level of alleles and genotypes, as well as deviation of the genotype counts from Hardy-Weinberg equilibrium (HWE), were analyzed using PLINK software ver. 1.07 [31]. For HWE analysis the significance threshold $p < 0.05$ was applied. Differences in allele and genotype distributions between cases and controls were tested with Fisher's exact test. The SHEsis software platform was used for analysis of linkage disequilibrium (LD) and haplotype association [32]. Chi-square test was used to compare haplotype frequencies between studied groups. The odds ratio (OR) and its 95% confidence interval (95%CI) were computed as the measure of effect size. P value < 0.05 was considered significant.

3. Results

All tested polymorphisms in *ERAP1* and *ERAP2* genes were in Hardy-Weinberg equilibrium in patients. In contrast, there was a slight departure from HWE ($p = 0.02$, Supplementary Table 1) for rs26653 (*ERAP1*) in the control group, so we excluded it from further analysis. This SNP was recently described to have no effect on psoriasis susceptibility in India [33], but was associated with psoriasis in European populations [17,18]. However, no significant difference in its frequency between our patients and controls was observed (Supplementary Table 2). All four SNPs from the *PSORS1* region, determining the *HLA-C*06:02* allele, were in HWE in controls but not in cases (Supplementary Table 1).

3.1. Application of SNP genotyping to determine HLA-C*06:02 distribution, and comparison with commercial PCR-SSP typing

To reduce the high costs of commercial PCR-SSP systems for *HLA-C* typing, we applied the SNP genotyping method of Lai and coworkers, which is based on estimation of a four-SNP haplotype specific for the *HLA-C*06:02* allele [29]. SNP genotyping also gives information on *HLA-C*06:02* homo- or heterozygosity without needing to type for all *HLA-C* alleles. We detected 13 haplotypes. One of these – AGCT (rs1062470, rs887466, rs2894207 and rs10484554) – corresponded to