GENETIC EXAMINATIONS IN PSORIASIS

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Abbreviations

AS	ankylosing spondylitis
CASPAR	Classification Criteria for Psoriatic Arthritis
CD	Crohn's disease
DR3	death domain receptor 3
EO-PsA	early onset psoriatic arthritis patient group
EO-PsC	early onset cutaneous psoriasis patient group
EO-PsV	early onset psoriasis vulgaris patient group
ERAP1	endoplasmic reticulum-associated amino-peptidase 1
HLA	human leukocyte antigen
IBD	inflammatory bowel disease
LO-PsA	late onset psoriatic arthritis patient group
LO-PsC	late onset cutaneous psoriasis patient group
LO-PsV	late onset psoriasis vulgaris patient group
MHC	major histocompatibility complex
NB-UVB	narrowband ultraviolet B radiation
PASI	Psoriasis Area Severity Index
PsA	psoriatic arthritis patient group
PsC	cutaneous psoriasis patient group
PSORS	major psoriasis susceptibility factor
PsV	psoriasis vulgaris patient group
PUVA	psoralen with ultraviolet A radiation
SNP	single nucleotide polymorphism
TL1A	TNF superfamily ligand A
TNF	tumor necrosis factor
TNFSF15	tumor necrosis factor superfamily, member 15
UC	ulcerative colitis
VEGI	vascular endothelial cell growth inhibitor

1. Introduction

1.1. Psoriasis

Psoriasis is a chronic, multifactorial, T-cell mediated inflammatory skin disease. It is relatively common, affecting 2–3% of the population. Psoriasis typically manifests as thickened, scaly, red skin plaques. Besides plaque psoriasis, we define flexural psoriasis with scaling in the axillae, sub-mammary and genital areas, guttate psoriasis with acute eruption of papules on the trunk and erythroderma with erythematous rash [1]. Pustular psoriasis is a distinct phenotype with sterile pustules, which can either be acute generalized (generalized pustular psoriasis) or localized only on digits (acrodermatitis continua of Hallopeau) or palms and soles (palmoplantar pustulosis) [2].

Early onset psoriasis, or type I psoriasis, refers to patients with onset before 40 years of age, more serious disease course than late onset psoriasis, strong family history, and *HLA-C*06* positivity. Early onset psoriasis has been reported in 75% of patients with psoriasis; hence, it affects the majority of patients with psoriasis. Late onset psoriasis, or type II psoriasis, in contrast, is characterized by an onset at or after 40 years, less severe clinical symptoms and rare family-inheritance [3,4]. The age at disease onset (with a cut-off value at 40 years) was found similarly important in the characterization of disease phenotype in psoriatic arthritis as well [5].

Both genetic and environmental factors play a role in the etiology of the disease [6]. The major psoriasis susceptibility factor (*PSORS1*) is located on chromosome 6. This locus includes the *HLA-B13* and *HLA-Cw6* alleles, which are the most important determinants [7].

Single nucleotide polymorphisms (SNPs) of several genes have been identified as contributing to psoriasis susceptibility [8,9]. We and others have reported significant association with variants of the *HLA-Cw*0602*, *IL23R*, *LCE3C*, *LCE3B-del* [10,11], *TNFSF15* [12], *ERAP1* genes [13,14,15]. Other candidate genes are involved in NF-kappa B signaling (*TNIP1*), type 1 interferon pathway (*RNF113* and *IFIH1)*, interleukin (IL)-23/Th17 axis (*IL23R, IL12B* and *TYK2*) [16], which is considered to be the central driver of immune activation, chronic inflammation and keratinocytes proliferation [17]. However, pustular psoriasis seems to be genetically distinct, mainly has strong associations with *IL36RN, AP1S3* and *CARD14* genes [18]. The effectiveness of biologic treatments in psoriasis but less effect in pustular psoriasis have also verified these findings [1].

Besides topical (corticosteroids, calcipotriol) therapies and phototherapy (narrowband ultraviolet B radiation – NB-UVB, psoralen with ultraviolet A radiation – PUVA) conventional systemic agents (methotrexate, cyclosporine, acitretin) can be used as second line therapy [19].

In moderate to severe psoriasis monoclonal antibodies and soluble receptors as biological therapies are approved for use as TNF (adalimumab [20], etanercept [21], infliximab [22] and certolizumab [23]), IL-12/23p40 (ustekinumab [24]), IL-23p19 (rizankizumab [25], guselkumab [26] and tildrakizumab [27]), IL-17 (ixekizumab [28] and secukinumab [29]), IL-17 receptor (brodalumab [30]) and JAK (tofacitinid [31]) inhibitors. The biologic therapy should be tailored to each patient, which is influenced by psoriasis factors (disease phenotype, presence of PsA and outcomes of previous biologic treatment), comorbidities (demyelinating disease and inflammatory bowel disease) [32,33] and genomic information (*HLA-C*06:02* status) [34].

Pustular psoriasis is recalcitrant to treatments used in plaque psoriasis; topical corticosteroids, PUVA, methotrexate, cyclosporine and acitretin have limited efficacy [19]. Loss-of-function mutations have been found in *IL36RN* in some generalized pustular psoriasis patients and in clinical trial an intravenous monoclonal antibody against the interleukin-36 receptor showed good efficacy [35].

1.2. Psoriatic arthritis

Psoriatic arthritis is a seronegative inflammatory arthritis present in nearly 25% of patients with psoriasis and develops after an average interval of about one decade [36]. Psoriatic arthritis is defined as a subtype of spondyloarthropathies, based on common human leukocyte antigen (HLA) associations. Clinically, this disease is characterized by changing degrees of oligoarthritis, polyarthritis and spondylitis and typically involves dactylitis, distal interphalangeal joint involvement, mutilating arthritis or enthesitis [12,36]. Given this heterogeneity, its diagnosis is difficult, but classification criteria such as CASPAR (classification criteria for psoriatic arthritis) [37] and screening tools such as ToPAS (Toronto Psoriatic Arthritis Screen), PASE (Psoriatic Arthritis Screening and Evaluation Questionnaire) and the PEST (Psoriasis Epidemiology Screening Tool) have facilitated the recognition of this disease among rheumatologists, dermatologists, and family physicians [38]. Several studies found an increased risk of psoriatic arthritis in patients with psoriatic scalp lesions, intertriginous/inverse psoriasis, and nail dystrophy [39,40,41,42].

Psoriatic arthritis is strongly associated with the *HLA-B13*, *HLA-B57*, *HLA-B39*, *HLA-Cw6* and *HLA-Cw7* alleles [9]. While psoriatic arthritis tends to a more severe disease course and appears earlier in *HLA-B*27* positive patients, the latency between the onset of psoriasis and onset of joint symptoms is longer in *HLA-Cw*0602* positive patients [5,43].

The pathophysiology of psoriatic arthritis is similar to that of psoriasis and involves important cytokines in the IL-23/IL-17 and tumor necrosis factor (TNF) pathway (e.g. IL-12, IL-17, IL-23,and TNF-a) [44,45]. Thus, many cytokine-targeting biologics and small-molecule inhibitors that provide effective skin clearance in psoriasis also improve joint symptoms and slow radiographic progression in PsA [46].

Non-pharmacological therapies includes physical, occupational therapy, smoking cessation, weight loss, massage therapy and exercise. For symptomatic therapy nonsteroidal anti-inflammatory drugs recommended for pain relief, oral corticosteroids are not recommended because of side effects and skin rebound when they are withdrawn [47]. Intra-articular corticosteroid injections may be used in persistent mono- and oligoarthritis [48]. Single disease modifying drugs (DMARDs) as methotrexate, sulfasalazine, cyclosporine, leflunomide and their combinations are recommended as treatment for peripheral arthritis, dactylitis and nail symptoms [49] but do not provide any disease-modifying effects on radiographic damage [46].

Biologics that inhibit TNF-a (adalimumab [50], certoliumab [51], etanercept [52], golimumab [53], infliximab [54]), IL-17A (secukinumab [55], ixekizumab [56]), IL-12/23 (ustekinumab [57]), Janus kinase (tofacitinib [58]) or the T-cells (abatacept [59]) are recommended across all psoriatic arthritis domains with some restrictions [49], and have significant benefits on arthritis, psoriasis, enthesitis and dactylitis [47].

1.3. Tumor necrosis factor superfamily

A disturbed cytokine network characterizes psoriasis. Tumor necrosis factor alpha (TNFα) seems to be one of the most important triggers for aberrant activation of lymphocytes and keratinocytes [60]. TNFα is a key cytokine both in psoriasis and in psoriatic arthritis: high levels of TNFα can be detected in psoriatic skin lesions as well as in the synovial fluid and synovial tissue of patients with psoriatic arthritis [61].

The tumor necrosis factor superfamily, member 15 protein (TNFSF15; also known as TNF superfamily ligand A, TL1A, and vascular endothelial cell growth inhibitor, VEGI) is a TNF-like factor expressed primarily in endothelial cells [62,63]. The TNFSF15 protein is

encoded by a gene on human chromosome 9q32 and consists of 251 amino acid residues with the characteristics of a type II transmembrane protein [64]. To date, no *PSORS* loci have been identified on human chromosome 9q32. TNFSF15 inhibits endothelial-cell and endothelial-progenitor-cell differentiation and stimulates T-cell activation, Th1 cytokine production and dendritic cell maturation [65]. TNFSF15 plays an important role in the pathogenesis of inflammatory bowel disease (IBD), atherosclerosis, rheumatoid arthritis and chronic inflammatory skin diseases [66,67,68,69]. Increased levels of TNFSF15 are found in the intestinal mucosa of patients with Crohn's disease (CD) and in the skin of patients with psoriasis [70,71]. Furthermore, single nucleotide polymorphisms (SNP) and certain haplotypes of the *TNFSF15* gene were reported to contribute to the development of IBD as an ethnicity-specific susceptibility factor [72].

1.4. Endoplasmic reticulum-associated amino-peptidase 1 (ERAP1)

Genetic variants of the *endoplasmic reticulum-associated amino-peptidase 1* (*ERAP1*) gene has been reported to be associated with ankylosing spondylitis (AS), psoriasis [73], and Behcet's disease [74]. The ERAP1 protein belongs to the M1 family of zinc metallopeptidase enzymes and is encoded by a gene on chromosome 5q15 [75]. ERAP1 trims peptides imported into the endoplasmic reticulum at their N-terminus and contributes to the shaping of the antigenic repertoire presented by class I major histocompatibility complex (MHC) molecules [76]. Depending on the peptide length and sequence composition, ERAP1 has the ability to both destroy and create peptide cargos for MHC class I molecules [77]. HLA-C*06:02 mediates an autoimmune response against melanocytes by autoantigen presentation. Arakawa et al. demonstrated that in psoriasis ERAP1 generates the causative melanocyte autoantigen through trimming NH2-terminal elongated peptide precursors to the appropriate length for presentation by HLA-C*06:02 [78]. The ERAP1 protein also contributes to the shedding of the membrane-bound receptors of inflammatory cytokines, such as IL-1R2, TNFR1 and IL-6R [79].

ERAP1 likely plays a pivotal role in the protection from infectious diseases by contributing to the maintenance of immune tolerance and control of inflammation [80]. The association of *ERAP1* variants with psoriasis has been investigated in populations of European and Chinese ancestry. Two *ERAP1* variants, rs27524 (noncoding) and rs30187 (Lys528ARG), were found to be genome-wide risk factors for psoriasis [13]. Furthermore, dominant epistasis between *HLA-Cw*0602* and the *ERAP1* rs30187 SNP was identified in

studies [13,14,15]. Another work identified the *ERAP1* rs27432 SNP (intronic) as a variant strongly associated with psoriasis [81]. In Han Chinese populations, *ERAP1* SNPs or gene variants in linkage disequilibrium with *ERAP1* SNPs were also found to be associated with psoriasis [82,83,84]. It is currently unclear, whether the effects of *ERAP1* on psoriasis can be explained by a single variant or by allotypic associations. The association of *ERAP1* with psoriatic arthritis has also been investigated; however, this study did not demonstrate an association between rs30187 and psoriatic arthritis or psoriatic arthritis subphenotypes [85]. A strong evidence of association for rs30187 and for CC rs30187/rs27044 haplotype has been shown in Romanian *HLA-B27* positive psoriatic arthritis population [86]. Overall metaanalysis showed an association between rs27524 and rs30187 polymorphisms and susceptibility to psoriasis [87].

2. Aims

The findings listed above in the introduction suggest that the *TNFSF15* gene plays a role in the pathomechanism of a wide range of immune-mediated human diseases, therefore in the first part of this study, we intended to examine the association between psoriasis, psoriatic arthritis and *TNFS15* gene.

As psoriasis is currently best stratified by disease onset and absence or presence of arthritis, in the second part of this study we hypothesized that stratifying psoriasis patients into early and late onset groups, as well as skin-only and arthritis subgroups leads to the identification of new *ERAP1* genotype–phenotype associations. Furthermore, as *ERAP1* seems to be associated with inflammatory diseases in a *HLA*-dependent manner, we also intended to explore whether a gene–gene interaction between *HLA-Cw*0602* and *ERAP1* exists in these well-stratified subgroups of psoriatic patients.

3. Subjects and methods

3.1. Subjects

This study was approved by the Internal Review Board of the University of Szeged, the approval number is PSO-GENET-001. Informed consent was obtained from all participating patients and volunteers, and the study was conducted in full accordance with the Principles of the Declaration of Helsinki. The study population consisted of 319 Hungarian Caucasian psoriasis vulgaris patients (designated as PsV) treated at the Department of Dermatology and Allergology and the Department of Rheumatology of the University of Szeged, and at the Department of Rheumatology of Pándy Kálmán Békés County Hospital, Gyula, as well as 200 ethnically matched healthy individuals with no known multifactorial inflammatory diseases. Of the 319 psoriasis vulgaris patients, 105 exhibited psoriatic arthritis (designated as PsA), fulfilling the Classification Criteria for Psoriatic Arthritis (CASPAR). The group of patients with psoriatic arthritis was further stratified into five homogenous clinical groups according to the Moll and Wright criteria [88]. None of the participating individuals had IBD. Patients with skin symptoms of psoriasis only were denoted as cutaneous psoriasis (designated as PsC) patients. Patients with onset before 40 years of age were classified as having early onset psoriasis (EO-PsV, EO-PsA, EO-PsC), whereas late onset psoriasis (LO-PsV, LO-PsA, LO-PsC) was defined by an onset at or after 40 years. The demographic and clinical characteristics of the study population are listed in Table 1.

Table 1
Demographic and clinical characteristics of the study population

Characteristic	Healthy controls (N=200)	PsV (N=319)	PsC (N=214)	PsA (N=105)
Age, mean (range) [years]	40.39 ± 19 (7–82)	52.13 ± 21 (17–82)	50.72 ± 19 (17–81)	54.34 ± 13 (27–82)
Males/females [N, (%)]	77 (39) / 123 (41)	177 (55) / 142 (45)	125 (58) / 89 (42)	52 (49) / 53 (51)
PASI, mean (range)	–	–	12.42 ± 16.54 (0.1–52.8)	–
Arthritis, distal [N, (%)]	–	–	–	13 (12)
Asymmetrical oligoarthritis [N, (%)]	–	–	–	28 (27)
Symmetrical polyarthritis [N, (%)]	–	–	–	45 (43)
Arthritis, axial [N, (%)]	–	–	–	19 (18)
Arthritis mutilans [N, (%)]	–	–	–	0 (0)

3.2. Genotyping and haplotype analysis

Genomic DNA was isolated from venous blood of patients and controls using the BioRobot EZ and the EZ1 DNA Blood Kit from QIAGEN (Hilden, Germany), according to the instructions of the manufacturer. Genotyping of five SNPs of the *TNFSF15* gene (see Table 2), five SNPs of the *ERAP1* gene and two SNPs of the *HLA-Cw*0602* gene (see Table 3), previously reported as candidate genes and SNPs in the pathogenesis of psoriasis and AS [73,89], was carried out with the PCR-based Assay-by-Design method of Applied Biosystems (Foster City, CA), following the instructions of the manufacturer. After PCR amplification, end-point detection was performed with a CFX 96 real-time PCR machine from Bio-Rad (Hercules, CA). The five SNPs of the *TNFSF15* gene were used to construct three haplotypes (Table 4), previously reported in Japanese, Korean and Caucasian IBD populations [66,72,88,90]. Genotyping of the rs10484554 SNP was used to determine the *HLA-C* status, as previously reported [13,73]. Three SNPs of the *ERAP1* gene (rs30187, rs10050860, rs17482078) were used to construct four haplotypes: Haplotype A (rs17482078/rs10050860/rs30187-CCC), Haplotype B (rs17482078/rs10050860/rs30187-CCT), Haplotype C (rs17482078/rs10050860/rs30187-TTC) and Haplotype D (rs17482078/rs10050860/rs30187-TTC), previously reported by Ombrello et al. to be risk or protective factors in AS [91].

We analyzed the association of five *TNFSF15*, five *ERAP1* and two *HLA-Cw*0602* SNPs as well as different *TNSF15* and *ERAP1* haplotypes with PsV, EO-PsC, LO-PsC, EO-PsA and LO-PsA.

Table 2

TNFSF15 gene SNPs

SNP	Kit number	Position[a]	Nucleotide change
rs3810936	C___363308_10	15524	G/A
rs6478108	C___170492_10	9706	G/A
rs6478109	C___1305297_10	–358	T/C
rs7848647	C___11277159_10	–638	G/A
rs7869487	C___11277149_10	–12506	G/A

[a] The first nucleotide of exon 1 is designated as position 1 based on the GenBank reference sequence NM_005118.2.

Table 3
ERAP1 and *HLA-Cw*0602* gene SNPs

Chr.	Gene	SNP	Kit number	Nucleotide change	Variation
5	*ERAP1*	rs27524	C___3056837_10	A/G	None
5	*ERAP1*	rs27525	C___3056838_10	C/T	None
5	*ERAP1*	rs30187	C___3056885_10	C/T	Lys528ARG
5	*ERAP1*	rs17482078	C___3056871_10	C/T	Arg725Gln
5	*ERAP1*	rs10050860	C___3056876_10	C/T	Asp575Asn
6	*HLA-C*	rs10484545	C___29666895_10	C/G	None
6	*HLA-C*	rs10484554	C___29612773_20	C/T	None

Table 4

TNFSF15 gene haplotypes

Haplotype	rs3810936	rs6478108	rs6478109	rs7848647	rs7869487
A	G	A	C	G	A
B	A	G	T	A	G
C	A	G	T	A	A

3.3. Statistical analysis

Genotype frequencies, frequencies of the main haplotypes and SNP associations were calculated and compared between patient groups using $\chi 2$-test and Fisher's exact test, using SPSS 15.0 (Chicago, IL) and Plink software package (v1.9). Odds ratios were calculated with 95% confidence intervals. Multiple-testing correction was applied to all comparisons using the Benjamini-Hochberg 'FDR' method in R (v3.2.3), with a significance threshold of $p<0.05$. As previously suggested and successfully applied by other authors [92,93,94], the age of 40 years was used for stratification of age at disease onset. No further stratification of age at disease onset was attempted due to the relatively small number of patients.

4. Results

4.1.1. *TNFSF15* SNP genotypes in healthy controls and patients

The genotype frequencies of the five examined SNPs are summarized in Table 5. The genotype distribution of the rs6478109 SNP showed a significant difference (p=0.0052) when compared for the group of PsV and the group of healthy controls. When the stratified group of PsC was compared with the group of healthy controls, the value of significance was p=0.0046. The proportion of patients with psoriasis that are heterozygous for the rare allele of the *TNFSF15* rs6478109 SNP was higher than that of heterozygotes identified in the group of healthy controls. However, the genotype distribution of this SNP did not show a significant difference when compared with healthy controls and PsA patients. The genotype distribution of the other four SNPs (rs3810936, rs6478108, rs7848647, rs7869487) did not show significant difference between the healthy controls and PsV, PsC or PsA patients.

Table 5

Distribution of *TNFSF15* SNP genotypes in healthy controls and patients

	Healthy controls (N=200)			**PsV (N=319)**				**PsC (N=214)**				**PsA (N=105)**			
	WT	Het	Hom rare	WT	Het	Hom rare	p	WT	Het	Hom rare	p	WT	Het	Hom rare	p
rs3810936	102 (51)	79 (39.5)	19 (9.5)	171 (53.6)	123 (38.5)	25 (7.9)	0.7454	115 (53.7)	79 (36.9)	20 (9.4)	0.8473	56 (53.3)	44 (41.9)	5 (4.8)	0.3437
rs6478108	99 (49.5)	101 (50.5)	0 (0)	166 (52)	152 (47.7)	1 (0.3)	0.6104	115 (53.7)	99 (46.3)	0 (0)	0.3884	51 (48.6)	53 (50.5)	1 (0.9)	0.3834
rs6478109	38 (19)	162 (81)	0 (0)	33 (10.3)	286 (89.7)	0 (0)	**0.0052**	20 (9.4)	194 (90.6)	0 (0)	**0.0046**	13 (12.4)	92 (87.6)	0 (0)	0.1410
rs7848647	113 (56.5)	70 (35)	17 (8.5)	178 (55.8)	116 (36.4)	25 (7.8)	0.9317	119 (55.6)	75 (35)	20 (9.4)	0.9524	59 (56.2)	41 (39)	5 (4.8)	0.4393
rs7869487	66 (33)	81 (40.5)	53 (26.5)	102 (32)	124 (38.9)	93 (29.1)	0.8064	71 (33.2)	78 (36.4)	65 (30.4)	0.6104	31 (29.5)	46 (43.8)	28 (26.7)	0.8011

WT, both alleles are wild type; Het, only one allele is wild type; Hom rare, both alleles are not wild type; listed as [N, (%)]

4.1.2. The main *TNFSF15* haplotypes in healthy controls and patients

Approximately half of the investigated individuals (from both patient and control groups) were found to have haplotypes previously described in Japanese, Korean and Caucasian populations [66,72,88,90]. The distribution of the three examined haplotypes is summarized in Table 6. No significant difference was found in the distribution of Haplotype A and B when healthy controls and patients with psoriasis and psoriatic arthritis were compared. The haplotype distribution of Haplotype C, however, showed a significant difference (p=0.0041) between the group of healthy individuals and the group of PsV. When the stratified group of PsC was compared with the group of healthy controls, the value of significance was p=0.0250 and a nearly significant value (p=0.0524) was found when the stratified group of PsA was compared with the group of healthy controls. Note that, due to the low frequencies of the examined haplotypes in this study, the statistical reliability of the tests was relatively low.

Table 6

Distribution of the main *TNFSF15* haplotypes in patients and in healthy controls

	Healthy controls (N=200)	**PsV (N=319)**		**PsC (N=214)**		**PsA (N=105)**	
Haplotype	**Frequency**	**Frequency**	**p**	**Frequency**	**p**	**Frequency**	**p**
A	83 (41.5)	130 (40.8)	0.8661	85 (39.7)	0.7123	45 (42.9)	0.8194
B	19 (9.5)	24 (7.5)	0.4266	14 (6.5)	0.2668	10 (9.5)	0.9946
C	7 (3.5)	1 (0.3)	**0.0041**	1 (0.5)	**0.0250**	0 (0)	0.0524

Frequency is listed as [N, (%)].

4.2.1. *ERAP1* gene and *HLA-C* SNP genotypes in healthy controls and patients

The genotype frequencies of the *HLA-C* and *ERAP1* SNPs are summarized in Table 7. Genotype frequencies in patients and controls were in Hardy-Weinberg equilibrium. Control genotype frequencies were comparable to those published in the literature. The genotype distribution of one *HLA-C* SNP (rs10484554) was found to be significantly different between the PsV patients and the group of healthy individuals ($p=5.9x10^{-5}$ respectively). The proportion carrying the mutant *HLA-Cw*0602* allele (rs10484554 SNP) was significantly higher among PsV patients than in the group of healthy controls (58.3% and 36.5%, respectively), and there was no difference in this respect between patients with skin only or skin and joint symptoms (57.9% and 59%, respectively). Taken together, the *HLA-Cw*0602* rs10484554 SNP seems to be a strong susceptibility factor for psoriasis (Table 7). In the case of the five other *ERAP1* SNPs (rs27524, rs27525, rs30187, rs17482078 and rs10050860) and rs10484545 *HLA-C* SNP, there were no statistically detectable differences in the genotype distributions between healthy individuals and the PsV patients.

Subsequently, psoriasis vulgaris patients were subdivided into groups according to the presence or absence of arthritis (Table 7). In line with earlier publications [89,93,95], the genotype distribution of the *HLA-C* rs10484554 SNP was found to be significantly different in both the PsC and the PsA subpopulations (p=0.0007 and p=0.0007, respectively), compared to healthy controls. In PsA (but not in PsC) patients, the genotype distribution of three *ERAP1* SNPs (rs10050860, rs27525 and rs17482078) was also significantly different (p=0.0252, p=0.0453 and p=0.0453, respectively) (Table 7). For all three SNPs, the proportion of patients carrying the wild type allele was higher than in the group of healthy individuals, suggesting that the rare allele of these SNPs might provide protection against the development of psoriatic arthritis. As no differences were detected in the genotype distribution of the studied *ERAP1* SNPs in PsC patients, it is likely that the trend in the difference observed for the rs10050860 SNP in the PsV patients is caused by the presence of the PsA patients. No difference was detected for the *ERAP1* rs27524 and rs30187 SNPs and the *HLA-C* rs10484545 SNP between the healthy controls and the psoriasis groups, even after stratification for arthritis.

We further stratified patients according to disease onset (early and late onset). Significant differences were observed only for the *HLA-C* rs10484554 SNP in the early onset groups, which is in agreement with earlier publications [14,15,89,95]. This result suggests that *HLA-C* positivity is a susceptibility factor only for early onset psoriasis. Stratification led

to no significant difference for the rs10484545 *HLA-C* SNP. The *ERAP1* SNPs (rs10050860, rs27525 and rs17482078) known to be associated with psoriatic arthritis were found to have only a tendency in the association with EO-PsA patients (p=0.0663, p=0.0663 and p=0.0997, respectively) (Table 7). This lends further support to the notion that the differences in distribution of these SNPs detected in the total PsA group are primarily driven by the early onset subpopulation, and that these *ERAP1* SNPs presumably provide protection against early onset psoriatic arthritis.

Table 7
Distribution of *ERAP1* and *HLA-Cw* SNP genotypes in healthy controls and psoriasis patients

		Healthy controls (N=200)			PsV (N=319)						PsC (N=214)						PsA (N=105)					
Gene	**SNP**	**WT**	**Het**	**Hom rare**	**Disease onset**	**WT**	**Het**	**Hom rare**	**p***	**FDR**	**Disease onset**	**WT**	**Het**	**Hom rare**	**p***	**FDR**	**Disease onset**	**WT**	**Het**	**Hom rare**	**p***	**FDR**
HLA-C	**rs10484554**	127(63.5)	52 (26)	21 (10.5)	Total (N=319)	133 (41.7)	143 (44.8)	43 (13.5)	**$5.7x10^{-6}$**	**$5.9x10^{-5}$**	Total (N=214)	90 (42.1)	91 (42.5)	33 (15.4)	**0.00007**	**0,0007**	Total (N=105)	43 (41)	52 (49.5)	10 (9.5)	**0.0001**	**0,0007**
					Early (N=195)	68 (34.9)	96 (49.2)	31 (15.9)	**$7.5x10^{-8}$**	**$1.5x10^{-6}$**	Early (N=132)	46 (34.8)	62 (47)	24 (18.2)	**$2.1x10^{-6}$**	**0,**0001	Early (N=63)	22 (34.9)	34 (54)	7 (11.1)	**0.0001**	**0,0014**
					Late (N=124)	65 (52.4)	47 (37.9)	12 (9.7)	0.0707	0.2355	Late (N=82)	44 (53.7)	29 (35.4)	9 (10.9)	0.2570	0,6746	Late (N=42)	21 (50)	18 (42.9)	3 (7.1)	0.1004	0,1757
	rs10484545	170 (85)	30 (15)	0 (0)	Total (N=319)	272 (85.2)	44 (13.8)	3 (1)	0.4697	0.5480	Total (N=214)	183 (85.5)	30 (14)	1 (0.5)	0.9426	0,9426	Total (N=105)	89 (84.7)	14 (13.3)	2 (2)	0.1939	0,2026
					Early (N=195)	165 (84.6)	29 (14.9)	1 (0.5)	0.9433	0.9433	Early (N=132)	109 (82.6)	23 (17.4)	0 (0)	0.5550	0,7343	Early (N=63)	56 (88.9)	6 (9.5)	1 (1.6)	0.1400	0,1960
					Late (N=124)	107 (86.3)	15 (12.1)	2 (1.6)	0.1899	0.2849	Late (N=82)	74 (90.2)	7 (8.6)	1 (1.2)	0.0886	0,4442	Late (N=42)	33 (78.6)	8 (19)	1 (2.4)	0.1332	0,1960
ERAP1	**rs30187**	84 (42)	102 (51)	14 (7)	Total (N=319)	125 (39.2)	158 (49.5)	36 (11.3)	0.2673	0.3742	Total (N=214)	82 (38.3)	111 (51.9)	21 (9.8)	0.5136	0,7343	Total (N=105)	43 (40.9)	47 (44.8)	15 (14.3)	0.1102	0,1543
					Early (N=195)	81 (41.5)	94 (48.2)	20 (10.3)	0.5024	0.5553	Early (N=132)	52 (39.4)	67 (50.8)	13 (9.8)	0.6294	0,7343	Early (N=63)	29 (46)	27 (42.9)	7 (11.1)	0.3979	0,4642
					Late (N=124)	44 (35.5)	64 (51.6)	16 (12.9)	0.1565	0.2739	Late (N=82)	30 (36.6)	44 (53.7)	8 (9.7)	0.5864	0,7343	Late (N=42)	14 (33.4)	20 (47.6)	8 (19)	**0.0436**	0,1017
	rs10050860	118 (59)	79 (39.5)	3 (1.5)	Total (N=319)	207 (64.9)	97 (30.4)	15 (4.7)	**0.0249**	0.1743	Total (N=214)	132 (61.7)	72 (33.6)	10 (4.7)	0.1103	0,4442	Total (N=105)	75 (71.4)	25 (23.8)	5 (4.8)	**0.0072**	**0,0252**
					Early (N=195)	121 (62.1)	63 (32.3)	11 (5.6)	**0.0418**	0.2195	Early (N=132)	75 (56.8)	49 (37.1)	8 (6.1)	0.0877	0,4442	Early (N=63)	46 (73)	14 (22.2)	3 (4.8)	**0.0142**	0,0663
					Late (N=124)	86 (69.4)	34 (27.4)	4 (3.2)	0.0542	0.2276	Late (N=82)	57 (69.5)	23 (28)	2 (2.5)	0.1269	0,4442	Late (N=42)	29 (69)	11 (26.2)	2 (4.8)	0.0980	0,1757
	rs17482078	121 (60.5)	74 (37)	5 (2.5)	Total (N=319)	210 (65.8)	93 (29.2)	16 (5)	0.0897	0.2355	Total (N=214)	135 (63.1)	69 (32.2)	10 (4.7)	0.3436	0,7343	Total (N=105)	75 (71.4)	24 (22.9)	6 (5.7)	**0.0195**	**0,0453**
					Early (N=195)	123 (63.1)	61 (31.3)	11 (5.6)	0.1777	0.2849	Early (N=132)	78 (59.1)	47 (35.6)	7 (5.3)	0.4391	0,7343	Early (N=63)	45 (71.4)	14 (22.2)	4 (6.4)	**0.0356**	0,0997
					Late (N=124)	87 (70.2)	32 (25.8)	5 (4)	0.0850	0.2355	Late (N=82)	57 (69.5)	22 (26.8)	3 (3.7)	0.2302	0,6746	Late (N=42)	30 (71.4)	10 (23.8)	2 (4.8)	0.1608	0,2047
	rs27524	88 (44)	93 (46.5)	19 (9.5)	Total (N=319)	125 (39.2)	152 (47.6)	42 (13.2)	0.3439	0.4248	Total (N=214)	85 (39.7)	104 (48.6)	25 (11.7)	0.6028	0,7343	Total (N=105)	40 (38.1)	48 (45.7)	17 (16.2)	0.2026	0,2026
					Early (N=195)	81 (41.5)	92 (47.2)	22 (11.3)	0.7979	0.8378	Early (N=132)	55 (41.7)	62 (47)	15 (11.3)	0.8295	0,8710	Early (N=63)	26 (41.3)	30 (47.6)	7 (11.1)	0.8953	0,8953
					Late (N=124)	44 (35.5)	60 (48.4)	20 (16.1)	0.1215	0.2677	Late (N=82)	30 (36.6)	42 (51.2)	10 (12.2)	0.4854	0,7343	Late (N=42)	14 (33.3)	18 (42.9)	10 (23.8)	**0.0307**	0,0997
	rs27525	55 (27.5)	145 (72.5)	0 (0)	Total (N=319)	108 (33.9)	211 (66.1)	0 (0)	0.1289	0.2677	Total (N=214)	66 (30.8)	148 (69.2)	0 (0)	0.4551	0,7343	Total (N=105)	42 (40)	63 (60)	0 (0)	**0.0259**	**0,0453**
					Early (N=195)	67 (34.4)	128 (65.6)	0 (0)	0.1402	0.2677	Early (N=132)	39 (29.5)	93 (70.5)	0 (0)	0.6856	0,7578	Early (N=63)	28 (44.4)	35 (55.6)	0 (0)	**0.0116**	0,0663
					Late (N=124)	41 (33.1)	83 (66.9)	0 (0)	0.2864	0.3759	Late (N=82)	27 (32.9)	55 (67.1)	0 (0)	0.3621	0,7343	Late (N=42)	14 (33.3)	28 (66.7)	0 (0)	0.4465	0,4808

WT, both alleles are wild type; Het, patient carrying the rare allele in one copy; Hom rare, patient carrying the rare allele in two copies; listed as [N, (%)]

* Significant compared to healthy controls

4.2.2. *ERAP1* haplotypes in healthy controls and patients

The *ERAP1* rs17482078, rs10050860, rs30187 and rs2287987 SNPs were found to be in strong linkage disequilibrium, and the association between haplotypes including these SNPs and AS was reported [96]. A linkage disequilibrium block containing the rs17482078/rs10050860/rs30187 SNPs was also identified in our dataset (Figure 1). Thus, we examined whether the rs17482078/rs10050860/rs30187 haplotypes were associated with psoriasis susceptibility (Table 8). We found that Haplotype B (rs17482078/rs10050860/rs30187-CCT) was a risk factor only for LO-PsV (p=0.0409) and for LO-PsA (p=0.0413).

Figure 1
Linkage disequilibrium (LD) for the *ERAP1* SNPs in the study population

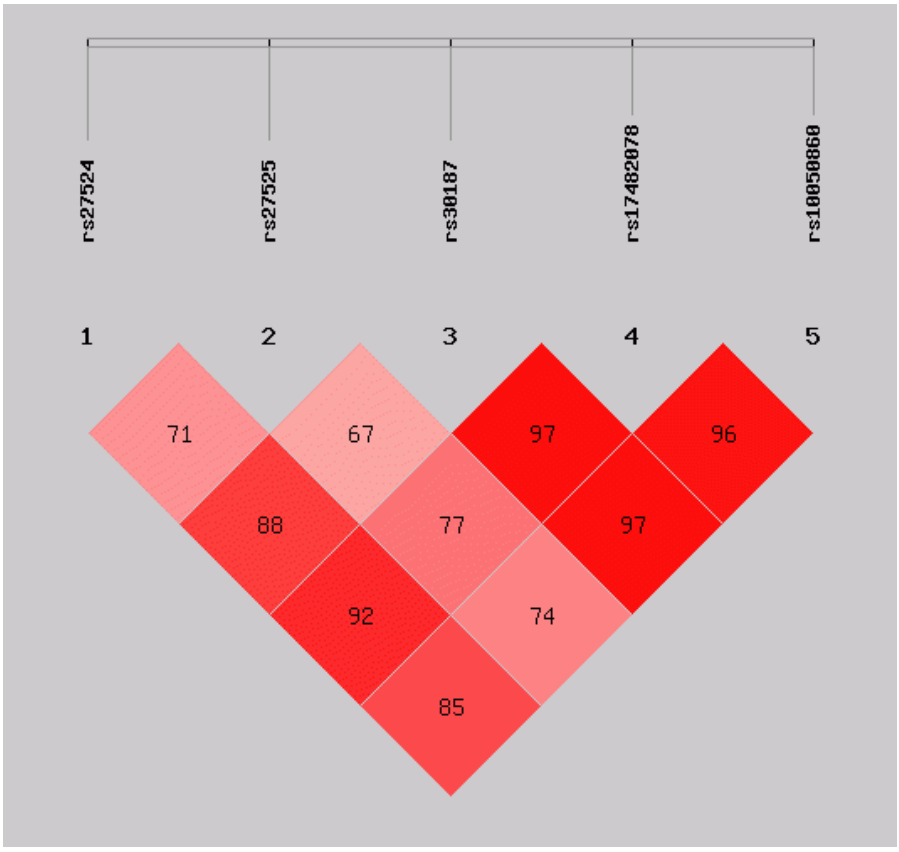

Table 8
Distribution of *ERAP1* haplotypes in healthy controls and psoriasis patients

	SNPs			Healthy controls (N=200)	PsV (N=319)			PsC (N=214)			PsA (N=105)		
Haplotype	**rs17482078**	**rs10050860**	**rs30187**	**Frequency**	**Disease onset**	**Frequency**	**p***	**Disease onset**	**Frequency**	**p***	**Disease onset**	**Frequency**	**p***
Haplotype A	C	C	C	38 (19)	Total (N=319)	58 (18.2)	0.8153	Total (N=214)	35 (16.4)	0.4804	Total (N=105)	23 (21.9)	0.5468
					Early (N=195)	36 (18.5)	0.8909	Early (N=132)	18 (13.6)	0.2015	Early (N=63)	18 (28.6)	0.1056
					Late (N=124)	22 (17.7)	0.7769	Late (N=82)	17 (20.7)	0.7389	Late (N=42)	5 (11.9)	0.2741
Haplotype B	C	C	T	80 (40)	Total (N=319)	148 (46.4)	0.1531	Total (N=214)	97 (45.3)	0.2736	Total (N=105)	51 (48.6)	0.1508
					Early (N=195)	84 (43)	0.5349	Early (N=132)	57 (43.2)	0.5644	Early (N=63)	27 (42.9)	0.6873
					Late (N=124)	64 (51.6)	**0.0409**	Late (N=82)	40 (48.8)	0.1756	Late (N=42)	24 (57.1)	**0.0413**
Haplotype C	T	T	C	47 (23.5)	Total (N=319)	64(20)	0.5220	Total (N=214)	45 (21)	0.7184	Total (N=105)	19 (18.1)	0.4624
					Early (N=195)	43(22.1)	0.8875	Early (N=132)	32 (24.2)	1	Early (N=63)	11 (17.6)	0.5169
					Late (N=124)	21 (16.9)	0.3125	Late (N=82)	13 (15.9)	0.3149	Late (N=42)	8 (19)	0.7642
Haplotype D	T	T	T	33 (16.5)	Total (N=319)	44 (13.8)	0.3985	Total (N=214)	34 (15.9)	0.8658	Total (N=105)	10 (9.5)	0.0962
					Early (N=195)	28 (14.4)	0.5560	Early (N=132)	22 (16.7)	0.9681	Early (N=63)	6 (9.5)	0.1742
					Late (N=124)	16 (12.9)	0.3798	Late (N=82)	12 (14.6)	0.6976	Late (N=42)	4 (9.5)	0.1842

Frequency is listed as [N, (%)]

* Significant compared to healthy controls carrying the given haplotype

4.2.3. *HLA-C* and *ERAP1* interactions in patients

As it was reported earlier that some *ERAP1* variants influence psoriasis susceptibility exclusively in individuals carrying the *HLA-C* risk allele [13,14,15], we analyzed *ERAP1* SNPs in *HLA-C* positive psoriasis and psoriatic arthritis patients. *ERAP1* SNP frequencies were compared between individuals carrying at least one copy of the risk allele of rs10484554 (*HLA-C* positive) and individuals not carrying the *HLA-C* sequence (*HLA-C* negative) (Table 9). Evidence for association in *HLA-C* positive individuals was observed for two loci. The *ERAP1* rs27524 SNP in *HLA-C* positive individuals exhibited a 1.74-fold increased risk for PsV (p=0.0454) and a 2.33-fold risk for PsA (p=0.0185), in agreement with the previous report [13]. Interestingly, rs27524 by itself was not associated in our dataset with either PsV or PsC and was found to have a tendency as a susceptibility factor only for LO-PsA (Table 7). The *ERAP1* rs27525 SNP decreased the risk for psoriatic arthritis development in *HLA-C* positive patients (odds ratio (OR) 0.42, p=0.0339). Thus, the presence of the *ERAP1* rs27525 SNP seems to protect *HLA-C* positive individuals from developing psoriatic arthritis. No other interaction was found for the *ERAP1* rs30187, rs10050860 and rs17482078 SNPs in *HLA-C* positive individuals, even after further stratifying the patients into early and late onset subgroups.

Table 9
HLA-C and *ERAP1* interactions in psoriasis and psoriatic arthritis

	PsV (N=319)				PsC (N=214)				PsA (N=105)			
ERAP1 SNP	**Disease onset**	**p***	**OR**	**95% CI**	**Disease onset**	**p***	**OR**	**95% CI**	**Disease onset**	**p***	**OR**	**95% CI**
rs30187	Total (N=319)	0.0506	1.72	0.99-2.97	Total (N=214)	0.0869	1.66	0.93-2.98	Total (N=105)	0.0818	1.84	0.92-3.67
	Early (N=195)	0.0643	1.73	0.97-3.09	Early (N=132)	0.0837	1.74	0.93-3.28	Early (N=63)	0.1812	1.70	0.78-3.69
	Late (N=124)	0.1342	1.70	0.85-3.41	Late (N=82)	0.3197	1.49	0.68-3.29	Late (N=42)	0.1302	2.17	0.79-6.00
s10050860	Total (N=319)	0.2402	0.72	0.41-1.25	Total (N=214)	0.3092	0.73	0.40-1.33	Total (N=105)	0.2893	0.68	0.34-1.39
	Early (N=195)	0.2550	0.71	0.39-1.28	Early (N=132)	0.4206	0.77	0.40-1.46	Early (N=63)	0.2090	0.59	0.26-1.34
	Late (N=124)	0.3967	0.74	0.36-1.50	Late (N=82)	0.3266	0.66	0.29-1.51	Late (N=42)	0.8050	0.88	0.33-2.39
rs17482078	Total (N=319)	0.2080	0.70	0.40-1.22	Total (N=214)	0.2568	0.71	0.39-1.29	Total (N=105)	0.2893	0.68	0.34-1.39
	Early (N=195)	0.2550	0.71	0.39-1.28	Early (N=132)	0.3374	0.73	0.38-1.39	Early (N=63)	0.3209	0.67	0.30-1.49
	Late (N=124)	0.2931	0.68	0.33-1.40	Late (N=82)	0.3266	0.66	0.29-1.51	Late (N=42)	0.5211	0.72	0.26-1.99
rs27524	Total (N=319)	**0.0454**	**1.74**	**1.01-3.01**	Total (N=214)	0.1572	1.52	0.85-2.72	Total (N=105)	**0.0185**	**2.33**	**1.15-4.72**
	Early (N=195)	0.0592	1.75	0.98-3.13	Early (N=132)	0.1587	1.57	0.84-2.95	Early (N=63)	0.0500	2.21	0.99-4.94
	Late (N=124)	0.1238	1.73	0.86-3.48	Late (N=82)	0.3905	1.41	0.64-3.12	Late (N=42)	0.0730	2.57	0.90-7.36
rs27525	Total (N=319)	0.1502	0.64	0.35-1.18	Total (N=214)	0.3682	0.74	0.38-1.43	Total (N=105)	0.0516	0.48	0.23-1.01
	Early (N=195)	0.1142	0.60	0.31-1.14	Early (N=132)	0.3473	0.72	0.36-1.44	Early (N=63)	**0.0339**	**0.42**	**0.19-0.94**
	Late (N=124)	0.4530	0.75	0.35-1.61	Late (N=82)	0.6254	0.80	0.33-1.94	Late (N=42)	0.4278	0.65	0.23-1.87

* Individuals carrying at least one copy of the rare allele of the rs10484554 *HLA-C* SNP and both wild type alleles of the indicated *ERAP1* SNP (*HLA-C* positive/*ERAP1* negative) are compared with individuals carrying at least one copy of the rare allele of the rs10484554 *HLA-C* SNP and at least one copy of the rare allele of the indicated *ERAP1* SNP (*HLA-C* positive/*ERAP1* positive).

5. Discussion

Genetic examinations are important in polygenic or multifactorial diseases like psoriasis, psoriatic arthritis, as from permutations and combinations of common gene variants, where each variant alone confers only a small risk but together or with other extraneous influences becomes pathogenic. Recent therapeutic recommendations regarding the place of biotherapies are based directly on immunological mechanisms and genetic backgrounds that have recently been elucidated.

We reported the first examination of *TNFSF15* gene variants in the Hungarian population. Our results suggest that the rs6478109 SNP of *TNFSF15* gene contributes to psoriasis susceptibility and this association is more apparent when the group of psoriatic patients with and without arthritis are analyzed separately. A significant difference was also found in the distribution of Haplotype C in the groups of healthy controls and PsV patients. The proportion of healthy individuals with Haplotype C was higher in the control group than in the group of patients with psoriasis. This result suggests that individuals with Haplotype C might be protected against psoriasis. However, due to the relatively low frequency of Haplotype C in our survey, it would be necessary to enroll more patients, preferably including multiple ethnicities, before it would be possible draw a conclusion about the protective nature haplotype C with respect to psoriasis.

The rs6478109 SNP is situated in the promoter region of the *TNFSF15* gene, 474 nt upstream of the transcription start site. According to *in silico* analysis (http://www.cbrc.jp/research/db/TFSEARCH.html), the region harboring the SNP potentially binds five transcription factors (GATA-1, GATA-2, GATA-3, Jk2, and c/EBPb). This observation suggests that the rs6478109 SNP might influence the expression of the *TNFSF15* gene and, by altering expression of the gene product, contributes to the pathogenesis of immune-mediated diseases.

TNFSF15 can be considered a novel common gene in the pathomechanism of IBD and psoriasis. Lymphocytes, monocytes and dendritic cells are the major sources of TNFSF15, which associates with death domain receptor 3 (DR3) [64,70]. Upregulation of the TNFSF/DR3 system is involved in the pathogenesis of chronic inflammatory diseases, such as IBDs, rheumatoid arthritis and psoriasis [66,68,69]. Expression of the TNFSF/DR3 system is enhanced in psoriatic skin at mRNA and protein levels [71,97]. TNFSF15/DR3 may contribute to psoriatic inflammation by enhancing Th1 and Th17 responses [98,99].

Yamazaki et al reported the association between *TNFSF1*5 gene variants and IBDs: five SNPs were associated with IBD phenotypes in Japanese and UK patients. These five polymorphic SNPs formed two frequent disease-associated haplotypes; the 'high-risk' Haplotype A and the 'low-risk' Haplotype B [100]. Picornell et al were unable to confirm the above association in a Jewish population; however, Haplotype B was found to be protective against CDs in non-Jewish patients [72]. In another study, the role of 'high risk' Haplotype A was confirmed in CD for a Korean population [90]. All these data suggest that the *TNFSF15* gene variants contribute to immune-mediated diseases in an ethnicity-specific manner.

The increased risk of psoriasis in patients with IBD has been reported [101,102,103]. This clinical overlap is supported by several genetic findings. Safrany et al have reported the association of *interleukin-23 receptor* gene SNPs in Hungarian patients with ulcerative colitis (UC) and psoriasis [104]. *IL12B* has also been confirmed as a susceptibility gene for both IBD and psoriasis [105].

Psoriasis has been associated with the *PSORS1* disease susceptibility locus of the MHC class I region on chromosome 6. Within *PSORS1*, the *HLA-Cw6* gene has shown the strongest association with psoriasis [9]: about 60% of psoriasis patients carry the *HLA-Cw*0602* allele. *HLA-C* positive patients exhibit earlier disease onset, higher incidence of guttate and eruptive type of psoriasis, more extensive disease symptoms, and more frequent exacerbations caused by throat infections than *HLA-C* negative individuals. The frequency of *HLA-Cw*0602* is significantly lower in patients with psoriatic arthritis compared with those with psoriasis alone, and its presence is associated with a longer psoriasis–arthritis interval and milder arthritis forms [95,106,107].

In the second part of this study, a well-characterized cohort of psoriasis patients was available for investigating whether *HLA-Cw6* or *ERAP1* gene polymorphisms are associated with different clinical phenotypes of psoriasis and psoriatic arthritis. Our dataset included patients with early and late onset psoriasis vulgaris, as well as with cutaneous psoriasis and psoriatic arthritis.

Our results confirmed the previously reported genetic association of psoriasis with both *HLA-Cw6* and *ERAP1* genes [13,14,15]. The *HLA-Cw*0602* rs10484554 SNP was found to have very strong association with PsV, and the association was highly significant with both PsC and PsA groups as well (although somewhat stronger with PsC). Interestingly, only EO-PsV was associated with *HLA-C* in our dataset. Previously, the *HLA-Cw*06* allele was not found to be a risk factor for late onset psoriasis in a Northern Polish population [109], and late onset psoriasis demonstrated only a weak association with *HLA-Cw*06* alleles in another

studies [14,109]. However, a study using dense genotyping revealed that *HLA-Cw*06* is associated with late onset psoriasis [110].

Genetic variants of *ERAP1* have been reported to be associated with psoriasis [14,15,73]. ERAP1 trims peptides imported into the endoplasmic reticulum at their N-terminus and contributes to the shaping of the antigenic repertoire presented by class I MHC molecules [76]. The association of *ERAP1* with psoriatic arthritis has previously been investigated; however, no association between *ERAP1* SNPs and psoriatic arthritis or psoriatic arthritis subphenotypes was identified [85]. *ERAP1* association with psoriasis was detected in patients with an age of onset between 10 and 20 years, and no association was detected in cases with onset below 10 years [73].

Our results suggest that the *HLA-C* rs10484554 SNP contributes to psoriasis susceptibility. The proportions of the rare alleles of the *ERAP1* rs27525, rs17482078 and rs10050860 SNPs were higher in the group of healthy individuals, suggesting that individuals with the rare alleles of these SNPs might be protected against psoriasis. These associations were also apparent when groups of psoriatic patients were analyzed separately according to the presence or absence of arthritis. The stratification of the patients for early (<40 years) or late (≥40 years) disease onset revealed an age-dependent difference in the genetic background of psoriasis: the associations with these SNPs tend to be stronger in patients with early disease onset.

Although *ERAP1* was neither dependent on nor interacting with *HLA-C*06:02* in certain populations, an interaction between *HLA-C* and *ERAP1* was reported and confirmed several times subsequently [10,13,14,15]. Interestingly, dominant epistasis between *HLA-Cw*0602* and one of the *ERAP1* SNPs was identified in psoriasis [81].

Studies of the *ERAP1* rs27524 SNP reported association with the pathogenesis of psoriasis, especially in the group with age of disease onset between 10 and 20 years [73,111]. Our results revealed only a trend in association with the late disease onset group, for which it might increase the likelihood of psoriasis development. No association with psoriasis was observed for the *ERAP1* rs30187 SNP in this study, which is in contrast with previous studies [13,73]; however, Jadon et al. also reported that there was no association with psoriatic arthritis [85]. In addition, this psoriasis-risk allele, which is common in the European population, was not associated with the disease in an East Asian population [82].

Psoriatic arthritis patients, especially in the early onset group, carrying the *ERAP1* rs27525 and rs17482078 SNPs seem to be protected from the subsequent development of the disease. Individuals carrying the rare allele of the *ERAP1* rs10050860 SNPs might be

protected against psoriasis, but this effect was more prominent among patients with psoriatic arthritis, especially in the early onset group. These findings suggest that these three SNPs (rs27525, rs17482078, rs10050860) might be protective against psoriatic arthritis.

The two coding *ERAP1* SNPs (rs17482078 and rs10050860) and the rs30187 SNP are in linkage disequilibrium (Figure 1) and were reported as a protective haplotype (rs17482078/rs100508607rs30187/rs2287987–TTCC) in *HLA-B* positive AS patients [96]. Also the rs17482078/rs10050860/rs30187-CCT haplotype was confirmed as a risk factor for AS [112] in an AS population in Belgium, which we examined as Haplotype B (rs17482078/rs10050860/rs30187-CCT) in psoriasis susceptibility similarly to AS [96], and it was found to be a risk factor for only LO-PsV (p=0.0409) and LO-PsA (p=0.0413). We were unable to find any association with psoriasis or with psoriatic arthritis for Haplotype A (rs17482078/rs10050860/rs30187-CCC), Haplotype C (rs17482078/rs10050860/rs30187-TTC) and Haplotype D (rs17482078/rs10050860/rs30187-TTC). These data suggest that the Haplotype B conferring disease risk in AS also influences susceptibility to joint involvement in psoriasis.

Genome-wide association studies identified interaction between the *ERAP1* rs27524 SNP and the *HLA-C* rs10484554 SNP; this interaction was the most prominent among individuals carrying one or two copies of the risk allele at rs10484554 [13]. In subsequent analysis, the association with *ERAP1* was not restricted to individuals carrying *HLA-Cw*0602*; however the genetic association with *ERAP1* (rs207524, rs30187, rs26653) in psoriasis was confined to individuals with an age of disease onset between 10 and 20 years [73]. In a Polish population *ERAP1* rs27524 SNP was a susceptibility factor for *HLA-C* positive patients with late-onset psoriasis [14]. In our study, the *ERAP1* rs27524 SNP in *HLA-C* positive individuals caused a 1.74-fold increased risk for PsV (p=0.0454) and a 2.33-fold increased risk for PsA (p=0.0185). Notably, the rs27524 SNP by itself was not associated either with PsV or with PsC in this dataset and was found to have only a tendency as a susceptibility factor for LO-PsA (Table 7). The *ERAP1* rs27525 SNP in *HLA-C* positive patients decreased the risk for psoriatic arthritis development (OR 0.42, p=0.0339); thus, the presence of these SNPs seems to protect against developing psoriatic arthritis.

6. Summary

Taken together with previous reports, our results suggest that the genetic variants of the *TNFSF15* gene contribute to the pathogenesis of the immune-mediated, multifactorial skin disease psoriasis and the genetic variants of the *ERAP1* and *HLA-C* genes contribute to the pathogenesis of psoriasis in a manner that is dependent on age of onset. Individuals with *HLA-Cw*0602* are more prone to early onset of disease (before 40 years), confirming that onset after 40 years represents a biologically valid approximation for a genetically distinct subgroup of psoriasis. The overall psoriasis group was stratified by various clinical aspects, including the age of onset and the presence or absence of psoriatic arthritis. This novel and careful stratification of patients according to the symptoms and age of onset lead to important insights for psoriasis, a heterogeneous, multifactorial disease, and might become more important for further research as well as for personalized medicine.

Finally, the interpretation of our results in view of the literature suggests that polymorphisms of immune-regulatory genes are key in several inflammatory diseases, and that ethnicity-specific aspects must also be considered.

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8. References

1. Raharja A, Mahil SK, Barker JN. Psoriasis: a brief overview. *Clinical Medicine* 2021;21(3):170–3.
2. Benjegerdes K, Hyde K, Kivelevitch D, et al. Pustular psoriasis: pathophysiology and current treatment perspectives. *Psoriasis Auckl* 2016;6:131–44.
3. Henseler T, Christophers E. Psoriasis of early and late onset: Characterization of two types of psoriasis vulgaris. *J Am Acad Dermatol* 1985;13:450-6.
4. Nair RP, Stuart P, Henseler T, et al. Localization of Psoriasis-Susceptibility Locus PSORS1 to a 60-kb Interval Telomeric to HLA-C. *Am J Hum Genet* 2000;66(6):1833-44.
5. Alonso S, Tejón P, Sarasqueta C, et al. Age at disease onset may help to further characterize the disease phenotype in psoriatic arthritis. *Joint Bone Spine* 2016;83(5):533-7.
6. Griffiths CE, Baker JN. Pathogenesis and clinical feathers of psoriasis. *Lancet* 2007;370:263-271.
7. Nickoloff BJ, Nestle FO. Recent insight into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *J Clin Invest* 2004;113: 1664-1675.
8. Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. *Ann Rev Immunol* 2014;32:227-55.
9. Chandran V. The genetics of psoriasis and psoriatic arthritis. *Clin Rev Allergy Immunol* 2013;44(2):149-56.
10. Bergboer JG, Zeeuwen PL, Schalkwijk J. Genetics of psoriasis: evidence for epistatic interaction between skin barrier abnormalities and immune deviation. *J Invest Dermatol* 2012;132(10):2320-31.
11. de Cid R, Riveira-Munoz E, Zeeuwen PL, et al. Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. *Nat Genet* 2009;41(2):211-5.
12. Kepiro L, Szell M, Kovacs L, et al. Genetic risk and protective factors of TNFSF15 gene variants detected using single nucleotide polymorphisms in Hungarians with psoriasis and psoriatic arthritis. *Hum Immunol* 2014;75(2):159-62.
13. Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium, Strange A, Capon F, et al. A genome-wide association study identifies

new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet* 2010;42(11):985-90.

14. Wiśniewski A, Matusiak L, Szczerkowska-Dobosz A, et al. 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. *Hum Immunol* 2017;17:30551-7.
15. Das A, Chandra A, Chakraborty J, et al. Associations of ERAP1 coding variants and domain specific interaction with HLA-C∗06 in the early onset psoriasis patients of India. *Hum Immunol* 2017;78:724-730.
16. Dand N, Mahil SK, Capon F, et al. Psoriasis and genetics. *Acta Derm Venereol* 2020;100:adv00030.
17. Schön MP, Erpenbeck L. The interleukin-23/interleukin-17 axis links adaptive and innate immunity in psoriasis. *Front Immunol* 2018;9:1323.
18. Onoufriadis A, Simpson MA, Pink AE, et al. Mutations in IL36RN/ IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. *Am J Hum Genet* 2011;89:432–437.
19. National Institute for Health and Care Excellence. Psoriasis: assessment management: Clinical guidline [CG153]. *NICE* 2017.
20. Alwawi EA, Mehlis SL, Gordon KB. Treating Psoriasis with Adalimumab. *Ther Clin Risk Manag* 2008;4:345–351.
21. Campanati A, Diotallevi F, Martina E, et al. Safety update of etanercept treatment for moderate to severe plaque psoriasis. *Expert Opin Drug Saf* 2020;PMID: 32178543 Review.
22. Gall JS, Kalb RE, Infliximab for the Treatment of Plaque Psoriasis. *Biologics* 2008;2:115–124.
23. Blauvelt A, Reich K, Lebwohl M, et al. Certolizumab Pegol for the Treatment of Patients with Moderate-to-severe Chronic Plaque Psoriasis: Pooled Analysis of Week 16 Data from Three Randomized Controlled Trials. *J Eur Acad Dermatol Venereol* 2018;33:546–552.
24. Farhi D. Ustekinumab for the treatment of psoriasis: Review of three multicenter clinical trials. *Drugs Today* 2010;46:259–264.
25. Witjes H, Khatri A, Diderichsen PM, et al. Meta-Analyses of Clinical Ecacy of Risankizumab and Adalimumab in Chronic Plaque Psoriasis: Supporting Evidence of Risankizumab Superiority. *Clin Pharmacol Ther* 2019;107:435–442.

26. Nogueira M, Torres T. Guselkumab for the treatment of psoriasis—evidence to date. *Drugs Context* 2019;8:1–11.
27. Blauvelt A, Sofen H, Papp K, et al. Tildrakizumab efficacy and impact on quality of life up to 52 weeks in patients with moderate-to-severe psoriasis: A pooled analysis of two randomized controlled trials. *J Eur Acad Dermatol Venereol* 2019;33:2305–2312.
28. Papp KA, Leonardi CL, Blauvelt A, et al. Ixekizumab Treatment for Psoriasis: Integrated Ecacy Analysis of Three Double-Blinded, Controlled Studies (UNCOVER-1, UNCOVER-2, UNCOVER-3). *Br J Dermatol* 2018;178:674–681.
29. López-Ferrer A, Vilarrasa E, Puig L. Secukinumab (AIN457) for the treatment of psoriasis. *Expert Rev Clin Immunol* 2015;11:1177–1188.
30. Foulkes AC, Warren RB. Brodalumab in Psoriasis: Evidence to Date and Clinical Potential. *Drugs Context* 2019;8:1–11.
31. Papp KA, Menter MA, Abe M, et al. Tofacitinib, an Oral Janus Kinase Inhibitor, for the Treatment of Chronic Plaque Psoriasis: Results from Two Randomized, Placebo-controlled, Phase III Trials. *Br J Dermatol* 2015;173:949–961.
32. Nast A, Smith C, Spuls PI, et al. EuroGuiDerm Guideline on the systemic treatment of Psoriasis vulgaris – Part 2: specific clinical and comorbid situations. *J Eur Acad Dermatology Venereol* 2021;35:281–317.
33. Smith CH, Yiu ZZN, Bale T, et al. British Association of Dermatologists guidelines for biologic therapy for psoriasis 2020: a rapid update. *Br J Dermatol* 2020;183:628–37.
34. Dand N, Duckworth M, Baudry D, et al. HLA-C*06:02 genotype is a predictive biomarker of biologic treatment response in psoriasis. *J Allergy Clin Immunol* 2019;143:2120–30.
35. Bachelez H, Choon S, Marrakchi S et al. Inhibition of the Interleukin-36 Pathway for the Treatment of Generalized Pustular Psoriasis. *N Engl J Med* 2019;380:981–983.
36. Gladman DD, Antoni C, Mease P, et al. Psoriatic arthritis: epidemiology, clinical features, course, and outcome. *Ann Rheum Dis* 2005;64:i14-7.
37. Taylor W, Gladman D, Helliwell P, et al.: Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006;54(8):2665–73.
38. Ocampo VD, Gladman D. Psoriatic arthritis. *F1000Research* 2019; 8:F1000 Faculty Rev-1665.

39. Wilson FC, Icen M, Crowson CS, et al. Incidence and clinical predictors of psoriatic arthritis in patients with psoriasis: a population-based study. *Arthritis Rheum* 2009;61:233–239.
40. Patrizi A, Venturi M, Scorzoni R, et al. Nail dystrophies, scalp and intergluteal/perianal psoriatic lesions: risk factors for psoriatic arthritis in mild skin psoriasis? *G Ital Dermatol Venereol* 2014;149:177–184.
41. Takata T, Takahashi A, Taniguchi Y, et al. Detection of asymptomatic enthesitis in psoriasis patients: an onset of psoriatic arthritis? *J Dermatol* 2016;43:650–654.
42. Papadavid E, Katsimbri P, Kapniari I, et al. Prevalence of psoriatic arthritis and its correlates among patients with psoriasis in Greece: results from a large retrospective study. *J Eur Acad Dermatol Venereol* 2016;30:1749–1752.
43. Queiro R, Tejón P, Alonso S, et al. Age at disease onset: a key factor for understanding psoriatic disease. *Rheumatology (Oxford)* 2014;53(7):1178-85.
44. Fitzgerald O, Winchester R. Psoriatic arthritis: from pathogenesis to therapy. *Arthritis Res Ther* 2009;11:214.
45. Suzuki E, Mellins ED, Gershwin ME, et al. The IL-23/IL-17 axis in psoriatic arthritis. *Autoimmun Rev* 2014;13:496–502.
46. Gottlieb A, Merola JF. Psoriatic arthritis for dermatologists. *J Dermatolog Treat* 2020;31(7):662-679.
47. Coates LC, Helliwell PS. Psoriatic arthritis: state of the art review. *Clinical Medicine* 2017;17(1):65–70.
48. Coates LC, Kavanaugh A, Mease PJ, et al. Group for Research and Assessment of Psoriasis and Psoriatic Arthritis 2015 Treatment Recommendations for Psoriatic Arthritis. *Arthritis Rheumatol* 2016;68:1060-71.
49. Ogdie A, Coates LC, Gladman DD. Treatment guidelines in psoriatic arthritis. *Rheumatology* 2020;59:37-46.
50. Elyoussfi S, Thomas BJ, Ciurtin C. Tailored treatment options for patients with psoriatic arthritis and psoriasis: Review of established and new biologic and small molecule therapies. *Rheumatol Int* 2016;36(5):603–12.
51. Mease PJ, Fleischmann R, Deodhar AA, et al. Effect of certolizumab pegol on signs and symptoms in patients with psoriatic arthritis: 24-week results of a Phase 3 double-blind randomised placebo-controlled study (RAPID-PsA). *Ann Rheum Dis* 2013;73(1):48–55.

52. Mease PJ, Gladman DD, Collier DH, et al. Etanercept and Methotrexate as Monotherapy or in Combination for Psoriatic Arthritis: Primary Results From a Randomized, Controlled Phase III Trial. *Arthritis Rheumatol* 2019;71(7):1112–1124.
53. Kavanaugh A, Husni ME, Harrison DD, et al. Safety and Efficacy of Intravenous Golimumab in Patients With Active Psoriatic Arthritis: Results Through Week Twenty-Four of the GO-VIBRANT Study. *Arthritis Rheumatol* 2017;69(11):2151–61.
54. Antoni C, Krueger GG, de Vlam K, et al. Infliximab improves signs and symptoms of psoriatic arthritis: results of the IMPACT 2 trial. *Ann Rheum Dis* 2005;64(8):1150–7.
55. Mease PJ, McInnes IB, Kirkham B, et al. Secukinumab Inhibition of Interleukin-17A in Patients with Psoriatic Arthritis. *N Engl J Med* 2015;373(14):1329–39.
56. Mease PJ, van der Heijde D, Ritchlin CT, et al. Ixekizumab, an interleukin-17A specific monoclonal antibody, for the treatment of biologic-naive patients with active psoriatic arthritis: results from the 24-week randomised, double-blind, placebo-controlled and active (adalimumab)-controlled period of the phase III trial SPIRIT-P1. *Ann Rheum Dis* 2017;76(1):79–87.
57. Kavanaugh A, Ritchlin C, Rahman P, et al. Ustekinumab, an anti-IL-12/23 p40 monoclonal antibody, inhibits radiographic progression in patients with active psoriatic arthritis: results of an integrated analysis of radiographic data from the phase 3, multicentre, randomised, double-blind, placebo-controlled PSUMMIT-1 and PSUMMIT-2 trials. *Ann Rheum Dis* 2014;73(6):1000–6.
58. Gladman D, Rigby W, Azevedo VF, et al. Tofacitinib for Psoriatic Arthritis in Patients with an Inadequate Response to TNF Inhibitors. *N Engl J Med* 2017;377(16):1525–36.
59. Mease PJ, Gottlieb AB, van der Heijde D, et al. Efficacy and safety of abatacept, a T-cell modulator, in a randomised, double-blind, placebo-controlled, phase III study in psoriatic arthritis. *Ann Rheum Dis* 2017;76(9):1550–8.
60. Nickoloff BJ. The cytokine network in psoriasis. *Arch Dermatol* 1991;127:871-884.
61. Veale DJ, Ritchlin C, Fitzgerald O. Immunopathology of psoriasis and psoriatic arthritis. *Ann Rheum Dis* 2005;64:26-29.
62. Yue TL, Ni J, Romanic AM, Gu JL, et al. TL1, a novel tumor necrosis f actor-like cytokine, induces apoptosis in endothelial cells. Involvement of activation of stress protein kinases (stress-activated protein kinase and p38 mitogen-activated protein kinase) and caspase-3-like protease. *J Biol Chem* 1999;274:1479-1486.

63. Zhai Y, Ni J, Jiang GW, et al. VEGI, a novel cytokine of the tumor necrosis factor family, is an angiogenesis inhibitor that suppresses the growth of colon carcinomas in vivo. *FASEB J* 1999;13:181–189.
64. Migone TS, Zhang J, Luo X, et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. *Immunity* 2002;16:479-92.
65. Zhang Z, Li LY. TNFSF15 Modulates Neovascularization and Inflammation. *Cancer Microenviron* 2012;5(3):237-47.
66. Thiébaut R, Kotti S, Jung C, et al. TNFSF15 polymorphisms are associated with susceptibility to inflammatory bowel disease in a new European cohort. *Am J Gastroenterol* 2009;104(2):384-91.
67. Kang YJ, Kim WJ, Bae HU, et al. Involvement of TL1A and DR3 in induction of pro-inflammatory cytokines and matrix metalloproteinase-9 in atherogenesis. *Cytokine* 2005;29(5):229-35.
68. Cassatella MA, Pereira-da-Silva G, Tinazzi I, et al. Soluble TNF-like cytokine (TL1A) production by immune complexes stimulated monocytes in rheumatoid arthritis. *J Immunol* 2007;178(11):7325-33.
69. Bamias G, Evangelou K, Vergou T, et al. Upregulation and nuclear localization of TNF-like cytokine 1A (TL1A) and its receptors DR3 and DcR3 in psoriatic skin lesions. *Exp Dermatol* 2011;20(9):725-31.
70. Bamias G, Martin C 3rd, Marini M, et al. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. *J Immunol* 2003;171(9):4868-74.
71. Bamias G, Evangelou K, Vergou T, et al. Upregulation and nuclear localization of TNF-like cytokine 1A (TL1A) and its receptors DR3 and DcR3 in psoriatic skin lesions. *Exp Dermatol* 2011;20(9):725-31.
72. Picornell Y, Mei L, Taylor K, et al. TNFSF15 is an ethnic-specific IBD gene. *Inflamm Bowel Dis* 2007;13(11):1333-8.
73. Lysell J, Padyukov L, Kockum I,et al. Genetic association with ERAP1 in psoriasis is confined to disease onset after puberty and not dependent on HLA-C*06. *J Invest Dermatol* 2013;133(2):411-7.
74. Kirino Y1, Bertsias G, Ishigatsubo Y, et al. Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between HLA-B*51 and ERAP1. *Nat Genet* 2013;45(2):202-7.

75. Tanioka T, Hattori A, Masuda S, et al. Human leukocyte-derived arginine aminopeptidase. The third member of the oxytocinase subfamily of aminopeptidases. *J Biol Chem* 2003;278(34):32275-83.
76. Jensen PE. Recent advances in antigen processing and presentation. *Nat Immunol* 2007;8(10):1041-8.
77. Wearsch PA, Cresswell P. The quality control of MHC class I peptide loading. *Curr Opin Cell Biol* 2008;20(6):624-31.
78. Arakawa A, Reeves E, Vollmer S, et al. ERAP1 Controls the Autoimmune Response against Melanocytes in Psoriasis by Generating the Melanocyte Autoantigen and Regulating its Amount for HLA-C*06:02 Presentation. *J Immunol* 2021;207(9):2235–2244.
79. Haroon N, Inman RD. Endoplasmic reticulum aminopeptidases: Biology and pathogenic potential. *Nat Rev Rheumatol* 2010;6(8):461-7.
80. Guerini FR, Cagliani R, Forni D, et al. A functional variant in ERAP1 predisposes to multiple sclerosis. *PLoS One* 2012;7(1):e29931.
81. Tsoi LC, Spain SL, Knight J, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat Genet* 2012;44(12):1341-8.
82. Tang H, Jin X, Li Y, et al. A large-scale screen for coding variants predisposing to psoriasis. *Nat Genet* 2014;46(1):45-50.
83. Sun LD, Cheng H, Wang ZX, et al. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. *Nat Genet* 2010;42(11):1005-9.
84. Sheng Y, Jin X, Xu J, et al. Sequencing-based approach identified three new susceptibility loci for psoriasis. *Nat Commun* 2014;5:4331.
85. Jadon D, Tillett W, Wallis D, et al. Exploring ankylosing spondylitis-associated ERAP1, IL23R and IL12B gene polymorphisms in subphenotypes of psoriatic arthritis. *Rheumatology (Oxford)* 2013;52(2):261-6.
86. Popa OM, Cherciu M, Cherciu LI, et al. ERAP1 and ERAP2 Gene Variations Influence the Risk of Psoriatic Arthritis in Romanian Population. *Arch Immunol Ther Exp* 2016;64(1):123-129.
87. Zavattaro E, Ramezani M, Sadeghi M. Endoplasmic reticulum aminopeptidase 1 (ERAP1) polymorphisms and psoriasis susceptibility: A systematic review and meta-analysis. *Gene* 2020;30:736:144416.
88. Helliwell PS, Taylor WJ. Classification and diagnostic criteria for psoriatic arthritis. *Ann Rheum Dis* 2005;64 Suppl 2:ii3-8.

89. Evans DM, Spencer CC, Pointon JJ, et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat Genet* 2011;43(8):761-7.
90. Yang SK, Lim J, Chang HS, et al. Association of TNFSF15 with Crohn's disease in Koreans. *Am J Gastroenterol* 2008;103(6):1437-42.
91. Ombrello MJ, Kastner DL, Remmers EF. Endoplasmic reticulum-associated amino-peptidase 1 and rheumatic disease: genetics. *Curr Opin Rheumatol* 2015;27(4):349-56.
92. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J Am Acad Dermatol* 1985;13(3):450-6.
93. Gudjonsson JE, Karason A, Runarsdottir EH, et al. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients--an analysis of 1019 HLA-C- and HLA-B-typed patients. *J Invest Dermatol* 2006;126(4):740-5.
94. Zhang X, Wang H, Te-Shao H, et al. The genetic epidemiology of psoriasis vulgaris in Chinese Han. *Int J Dermatol* 2002;41(10):663-9.
95. Ho PY, Barton A, Worthington J, et al. HLA-Cw6 and HLA-DRB1*07 together are associated with less severe joint disease in psoriatic arthritis. *Ann Rheum Dis* 2007;66(6):807-11.
96. Bettencourt BF, Rocha FL, Alves H, et al. Protective effect of an ERAP1 haplotype in ankylosing spondylitis: investigating non-MHC genes in HLA-B27-positive individuals. *Rheumatology (Oxford)* 2013;52(12):2168-76.
97. Bebes A, Német IB, Vas K, et al. The soluble form of TL1A cytokine produced by human keratinocytes may contribute to psoriasis pathogenesis. *J Invest Dermatol* 2012;132(2): S1-161.
98. Kamada N, Hisamatsu T, Honda H, et al. TL1A produced by lamina propria macrophages induces Th1 and Th17 immune responses in cooperation with IL-23 in patients with Crohn's disease. *Inflamm Bowel Dis* 2010;16(4):568-75.
99. Pappu BP, Borodovsky A, Zheng TS, et al. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. *J Exp Med* 2008;205(5):1049-62.
100. Yamazaki K, McGovern D, Ragoussis J, et al. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. *Hum Mol Genet* 2005;14(22):3499-506.
101. Bernstein CN, Wajda A, Blanchard JF. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. *Gastroenterology* 2005;129(3):827-36.

102. Weng X, Liu L, Barcellos LF, et al. Clustering of inflammatory bowel disease with immune mediated diseases among members of a northern california-managed care organization. *Am J Gastroenterol* 2007;102(7):1429-35.
103. Yates VM, Watkinson G, Kelman A. Further evidence for an association between psoriasis, Crohn's disease and ulcerative colitis. *Br J Dermatol* 1982;106(3):323-30.
104. Safrany E, Szabo M, Szell M, et al. Difference of interleukin-23 receptor gene haplotype variants in ulcerative colitis compared to Crohn's disease and psoriasis. *Inflamm Res* 2013;62(2):195-200.
105. Cargill M, Schrodi SJ, Chang M, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007;80(2):273-90.
106. Queiro R, Gonzalez S, López-Larrea C, et al. HLA-C locus alleles may modulate the clinical expression of psoriatic arthritis. *Arthritis Res Ther* 2006;8:R185.
107. Winchester R1, Minevich G, Steshenko V, et al. HLA associations reveal genetic heterogeneity in psoriatic arthritis and in the psoriasis phenotype. *Arthritis Rheum* 2012;64:1134–1144.
108. Szczerkowska-Dobosz A, Niespodziana K, Rebala K, et al. Lack of association of HLA-C alleles with late-onset psoriasis in the northern Polish population. *J Appl Genet* 2007;48(3):273-5.
109. Allen MH, Ameen H, Fisher SA, et al. The major psoriasis susceptibility locus PSORS1 is not a risk factor for late-onset psoriasis. *J Invest Dermatol* 2005;124(1):103-6.
110. Hébert HL, Bowes J, Smith RL, et al. Identification of loci associated with late-onset psoriasis using dense genotyping of immune-related regions. *Br J Dermatol* 2015;172(4):933-*9.*
111. Bergboer JG, Oostveen AM, de Jager ME, et al. Paediatric-onset psoriasis is associated with ERAP1 and IL23R loci, LCE3C_LCE3B deletion and HLA-C*06. *Br J Dermatol* 2012;167(4):922-5.
112. Kadi A, Izac B, Said-Nahal R, Leboime A, et al. Investigating the genetic association between ERAP1 and spondyloarthritis. *Ann Rheum Dis* 2013;72(4):608-13.

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Rapid Communication

Genetic risk and protective factors of *TNFSF15* gene variants detected using single nucleotide polymorphisms in Hungarians with psoriasis and psoriatic arthritis

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ABSTRACT

The aim of this study was to examine the role of single nucleotide polymorphisms (SNPs) and haplotypes of the tumor necrosis factor ligand superfamily member 15 (*TNFSF15*) gene in Hungarians with psoriasis and psoriatic arthritis. A case-control study was performed, and five *TNFSF15* SNPs (rs3810936, rs6478108, rs6478109, rs7848647, rs7869487) were genotyped in 319 patients with psoriasis, 105 of whom also have psoriatic arthritis, and in 200 healthy individuals. Three haplotypes (A, B, C) based on these five SNPs were also analyzed. Our findings suggest that the rs6478109 SNP may be a genetic risk factor in psoriasis ($p = 0.0046$), while haplotype C may be protective ($p = 0.0250$). These results suggest that certain variants of the *TNFSF15* gene contribute to the pathogenesis of the immune-mediated, multifactorial skin disease psoriasis, and that this difference is more readily apparent when groups of patients with and without psoriatic arthritis are examined separately.

1. Introduction

Psoriasis is a chronic, multifactorial, T-cell mediated inflammatory skin disease. It is relatively common, affecting 2–3% of the population. Psoriasis typically manifests as thickened, scaly, red skin plaques. Both genetic and environmental factors play a role in the etiology of the disease [1]. The major psoriasis susceptibility factor (*PSORS1*) is located on chromosome 6. This locus includes the HLA-B13 and HLA-Cw6 alleles, which are the most important determinants [2].

Psoriatic arthritis is a common seronegative inflammatory arthritis that characteristically occurs in individuals with psoriasis [3]. The prevalence of psoriatic arthritis in patients with psoriasis has been reported as 6% and 39% for different study populations [4]. Psoriatic arthritis is classified as a subtype of spondyloarthropathy based on common human leukocyte antigen (HLA) associations and characteristic inflammatory and clinical features [3], and is strongly associated with the HLA-B13, B-27 and HLA-Cw6 alleles [5].

Psoriasis is characterized by a disturbed cytokine network. Tumor necrosis factor alpha (TNFα) seems to be one of the most important triggers for aberrant activation of lymphocytes and keratinocytes [6]. TNFα is a key cytokine both in psoriasis and psoriatic arthritis: high levels of TNFα can be detected in psoriatic skin lesions as well as in the synovial fluid and synovial tissue of patients with psoriatic arthritis [7].

The tumor necrosis factor superfamily, member 15 protein (TNFSF15; also known as TNF superfamily ligand A, TL1A, and vascular endothelial cell growth inhibitor, VEGI) is a TNF-like factor expressed primarily in endothelial cells [8,9]. The TNFSF15 protein is encoded by a gene on human chromosome 9q32 and consists of 251 amino acid residues with the characteristics of a type II transmembrane protein [10]. To date, no *PSORS* loci have been indentified on human chromosome 9q32. TNFSF15 inhibits endothelial-cell and endothelial-progenitor-cell differentiation and stimulates T-cell

Abbreviations: CASPAR, Classification Criteria for Psoriatic Arthritis; HLA, human leukocyte antigen; TNFSF15, tumor necrosis factor superfamily, member 15; TNF, tumor necrosis factor; TL1A, TNF superfamily ligand A; VEGI, vascular endothelial cell growth inhibitor; SNP, single nucleotide polymorphism; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; DR3, death domain receptor 3; PASI, Psoriasis Area Severity Index.

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activation, Th1 cytokine production and dendritic cell maturation [11]. TNFSF15 plays an important role in the pathogenesis of inflammatory bowel disease (IBD), atherosclerosis, rheumatoid arthritis and chronic inflammatory skin diseases [12–15]. Increased levels of TNFSF15 are found in the intestinal mucosa of patients with Crohn's disease (CD) and in the skin of patients with psoriasis [16,15]. Furthermore, single nucleotide polymorphisms (SNP) and certain haplotypes of the *TNFSF15* gene were reported to contribute to the development of IBD as an ethnicity-specific susceptibility factor [17]. The above findings suggest that the *TNFSF15* gene plays a role in the pathomechanism of a wide range of immune-mediated human diseases. In this study, we examined the association between psoriasis, psoriatic arthritis, five SNPs and three haplotypes of the *TNFS15* gene.

2. Subjects and methods

2.1. Subjects

This study was approved by the Internal Review Board of University of Szeged. Informed consent approved by the Internal Review Board was obtained from all participating patients and volunteers, and the study was conducted in full accordance with the Principles of the Declaration of Helsinki. Enrolled in the study were 319 Hungarian Caucasian patients treated for psoriasis and psoriatic arthritis at the Department of Dermatology and Allergology, at the Department of Rheumatology of the University of Szeged and at the Department of Rheumatology of Pándy Kálmán Békés County Hospital, Gyula, and 200 ethnically matched healthy individuals without any multifactorial inflammatory diseases. Of the 319 psoriasis patients, 105 exhibited psoriatic arthritis fulfilling the Classification Criteria for Psoriatic Arthritis (CASPAR). The group of patients with psoriatic arthritis was stratified into five homogenous phenotypic groups according to the Moll and Wright criteria [18]. None of the participating individuals had IBD. The demographic and clinical characteristics of the study population are shown in Table 1.

2.2. Genotyping and haplotype analysis

Genomic DNA was isolated from venous blood of the patients and controls using the BioRobot EZ from QIAGEN (Hilden, Germany) with the EZ1 DNA Blood Kit, according to the instructions of the manufacturer. Genotyping of five SNPs of the *TNFSF15* gene (see Table 2a) was carried out with the PCR-based Assay-by-Design method of Applied Biosystems (Foster City, CA), following the instructions of the manufacturer. After PCR amplification, end-point detection was performed with an iQ5 real-time PCR machine from Bio-Rad (Hercules, CA). These SNPs were used to construct three haplotypes (Table 2b), previously reported in Japanese, Korean and Caucasian IBD populations [12,17–19].

Table 2a
TNFSF15 gene SNPs.

SNP	Kit number	Position[a]	Nucleotide change
rs3810936	C___363308_10	15524	G/A
rs6478108	C___170492_10	9706	G/A
rs6478109	C___1305297_10	−358	T/C
rs7848647	C___11277159_10	−638	G/A
rs7869487	C___11277149_10	−12506	G/A

[a] The first nucleotide of exon 1 is designated as position 1 based on the GenBank reference sequence NM_005118.2.

Table 2b
TNFSF15 gene haplotypes.

Haplotype	rs3810936	rs6478108	rs6478109	rs7848647	rs7869487
A	G	A	C	G	A
B	A	G	T	A	G
C	A	G	T	A	A

2.3. Statistical analysis

Genotype frequencies and the distribution of the three main haplotypes were compared for patients and controls by means of χ^2-test or the Fisher's exact test when it was necessary (expected values <0.05). Odds ratios with 95% confidence intervals were also calculated. Statistical analysis was performed using SPSS 15.0 (Chicago, IL).

3. Results

The genotype frequencies of the five examined SNPs are summarized in Table 3. The genotype distribution of the rs6478109 SNP showed a significant difference ($p = 0.0052$) when compared for the group of patients with psoriasis and the group of healthy controls. When the stratified groups of psoriatic patients without arthritis was compared with the group of healthy controls, the value of significance was $p = 0.0046$. The proportion of patients with psoriasis that are heterozygous for the rare allele of the *TNFSF15* rs6478109 SNP was higher than that of heterozygotes identified in the group of healthy controls. However, the genotype distribution of this SNP did not show a significant difference when compared with healthy controls and patients with psoriatic arthritis. The genotype distribution of the other four SNPs (rs3810936, rs6478108, rs7848647, rs7869487) did not show significant difference between the healthy controls and patients with psoriasis or psoriatic arthritis.

Approximately half of the investigated individuals (from both patient and control groups) were found to have haplotypes previously described in Japanese, Korean and Caucasian populations [12,17–19]. The distribution of the three examined haplotypes is summarized in Table 4. No significant difference was found in

Table 1
Demographic and clinical characteristics of the study population.

Characteristic	Controls ($N = 200$)	Psoriasis ($N = 319$)	Psoriasis without arthritis ($N = 214$)	Psoriatic arthritis ($N = 105$)
Age, mean (range) [years]	40.39 ± 19 (7–82)	52.13 ± 21 (17–82)	50.72 ± 19 (17–81)	54.34 ± 13 (27–82)
Males/females [N, (%)]	77 (39)/123 (41)	177 (55)/142 (45)	123 (57)/86 (43)	52 (49)/53 (51)
PASI, mean (range)	–	–	12.42 ± 16.54 (0.1–52.8)	–
Arthritis, distal [N, (%)]	–	–	–	13 (12)
Asymmetrical oligoarthritis [N, (%)]	–	–	–	28 (27)
Symmetrical polyarthritis [N, (%)]	–	–	–	45 (43)
Arthritis, axial [N, (%)]	–	–	–	19 (18)
Arthritis mutilans [N, (%)]	–	–	–	0 (0)

Table 3
Distribution of *TNFSF15* SNP genotypes in healthy controls and patients.

	Healthy controls ($N = 200$)			Psoriasis ($N = 319$)				Psoriasis without arthritis ($N = 214$)				Psoriatic arthritis ($N = 105$)			
	WT	Het	Hom rare	WT	Het	Hom rare	p	WT	Het	Hom rare	p	WT	Het	Hom rare	p
rs3810936	102 (51)	79 (39.5)	19 (9.5)	171 (53.6)	123 (38.5)	25 (7.9)	0.7454	115 (53.7)	79 (36.9)	20 (9.4)	0.8473	56 (53.3)	44 (41.9)	5 (4.8)	0.3437
rs6478108	99 (49.5)	101 (50.5)	0 (0)	166 (52)	152 (47.7)	1 (0.3)	0.6104	115 (53.7)	99 (46.3)	0 (0)	0.3884	51 (48.6)	53 (50.5)	1 (0.9)	0.3834
rs6478109	38 (19)	162 (81)	0 (0)	33 (10.3)	286 (89.7)	0 (0)	**0.0052**	20 (9.4)	194 (90.6)	0 (0)	**0.0046**	13 (12.4)	92 (87.6)	0 (0)	0.1410
rs7848647	113 (56.5)	70 (35)	17 (8.5)	178 (55.8)	116 (36.4)	25 (7.8)	0.9317	119 (55.6)	75 (35)	20 (9.4)	0.9524	59 (56.2)	41 (39)	5 (4.8)	0.4393
rs7869487	66 (33)	81 (40.5)	53 (26.5)	102 (32)	124 (38.9)	93 (29.1)	0.8064	71 (33.2)	78 (36.4)	65 (30.4)	0.6104	31 (29.5)	46 (43.8)	28 (26.7)	0.8011

WT, both alleles are wildtype; Het, only one allele is wildtype; Hom rare, both alleles are not wildtype; listed as [N, (%)].

Table 4
Distribution of the main *TNFSF15* haplotypes in patients and in healthy controls.

Haplotype	Healthy controls ($N = 200$)	Psoriasis ($N = 319$)		Psoriasis without arthritis ($N = 214$)		Psoriatic arthritis ($N = 105$)	
	Frequency	Frequency	p	Frequency	p	Frequency	p
A	83 (41.5)	130 (40.8)	0.8661	85 (39.7)	0.7123	45 (42.9)	0.8194
B	19 (9.5)	24 (7.5)	0.4266	14 (6.5)	0.2668	10 (9.5)	0.9946
C	7 (3.5)	1 (0.3)	**0.0041**	1 (0.5)	**0.0250**	0 (0)	0.0524

Frequency is listed as [N, (%)].

the distribution of Haplotype A and B when healthy controls and patients with psoriasis and psoriatic arthritis were compared. The haplotype distribution of Haplotype C, however, showed a significant difference ($p = 0.0041$) between the group of healthy individuals and the group of patients with psoriasis. When the stratified group of psoriatic patients without arthritis was compared with the group of healthy controls, the value of significance was $p = 0.0250$ and a nearly significant value ($p = 0.0524$) was found when the stratified group of psoriatic patients with arthritis was compared with the group of healthy controls. Note that, due to the low frequencies of the examined haplotypes in this study, the statistical reliability of the tests was relatively low.

4. Discussion

Here we report the first examination of *TNFSF15* gene variants in the Hungarian population. Our results suggest that the rs6478109 SNP of *TNFSF15* gene contributes to psoriasis susceptibility and this association is more apparent when groups of psoriatic patients with and without arthritis are analyzed separately. A significant difference was also found in the distribution of Haplotype C in the groups of healthy controls and psoriatic patients without arthritis. The proportion of healthy individuals with Haplotype C was higher in the control group than in the group of patients with psoriasis. This result suggests that individuals with Haplotype C might be protected against psoriasis. However, due to the relatively low frequency of Haplotype C in our survey, it would be necessary to enroll more patients, preferably including multiple ethnicities, before it would be possible draw a conclusion about the protective nature haplotype C with respect to psoriasis.

The rs6478109 SNP is situated in the promoter region of the *TNFSF15* gene, 474 nt upstream of the transcription start site. According to *in silico* analysis (http://www.cbrc.jp/research/db/TFSEARCH.html), the region harboring the SNP potentially binds five transcription factors (GATA-1, GATA-2, GATA-3, Jk2, and c/EBPb). This observation suggests that the rs6478109 SNP might influence the expression of the *TNFSF15* gene and, by altering expression of the gene product, contributes to the pathogenesis of immune-mediated diseases.

TNFSF15 can be considered a novel common gene in the pathomechanism of IBD and psoriasis. Lymphocytes, monocytes and dendritic cells are the major sources of TNFSF15, which associates with death domain receptor 3 (DR3) [10,16]. Upregulation of the TNFSF/DR3 system is involved in the pathogenesis of chronic inflammatory diseases, such as IBDs, rheumatoid arthritis and psoriasis [12,14,15]. Expression of the TNFSF/DR3 system is enhanced in psoriatic skin at mRNA and protein levels [15,23]. TNFSF15/DR3 may contribute to psoriatic inflammation by enhancing Th1 and Th17 responses [21,22].

Yamazaki et al. have recently reported the association between *TNFSF15* gene variants and IBDs: five SNPs were associated with IBD phenotypes in Japanese and UK patients. These five polymorphic SNPs formed two frequent disease-associated haplotypes; the 'high-risk' Haplotype A and the 'low-risk' Haplotype B [20]. Picornell et al. were unable to confirm the above association in a Jewish population; however Haplotype B was found to be protective against CDs in non-Jewish patients [17]. In another study, the role of 'high risk' Haplotype A was confirmed in CD for a Korean population [19]. All these data suggest that the *TNFSF15* gene variants contribute to immune-mediated diseases in an ethnicity-specific manner.

The increased risk of psoriasis in patients with IBD has been reported [24–26]. This clinical overlap is supported by several genetic findings. Safrany et al. have recently reported the association of *interleukin-23 receptor* gene SNPs in Hungarian patients with ulcerative colitis (UC) and psoriasis [27]. *IL12B* has also been confirmed as a susceptibility gene for both IBD and psoriasis [28].

Taken together with previous reports, our results suggest that the genetic variants of the *TNFSF15* gene contribute to the pathogenesis of the immune-mediated, multifactorial skin disease psoriasis. Our findings also call attention to the importance of the careful stratification of patient groups according to symptoms. Finally, the interpretation of our results in view of the literature suggests that polymorphisms of immune-regulatory genes are key in several inflammatory diseases, and that ethnicity-specific aspects must also be considered.

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References

[1] Griffiths CE, Baker JN. Pathogenesis and clinical feathers of psoriasis. Lancet 2007;370:263–71.
[2] Nickoloff BJ, Nestle FO. Recent insight into the immunopathogenesis of psoriasis provide new therapeutic opportunities. J Clin Invest 2004;113:1664–75.
[3] Gladman D, Antoni C, Mease P, Clegg DO, Nash P. Psoriatic arthritis: epidemiology, clinical features, course and outcome. Ann Rheum Dis 2005;64(Suppl. 2):ii14–7.
[4] Sheeb M, Uramoto KM, Gibson LE, O'Fallon WM, Gabriel SE. The epidemiology of psoriatic arthritis in Olmsted County, Minnesota, USA, 1982–1991. J Rheumatol 2000;27:1247–50.
[5] Gladman DD, Anhorn KA, Schachter RK, Mervart H. HLA antigens in psoriatic arthritis. J Rheumatol 1986;13:586–92.
[6] Nickoloff BJ. The cytokine network in psoriasis. Arch Dermatol 1991;127:871–84.
[7] Veale DJ, Ritchlin C, Fitzgerald O. Immunopathology of psoriasis and psoriatic arthritis. Ann Rheum Dis 2005;64:26–9.
[8] Yue TL, Ni J, Romanic AM, Gu JL, Keller P, Wang C, et al. TL1, a novel tumor necrosis factor-like cytokine, induces apoptosis in endothelial cells. Involvement of activation of stress protein kinases (stress-activated protein kinase and p38 mitogen-activated protein kinase) and caspase-3-like protease. J Biol Chem 1999;274:1479–86.
[9] Zhai Y, Ni J, Jiang GW, Lu J, Xing L, Lincoln C, et al. VEGI, a novel cytokine of the tumor necrosis factor family, is an angiogenesis inhibitor that suppresses the growth of colon carcinomas in vivo. FASEB J 1999;13:181–9.
[10] Migone TS, Zhang J, Luo X, Zhuang L, Chen C, Hu B, et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. Immunity 2002;16:479–92.
[11] Zhang Z, Li LY. TNFSF15 modulates neovascularization and inflammation. Cancer Microenviron 2012;5(3):237–47.
[12] Thiébaut R, Kotti S, Jung C, Merlin F, Colombel JF, Lemann M, et al. TNFSF15 polymorphisms are associated with susceptibility to inflammatory bowel disease in a new European cohort. Am J Gastroenterol 2009;104(2):384–91.
[13] Kang YJ, Kim WJ, Bae HU, Kim DI, Park YB, Park JE, et al. Involvement of TL1A and DR3 in induction of pro-inflammatory cytokines and matrix metalloproteinase-9 in atherogenesis. Cytokine 2005;29(5):229–35.
[14] Cassatella MA, Pereira-da-Silva G, Tinazzi I, Facchetti F, Scapini P, Calzetti F, et al. Soluble TNF-like cytokine (TL1A) production by immune complexes stimulated monocytes in rheumatoid arthritis. J Immunol 2007;178(11):7325–33.
[15] Bamias G, Evangelou K, Vergou T, Tsimaratou K, Kaltsa G, Antoniou C, et al. Upregulation and nuclear localization of TNF-like cytokine 1A (TL1A) and its receptors DR3 and DcR3 in psoriatic skin lesions. Exp Dermatol 2011;20(9):725–31.
[16] Bamias G, Martin 3rd C, Marini M, Hoang S, Mishina M, Ross WG, et al. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. J Immunol 2003;171(9):4868–74.
[17] Picornell Y, Mei L, Taylor K, Yang H, Targan SR, Rotter JI. TNFSF15 is an ethnic-specific IBD gene. Inflamm Bowel Dis 2007;13(11):1333–8.
[18] Helliwell P, Taylor W. Classification and diagnostic criteria for psoriatic arthritis. Ann Rheum Dis 2005;64(Suppl. 2):ii3–8.
[19] Yang SK, Lim J, Chang HS, Lee I, Li Y, Liu J, et al. Association of TNFSF15 with Crohn's disease in Koreans. Am J Gastroenterol 2008;103(6):1437–42.
[20] Yamazaki K, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, et al. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. Hum Mol Genet 2005;14(22):3499–506.
[21] Kamada N, Hisamatsu T, Honda H, Kobayashi T, Chinen H, Takayama T, et al. TL1A produced by lamina propria macrophages induces Th1 and Th17 immune responses in cooperation with IL-23 in patients with Crohn's disease. Inflamm Bowel Dis 2010;16(4):568–75.
[22] Pappu BP, Borodovsky A, Zheng TS, Yang X, Wu P, Dong X, et al. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. J Exp Med 2008;205(5):1049–62.
[23] Bebes A, Német IB, Vas K, Bata-Csörgő Z, Kemény L, Tubak V. The soluble form of TL1A cytokine produced by human keratinocytes may contribute to psoriasis pathogenesis. J Invest Dermatol 2012;132(2):S1–161.
[24] Bernstein CN, Wajda A, Blanchard JF. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. Gastroenterology 2005;129(3):827–36.
[25] Weng X, Liu L, Barcellos LF, Allison JE, Herrinton LJ. Clustering of inflammatory bowel disease with immune mediated diseases among members of a northern california-managed care organization. Am J Gastroenterol 2007;102(7):1429–35.
[26] Yates VM, Watkinson G, Kelman A. Further evidence for an association between psoriasis, Crohn' disease and ulcerative colitis. Br J Dermatol 1982;106(3):323–30.
[27] Safrany E, Szabo M, Szell M, Kemeny L, Sumegi K, Melegh BI, et al. Difference of interleukin-23 receptor gene haplotype variants in ulcerative colitis compared to Crohn's disease and psoriasis. Inflamm Res 2013;62(2):195–200.
[28] Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet 2007;80(2):273–90.

II.

Original paper

The association of HLA-C and ERAP1 polymorphisms in early and late onset psoriasis and psoriatic arthritis patients of Hungary

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Abstract

Introduction: Single nucleotide polymorphisms (SNPs) of the HLA-C and ERAP1 genes were recently determined to contribute to psoriasis susceptibility. However, data regarding the association of these genes with specific subgroups of psoriasis are scarce.

Aim: To examine the possible association of the HLA-C and ERAP-1 polymorphisms with early and late onset psoriasis and psoriatic arthritis.

Material and methods: Five ERAP1 SNPs and two HLA-C SNPs were genotyped in 105 psoriatic arthritis patients, 214 cutaneous psoriasis patients and 200 healthy individuals. Haplotypes were constructed for three ERAP1 SNPs (rs17482078, rs10050860, rs30187), and interaction between HLA-Cw*0602 and ERAP1 was also analysed.

Results: The HLA-Cw*0602 rs10484554 SNP was found to be a strong susceptibility factor for early onset cutaneous psoriasis and early onset psoriatic arthritis. ERAP1 SNPs (rs10050860, rs17482078, rs27525) appear to have a protective function for early onset psoriatic arthritis. The haplotype B was identified as a susceptibility factor for late onset psoriatic arthritis. In HLA-C positive individuals the rs27524 ERAP1 SNP was associated with a significantly increased risk of psoriatic arthritis development, whereas the rs27525 ERAP1 SNP had the opposite effect.

Conclusions: These results suggest that the HLA-C and ERAP1 genes contribute to the pathogenesis of psoriasis and psoriatic arthritis in an age-dependent manner.

Key words: *HLA-C*, *ERAP1*, psoriasis, psoriatic arthritis, polymorphism.

Introduction

Psoriasis is a common, chronic immune-mediated skin disease. Clinically, psoriasis is characterized by the presence of well-demarcated, scaly, erythematous skin lesions, and is frequently associated with arthritis [1]. Psoriatic arthritis is a seronegative inflammatory arthritis present in nearly 25% of patients with psoriasis and develops after an average interval of about one decade [2]. Psoriatic arthritis is defined as a subtype of spondyloarthropathies, based on common human leukocyte antigen (HLA) associations. Clinically, this disease is characterized by changing degrees of oligoarthritis, polyarthritis and spondylitis and typically involves dactylitis, distal interphalangeal joint involvement or mutilating arthritis [2, 3].

Early onset psoriasis, or type I psoriasis, refers to patients with an onset before 40 years of age, more serious disease course than the late onset psoriasis, strong family history, and *HLA-C*06* positivity. Early onset psoriasis has been reported in 75% of patients with psoriasis; hence, it affects the majority of patients with psoriasis. Late onset psoriasis, or type II psoriasis, in contrast, is characterized by an onset at or after 40 years, less severe clinical symptoms and rare family inheritance [4, 5].

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Recently, age at disease onset (with a cut-off value at 40 years) has been found similarly important in the characterization of the disease phenotype in psoriatic arthritis as well [6].

Single nucleotide polymorphisms (SNPs) of several genes have been identified as contributing to psoriasis susceptibility [7, 8]. We and others have previously reported a significant association with variants of the *HLA-Cw*0602*, *IL23R*, *LCE3C*, *LCE3B-del* [9, 10], *IL12R*, *IL23* [11], *TNFSF15* [3], and *ERAP1* genes [12–14]. Psoriatic arthritis is strongly associated with the *HLA-B13*, *HLA-B57*, *HLA-B39*, *HLA-Cw6* and *HLA-Cw7* alleles [8]. While psoriatic arthritis tends to have a more severe disease course and appears earlier in *HLA-B*27* positive patients, the latency between the onset of psoriasis and onset of joint symptoms is longer in *HLA-Cw*0602* positive patients [6, 15].

Genetic variants of the *endoplasmic reticulum-associated amino-peptidase 1* (*ERAP1*) gene has recently been reported to be associated with ankylosing spondylitis (AS), psoriasis [16], and Behcet's disease [17]. The ERAP1 protein belongs to the M1 family of zinc metallopeptidase enzymes and is encoded by a gene on chromosome 5q15 [18]. ERAP1 trims peptides imported into the endoplasmic reticulum at their N-terminus and contributes to the shaping of the antigenic repertoire presented by class I major histocompatibility complex (MHC) molecules [19]. Depending on the peptide length and sequence composition, ERAP1 has the ability to both destroy and create peptide cargos for MHC class I molecules [20]. The ERAP1 protein also contributes to the shedding of the membrane-bound receptors of inflammatory cytokines, such as IL-1R2, TNFR1 and IL-6R [21]. ERAP1 likely plays a pivotal role in the protection from infectious diseases by contributing to the maintenance of immune tolerance and control of inflammation [22]. The association of *ERAP1* variants with psoriasis has been investigated recently in populations of European and Chinese ancestry. Two *ERAP1* variants, rs27524 (noncoding) and rs30187 (Lys528ARG), were found to be genome-wide risk factors for psoriasis [12]. Furthermore, dominant epistasis between *HLA-Cw*0602* and the *ERAP1* rs30187 SNP was identified in studies [12–14]. Another recent work identified the *ERAP1* rs27432 SNP (intronic) as a variant strongly associated with psoriasis [23]. In Han Chinese populations, *ERAP1* SNPs or gene variants in linkage disequilibrium with *ERAP1* SNPs were also found to be associated with psoriasis [24–26]. It is currently unclear whether the effects of *ERAP1* on psoriasis can be explained by a single variant or by allotypic associations. Recently, the association of *ERAP1* with psoriatic arthritis has also been investigated; however, this study did not demonstrate an association between rs30187 and psoriatic arthritis or psoriatic arthritis subphenotypes [27].

As psoriasis is currently best stratified by disease onset and absence or presence of arthritis, we hypothesized that stratifying psoriasis patients into early and late onset groups, as well as skin-only and arthritis subgroups leads to the identification of new *ERAP1* genotype–phenotype associations. Furthermore, as *ERAP1* seems to be associated with inflammatory diseases in a *HLA*-dependent manner, we also intended to explore whether a gene–gene interaction between *HLA-Cw*0602* and *ERAP1* exists in these well-stratified subgroups of psoriatic patients.

Aim

Furthermore, as *ERAP1* seems to be associated with inflammatory diseases in a *HLA*-dependent manner, we also intended to explore whether a gene–gene interaction between *HLA-Cw*0602* and *ERAP1* exists in these well-stratified subgroups of psoriatic patients. We have analysed the association of five *ERAP1* and two *HLA-Cw*0602* SNPs as well as different *ERAP1* haplotypes with EOP, LOP and psoriatic arthritis.

Material and methods

Subjects

This study was approved by the Internal Review Board of the University of Szeged, the approval number is PSO-GENET-001. Informed consent was obtained from all participating patients and volunteers, and the study was conducted in full accordance with the principles of the Declaration of Helsinki. The study population consisted of 319 Hungarian Caucasian psoriasis vulgaris patients (designated as PsV) treated at the Department of Dermatology and Allergology and the Department of Rheumatology of the University of Szeged, and at the Department of Rheumatology of Pándy Kálmán Békés County Hospital, Gyula, as well as 200 ethnically-matched healthy individuals with no known multifactorial inflammatory diseases. Of the 319 psoriasis vulgaris patients, 105 exhibited psoriatic arthritis (designated as PsA), fulfilling the Classification Criteria for Psoriatic Arthritis (CASPAR). The group of patients with psoriatic arthritis was further stratified into five homogenous clinical groups according to the Moll and Wright criteria [28]. Patients with skin symptoms of psoriasis only were denoted as cutaneous psoriasis (designated as PsC) patients. Patients with onset before 40 years of age were classified as having early onset psoriasis (EO-PsV, EO-PsA, EO-PsC), whereas late onset psoriasis (LO-PsV, LO-PsA, LO-PsC) was defined by an onset at or after 40 years. The demographic and clinical characteristics of the study population are listed in Table 1.

Genotyping and haplotype analysis

Genomic DNA was isolated from venous blood of patients and controls using the BioRobot EZ and the EZ1 DNA Blood Kit from QIAGEN (Hilden, Germany), accord-

Table 1. Demographic and clinical characteristics of the study population

Characteristic	Healthy controls (*N* = 200)	PsV (*N* = 319)	PsC (*N* = 214)	PsA (*N* = 105)
Age, mean (range) [years]	40.39 ±19 (7–82)	52.13 ±21 (17–82)	50.72 ±19 (17–81)	54.34 ±13 (27–82)
Males/females [*N*, (%)]	77 (39)/123 (41)	177 (55)/142 (45)	125 (58)/89 (42)	52 (49)/53 (51)
PASI, mean (range)	–	–	12.42 ±16.54 (0.1–52.8)	–
Arthritis, distal [*N*, (%)]	–	–	–	13 (12)
Asymmetrical oligoarthritis [*N*, (%)]	–	–	–	28 (27)
Symmetrical polyarthritis [*N*, (%)]	–	–	–	45 (43)
Arthritis, axial [*N*, (%)]	–	–	–	19 (18)
Arthritis mutilans [*N*, (%)]	–	–	–	0 (0)

Table 2. *ERAP1* and *HLA-Cw*0602* gene SNPs

Chr.	Gene	SNP	Kit number	Nucleotide change	Variation
5	*ERAP1*	rs27524	C___3056837_10	A/G	None
5	*ERAP1*	rs27525	C___3056838_10	C/T	None
5	*ERAP1*	rs30187	C___3056885_10	C/T	Lys528ARG
5	*ERAP1*	rs17482078	C___3056871_10	C/T	Arg725Gln
5	*ERAP1*	rs10050860	C___3056876_10	C/T	Asp575Asn
6	*HLA-C*	rs10484545	C___29666895_10	C/G	None
6	*HLA-C*	rs10484554	C___29612773_20	C/T	None

ing to the instructions of the manufacturer. Genotyping of five SNPs of the *ERAP1* gene and two SNPs of the *HLA-Cw*0602* gene (Table 2), previously reported as candidate genes and SNPs in the pathogenesis of psoriasis and AS [16, 29], was carried out with the PCR-based Assay-by-Design method of Applied Biosystems (Foster City, CA), following the instructions of the manufacturer. After PCR amplification, end-point detection was performed with a CFX 96 real-time PCR machine from Bio-Rad (Hercules, CA). Genotyping of the rs10484554 SNP was used to determine the *HLA-C* status, as previously reported [12, 16]. Three SNPs (rs30187, rs10050860, rs17482078) were used to construct four haplotypes: Haplotype A (rs17482078/rs10050860/rs30187-CCC), Haplotype B (rs17482078/rs10050860/rs30187-CCT), Haplotype C (rs17482078/rs10050860/rs30187-TTC) and Haplotype D (rs17482078/rs10050860/rs30187-TTC, previously reported by Ombrello et al. to be risk or protective factors in AS [30].

Statistical analysis

Genotype frequencies, frequencies of the main haplotypes and SNP associations were calculated and compared between patient groups using Fisher's exact test, using the Plink software package (v1.9). Odds ratios were calculated with 95% confidence intervals. Multiple-testing correction was applied to all comparisons using the Benjamini-Hochberg 'FDR' method in R (v3.2.3), with a significance threshold of $p < 0.05$. As previously suggested and successfully applied by other authors [31–33], the age of 40 years was used for stratification of age at disease onset. No further stratification of age at disease onset was attempted due to the relatively small number of patients.

Results

The genotype frequencies of the *HLA-C* and *ERAP1* SNPs are summarized in Table 3. Genotype frequencies in patients and controls were in Hardy-Weinberg equilibrium. Control genotype frequencies were comparable to those published in the literature. The genotype distribution of one *HLA-C* SNP (rs10484554) was found to be significantly different between the PsV patients and the group of healthy individuals ($p = 5.9 \times 10^{-5}$ respectively). The proportion carrying the mutant *HLA-Cw*0602* allele (rs10484554 SNP) was significantly higher among PsV patients than in the group of healthy controls (58.3% and 36.5%, respectively), and there was no difference in this respect between patients with skin only or skin and joint symptoms (57.9% and 59%, respectively). Taken together, the *HLA-Cw*0602* rs10484554 SNP seems to be a strong susceptibility factor for psoriasis (Table 3). In the case of the five other *ERAP1* SNPs (rs27524, rs27525, rs30187, rs17482078 and rs10050860) and rs10484545 *HLA-C* SNP, there were no statistically detectable differences in the genotype distributions between healthy individuals and the PsV patients.

Table 3. Distribution of ERAP1 and HLA-Cw SNP genotypes in healthy controls and psoriasis patients

Healthy controls (*N* = 200)					PsV (*N* = 319)						PsC (*N* = 214)						PsA (*N* = 105)					
Gene	SNP	WT	Het	Hom rare	Disease onset	WT	Het	Hom rare	*P*-value*	FDR	Disease onset	WT	Het	Hom rare	*P*-value*	FDR	Disease onset	WT	Het	Hom rare	*P*-value*	FDR
HLA-C	rs10484554	127(63.5)	52 (26)	21 (10.5)	Total (*N* = 319)	133 (41.7)	143 (44.8)	43 (13.5)	5.7×10^{-6}	5.9 × 10–5	Total (*N* = 214)	90 (42.1)	91 (42.5)	33 (15.4)	0.00007	0.0007	Total (*N* = 105)	43 (41)	52 (49.5)	10 (9.5)	0.0001	0.0007
					Early (*N* = 195)	68 (34.9)	96 (49.2)	31 (15.9)	7.5×10^{-8}	1.5 × 10–6	Early (*N* = 132)	46 (34.8)	62 (47)	24 (18.2)	2.1 × 10-6	0.0001	Early (*N* = 63)	22 (34.9)	34 (54)	7 (11.1)	0.0001	0.0014
					Late (*N* = 124)	65 (52.4)	47 (37.9)	12 (9.7)	0.0707	0.2355	Late (*N* = 82)	44 (53.7)	29 (35.4)	9 (10.9)	0.2570	0.6746	Late (*N* = 42)	21 (50)	18 (42.9)	3 (7.1)	0.1004	0.1757
	rs10484545	170 (85)	30 (15)	0 (0)	Total (v = 319)	272 (85.2)	44 (13.8)	3 (1)	0.4697	0.5480	Total (*N* = 214)	183 (85.5)	30 (14)	1 (0.5)	0.9426	0.9426	Total (*N* = 105)	89 (84.7)	14 (13.3)	2 (2)	0.1939	0.2026
					Early (*N* = 195)	165 (84.6)	29 (14.9)	1 (0.5)	0.9433	0.9433	Early (*N* = 132)	109 (82.6)	23 (17.4)	0 (0)	0.5550	0.7343	Early (*N* = 63)	56 (88.9)	6 (9.5)	1 (1.6)	0.1400	0.1960
					Late (*N* = 124)	107 (86.3)	15 (12.1)	2 (1.6)	0.1899	0.2849	Late (*N* = 82)	74 (90.2)	7 (8.6)	1 (1.2)	0.0886	0.4442	Late (*N* = 42)	33 (78.6)	8 (19)	1 (2.4)	0.1332	0.1960
ERAP1	rs30187	84 (42)	102 (51)	14 (7)	Total (*N* = 319)	125 (39.2)	158 (49.5)	36 (11.3)	0.2673	0.3742	Total *N* = 214)	82 (38.3)	111 (51.9)	21 (9.8)	0.5136	0.7343	Total (*N* = 105)	43 (40.9)	47 (44.8)	15 (14.3)	0.1102	0.1543
					Early (*N* = 195)	81 (41.5)	94 (48.2)	20 (10.3)	0.5024	0.5553	Early (v = 132)	52 (39.4)	67 (50.8)	13 (9.8)	0.6294	0.7343	Early (*N* = 63)	29 (46)	27 (42.9)	7 (11.1)	0.3979	0.4642
					Late (*N* = 124)	44 (35.5)	64 (51.6)	16 (12.9)	0.1565	0.2739	Late (*N* = 82)	30 (36.6)	44 (53.7)	8 (9.7)	0.5864	0.7343	Late (*N* = 42)	14 (33.4)	20 (47.6)	8 (19)	0.0436	0.1017
	rs10050860	118 (59)	79 (39.5)	3 (1.5)	Total (*N* = 319)	207 (64.9)	97 (30.4)	15 (4.7)	0.0249	0.1743	Total (*N* = 214)	132 (61.7)	72 (33.6)	10 (4.7)	0.1103	0.4442	Total (*N* = 105)	75 (71.4)	25 (23.8)	5 (4.8)	0.0072	0.0252
					Early (*N* = 195)	121 (62.1)	63 (32.3)	11 (5.6)	0.0418	0.2195	Early (*N* = 132)	75 (56.8)	49 (37.1)	8 (6.1)	0.0877	0.4442	Early (*N* = 63)	46 (73)	14 (22.2)	3 (4.8)	0.0142	0.0663
					Late (*N* = 124)	86 (69.4)	34 (27.4)	4 (3.2)	0.0542	0.2276	Late (*N* = 82)	57 (69.5)	23 (28)	2 (2.5)	0.1269	0.4442	Late (*N* = 42)	29 (69)	11 (26.2)	2 (4.8)	0.0980	0.1757
	rs17482078	121 (60.5)	74 (37)	5 (2.5)	Total (*N* = 319)	210 (65.8)	93 (29.2)	16 (5)	0.0897	0.2355	Total (*N* = 214)	135 (63.1)	69 (32.2)	10 (4.7)	0.3436	0.7343	Total (*N* = 105)	75 (71.4)	24 (22.9)	6 (5.7)	0.0195	0.0453
					Early (*N* = 195)	123 (63.1)	61 (31.3)	11 (5.6)	0.1777	0.2849	Early (*N* = 132)	78 (59.1)	47 (35.6)	7 (5.3)	0.4391	0.7343	Early (*N* = 63)	45 (71.4)	14 (22.2)	4 (6.4)	0.0356	0.0997
					Late (*N* = 124)	87 (70.2)	32 (25.8)	5 (4)	0.0850	0.2355	Late (*N* = 82)	57 (69.5)	22 (26.8)	3 (3.7)	0.2302	0.6746	Late (*N* = 42)	30 (71.4)	10 (23.8)	2 (4.8)	0.1608	0.2047

Table 3. Cont.

Healthy controls (*N* = 200)			PsV (*N* = 319)					PsC (*N* = 214)					PsA (*N* = 105)				
Gene		**Disease onset**	**WT**	**Het**	**Hom rare**	***P*-value***	**FDR**	**Disease onset**	**WT**	**Het**	**Hom rare**	***P*-value***	**FDR**	**Disease onset**	**WT**	**Het**	**Hom rare**	***P*-value***	**FDR**
SNP	**WT**																		
Hom rare	**Het**																		
rs27524	88 (44)	Total (*N* = 319)	125 (39.2)	152 (47.6)	42 (13.2)	0.3439	0.4248	Total (*N* = 214)	85 (39.7)	104 (48.6)	25 (11.7)	0.6028	0.7343	Total (*N* = 105)	40 (38.1)	48 (45.7)	17 (16.2)	0.2026	0.2026
		Early (*N* = 195)	81 (41.5)	92 (47.2)	22 (11.3)	0.7979	0.8378	Early (*N* = 132)	55 (41.7)	62 (47)	15 (11.3)	0.8295	0.8710	Early (*N* = 63)	26 (41.3)	30 (47.6)	7 (11.1)	0.8953	0.8953
19 (9.5)	93 (46.5)	Late (*N* = 124)	44 (35.5)	60 (48.4)	20 (16.1)	0.1215	0.2677	Late (*N* = 82)	30 (36.6)	42 (51.2)	10 (12.2)	0.4854	0.7343	Late (*N* = 42)	14 (33.3)	18 (42.9)	10 (23.8)	0.0307	0.0997
rs27525	55 (27.5)	Total (*N* = 319)	108 (33.9)	211 (66.1)	0 (0)	0.1289	0.2677	Total (*N* = 214)	66 (30.8)	148 (69.2)	0 (0)	0.4551	0.7343	Total (*N* = 105)	42 (40)	63 (60)	0 (0)	0.0259	0.0453
0 (0)	145 (72.5)	Early (*N* = 195)	67 (34.4)	128 (65.6)	0 (0)	0.1402	0.2677	Early (*N* = 132)	39 (29.5)	93 (70.5)	0 (0)	0.6856	0.7578	Early (*N* = 63)	28 (44.4)	35 (55.6)	0 (0)	0.0116	0.0663
		Late (*N* = 124)	41 (33.1)	83 (66.9)	0 (0)	0.2864	0.3759	Late (*N* = 82)	27 (32.9)	55 (67.1)	0 (0)	0.3621	0.7343	Late (*N* = 42)	14 (33.3)	28 (66.7)	0 (0)	0.4465	0.4808

*WT – both alleles are wild type, Het – patient carrying the rare allele in one copy, Hom rare – patient carrying the rare allele in two copies; listed as [N, (%)]. *Significant compared to healthy controls.*

Subsequently, psoriasis vulgaris patients were subdivided into groups according to the presence or absence of arthritis (Table 3). In line with earlier publications [29, 32, 34], the genotype distribution of the *HLA-C* rs10484554 SNP was found to be significantly different in both the PsC and the PsA subpopulations ($p = 0.0007$ and $p = 0.0007$, respectively), compared to healthy controls. In PsA (but not in PsC) patients, the genotype distribution of three *ERAP1* SNPs (rs10050860, rs27525 and rs17482078) was also significantly different ($p = 0.0252$, $p = 0.0453$ and $p = 0.0453$, respectively) (Table 3). For all three SNPs, the proportion of patients carrying the wild type allele was higher than in the group of healthy individuals, suggesting that the rare allele of these SNPs might provide protection against the development of psoriatic arthritis. As no differences were detected in the genotype distribution of the studied *ERAP1* SNPs in PsC patients, it is likely that the trend in the difference observed for the rs10050860 SNP in the PsV patients is caused by the presence of the PsA patients. No difference was detected for the *ERAP1* rs27524 and rs30187 SNPs and the *HLA-C* rs10484545 SNP between the healthy controls and the psoriasis groups, even after stratification for arthritis.

We further stratified patients according to disease onset (early and late onset). Significant differences were observed only for the *HLA-C* rs10484554 SNP in the early onset groups, which is in agreement with earlier publications [13, 14, 29, 34]. This result suggests that *HLA-C* positivity is a susceptibility factor only for early onset psoriasis. Stratification led to no significant difference for the rs10484545 *HLA-C* SNP. The *ERAP1* SNPs (rs10050860, rs27525 and rs17482078) known to be associated with psoriatic arthritis were found to have only a tendency in the association with EO-PsA patients ($p = 0.0663$, $p = 0.0663$ and $p = 0.0997$, respectively) (Table 3). This lends further support to the notion that the differences in distribution of these SNPs detected in the total PsA group are primarily driven by the early onset subpopulation, and that these *ERAP1* SNPs presumably provide protection against early onset psoriatic arthritis.

The *ERAP1* rs17482078, rs10050860, rs30187 and rs2287987 SNPs were found to be in strong linkage disequilibrium, and the association between haplotypes including these SNPs and AS was recently reported [35]. A linkage disequilibrium block containing the rs17482078/rs10050860/rs30187 SNPs was also identified in our dataset (Figure 1). Thus, we examined whether the rs17482078/rs10050860/rs30187 haplotypes were associated with psoriasis susceptibility (Table 4). We found that Haplotype B (rs17482078/rs10050860/rs30187-CCT) was a risk factor only for LO-PsV ($p = 0.0409$) and for LO-PsA ($p = 0.0413$).

As it was reported earlier that some *ERAP1* variants influence psoriasis susceptibility exclusively in individuals carrying the *HLA-C* risk allele [12–14], we analysed

Table 4. Distribution of ERAP1 haplotypes in healthy controls and psoriasis patients

Haplotype	SNPs			Healthy controls (*N* = 200)	PsO (*N* = 319)			PsC (*N* = 214)			PsA (*N* = 105)		
	rs17482078	rs10050860	rs30187	Frequency	Disease onset	Frequency	*P*-value*	Disease onset	Frequency	*P*-value*	Disease onset	Frequency	*P*-value*
A	C	C	C	38 (19)	Total (*N* = 319)	58 (18.2)	0.8153	Total (*N* = 214)	35 (16.4)	0.4804	Total (*N* = 105)	23 (21.9)	0.5468
					Early (*N* = 195)	36 (18.5)	0.8909	Early (*N* = 132)	18 (13.6)	0.2015	Early (*N* = 63)	18 (28.6)	0.1056
					Late (*N* = 124)	22 (17.7)	0.7769	Late (*N* = 82)	17 (20.7)	0.7389	Late (*N* = 42)	5 (11.9)	0.2741
B	C	C	T	80 (40)	Total (*N* = 319)	148 (46.4)	0.1531	Total (*N* = 214)	97 (45.3)	0.2736	Total (*N* = 105)	51 (48.6)	0.1508
					Early (*N* = 195)	84 (43)	0.5349	Early (*N* = 132)	57 (43.2)	0.5644	Early (*N* = 63)	27 (42.9)	0.6873
					Late (*N* = 124)	64 (51.6)	0.0409	Late (*N* = 82)	40 (48.8)	0.1756	Late (*N* = 42)	24 (57.1)	0.0413
C	T	T	C	47 (23.5)	Total (*N* = 319)	64(20)	0.5220	Total (*N* = 214)	45 (21)	0.7184	Total (*N* = 105)	19 (18.1)	0.4624
					Early (*N* = 195)	43(22.1)	0.8875	Early (*N* = 132)	32 (24.2)	1	Early (*N* = 63)	11 (17.6)	0.5169
					Late (*N* = 124)	21 (16.9)	0.3125	Late (*N* = 82)	13 (15.9)	0.3149	Late (*N* = 42)	8 (19)	0.7642
D	T	T	T	33 (16.5)	Total (*N* = 319)	44 (13.8)	0.3985	Total (*N* = 214)	34 (15.9)	0.8658	Total (*N* = 105)	10 (9.5)	0.0962
					Early (*N* = 195)	28 (14.4)	0.5560	Early (*N* = 132)	22 (16.7)	0.9681	Early (*N* = 63)	6 (9.5)	0.1742
					Late (*N* = 124)	16 (12.9)	0.3798	Late (*N* = 82)	12 (14.6)	0.6976	Late (*N* = 42)	4 (9.5)	0.1842

*Frequency is listed as [N, (%)]. * Significant compared to healthy controls carrying the given haplotype.*

ERAP1 SNPs in *HLA-C* positive psoriasis and psoriatic arthritis patients. *ERAP1* SNP frequencies were compared between individuals carrying at least one copy of the risk allele of rs10484554 (*HLA-C* positive) and individuals not carrying the *HLA-C* sequence (*HLA-C* negative) (Table 5). Evidence for association in *HLA-C* positive individuals was observed for two loci. The *ERAP1* rs27524 SNP in *HLA-C* positive individuals exhibited a 1.74-fold increased risk of PsV ($p = 0.0454$) and a 2.33-fold risk of PsA ($p = 0.0185$), in agreement with the previous report [12]. Interestingly, rs27524 by itself was not associated in our dataset with either PsV or PsC and was found to have a tendency as a susceptibility factor only for LO-PsA (Table 3). The *ERAP1* rs27525 SNP decreased the risk of psoriatic arthritis development in *HLA-C* positive patients (odds ratio (OR) 0.42, $p = 0.0339$). Thus, the presence of the *ERAP1* rs27525 SNP seems to protect *HLA-C* positive individuals from developing psoriatic arthritis. No other interaction was found for the *ERAP1* rs30187, rs10050860 and rs17482078 SNPs in *HLA-C* positive individuals, even after further stratifying the patients into early and late onset subgroups.

Discussion

Psoriasis has been associated with the *PSORS1* disease susceptibility locus of the MHC class I region on chromosome 6. Within *PSORS1*, the *HLA-Cw6* gene has shown the strongest association with psoriasis [8]: about 60% of psoriasis patients carry the *HLA-Cw*0602* allele. *HLA-C* positive patients exhibit earlier disease onset, higher incidence of guttate and eruptive type of psoriasis, more extensive disease symptoms, and more frequent exacerbations caused by throat infections than *HLA-C* negative individuals. The frequency of *HLA-Cw*0602* is significantly lower in patients with psoriatic arthritis compared with those with psoriasis alone, and its presence is associated with a longer psoriasis–arthritis interval and milder arthritis forms [34, 36, 37].

In this study, a well-characterized cohort of psoriasis patients was available for investigating whether *HLA-Cw6* or *ERAP1* gene polymorphisms are associated with different clinical phenotypes of psoriasis and psoriatic arthritis. Our dataset included patients with early and late onset psoriasis vulgaris, as well as with cutaneous psoriasis and psoriatic arthritis.

Our results confirmed the previously reported genetic association of psoriasis with both *HLA-Cw6* and *ERAP1* genes [12–14]. The *HLA-Cw*0602* rs10484554 SNP was found to have a very strong association with PsV, and the association was highly significant with both PsC and PsA groups as well (although somewhat stronger with PsC). Interestingly, only EO-PsV was associated with *HLA-C* in our dataset. Previously, the *HLA-Cw*06* allele was not found to be a risk factor for late onset psoriasis in a Northern Polish population [38], and late onset psoriasis demonstrated only a weak association with *HLA-Cw*06* alleles in other studies [13, 39]. More recently, however, a study using dense genotyping revealed that *HLA-Cw*06* is associated with late onset psoriasis [40].

Table 5. HLA-C and *ERAP1* interactions in psoriasis and psoriatic arthritis

ERAP1 SNP	PsV (*N* = 319)				PsC (*N* = 214)				PsA (*N* = 105)			
	Disease onset	*P*-value*	OR	95% CI	Disease onset	*P*-value*	OR	95% CI	Disease onset	*P*-value*	OR	95% CI
rs30187	Total (*N* = 319)	0.0506	1.72	0.99–2.97	Total (*N* = 214)	0.0869	1.66	0.93–2.98	Total (*N* = 105)	0.0818	1.84	0.92–3.67
	Early (*N* = 195)	0.0643	1.73	0.97–3.09	Early (*N* = 132)	0.0837	1.74	0.93–3.28	Early (*N* = 63)	0.1812	1.70	0.78–3.69
	Late (*N* = 124)	0.1342	1.70	0.85–3.41	Late (*N* = 82)	0.3197	1.49	0.68–3.29	Late (*N* = 42)	0.1302	2.17	0.79–6.00
s10050860	Total (*N* = 319)	0.2402	0.72	0.41–1.25	Total (*N* = 214)	0.3092	0.73	0.40–1.33	Total (*N* = 105)	0.2893	0.68	0.34–1.39
	Early (*N* = 195)	0.2550	0.71	0.39–1.28	Early (*N* = 132)	0.4206	0.77	0.40–1.46	Early (*N* = 63)	0.2090	0.59	0.26–1.34
	Late (*N* = 124)	0.3967	0.74	0.36–1.50	Late (*N* = 82)	0.3266	0.66	0.29–1.51	Late (*N* = 42)	0.8050	0.88	0.33–2.39
rs17482078	Total (*N* = 319)	0.2080	0.70	0.40–1.22	Total (*N* = 214)	0.2568	0.71	0.39–1.29	Total (*N* = 105)	0.2893	0.68	0.34–1.39
	Early (*N* = 195)	0.2550	0.71	0.39–1.28	Early (*N* = 132)	0.3374	0.73	0.38–1.39	Early (*N* = 63)	0.3209	0.67	0.30–1.49
	Late (*N* = 124)	0.2931	0.68	0.33–1.40	Late (*N* = 82)	0.3266	0.66	0.29–1.51	Late (*N* = 42)	0.5211	0.72	0.26–1.99
rs27524	Total (*N* = 319)	0.0454	1.74	1.01–3.01	Total (*N* = 214)	0.1572	1.52	0.85–2.72	Total (*N* = 105)	0.0185	2.33	1.15–4.72
	Early (*N* = 195)	0.0592	1.75	0.98–3.13	Early (*N* = 132)	0.1587	1.57	0.84–2.95	Early (*N* = 63)	0.0500	2.21	0.99–4.94
	Late (*N* = 124)	0.1238	1.73	0.86–3.48	Late (*N* = 82)	0.3905	1.41	0.64–3.12	Late (*N* = 42)	0.0730	2.57	0.90–7.36
rs27525	Total (*N* = 319)	0.1502	0.64	0.35–1.18	Total (*N* = 214)	0.3682	0.74	0.38–1.43	Total (*N* = 105)	0.0516	0.48	0.23–1.01
	Early (*N* = 195)	0.1142	0.60	0.31–1.14	Early (*N* = 132)	0.3473	0.72	0.36–1.44	Early (*N* = 63)	0.0339	0.42	0.19–0.94
	Late (*N* = 124)	0.4530	0.75	0.35–1.61	Late (*N* = 82)	0.6254	0.80	0.33–1.94	Late (*N* = 42)	0.4278	0.65	0.23–1.87

**Individuals carrying at least one copy of the rare allele of the rs10484554 HLA-C SNP and both wild type alleles of the indicated ERAP1 SNP (HLA-C positive/ERAP1 negative) are compared with individuals carrying at least one copy of the rare allele of the rs10484554 HLA-C SNP and at least one copy of the rare allele of the indicated ERAP1 SNP (HLA-C positive/ERAP1 positive).*

Genetic variants of *ERAP1* have recently been reported to be associated with psoriasis [13, 14, 16]. ERAP1 trims peptides imported into the endoplasmic reticulum at their N-terminus and contributes to the shaping of the antigenic repertoire presented by class I MHC molecules [19]. The association of *ERAP1* with psoriatic arthritis has previously been investigated; however, no association between *ERAP1* SNPs and psoriatic arthritis or psoriatic arthritis subphenotypes was identified [27]. Recently, *ERAP1* association with psoriasis was detected in patients with an age of onset between 10 and 20 years, and no association was detected in cases with onset below 10 years [16].

Our results suggest that the *HLA-C* rs10484554 SNP contributes to psoriasis susceptibility. The proportions of the rare alleles of the *ERAP1* rs27525, rs17482078 and rs10050860 SNPs were higher in the group of healthy individuals, suggesting that individuals with the rare alleles of these SNPs might be protected against psoriasis. These associations were also apparent when groups of psoriatic patients were analysed separately according to the presence or absence of arthritis. The stratification of the patients for early (< 40 years) or late (≥ 40 years) disease onset revealed an age-dependent difference in the genetic background of psoriasis: the associations with these SNPs tend to be stronger in patients with early disease onset.

Although *ERAP1* was neither dependent on nor interacting with *HLA-C*06:02* in certain populations, an interaction between *HLA-C* and *ERAP1* was reported recently and confirmed several times subsequently [9, 12–14]. Interestingly, dominant epistasis between *HLA-Cw*0602* and one of the *ERAP1* SNPs was identified recently in psoriasis [23].

Recent studies of the *ERAP1* rs27524 SNP reported association with the pathogenesis of psoriasis, especially in the group with age of disease onset between 10 and 20 years [16, 41]. Our results revealed only a trend in association with the late disease onset group, for which it might increase the likelihood of psoriasis development. No association with psoriasis was observed for the *ERAP1* rs30187 SNP in this study, which is in contrast with previous studies [12, 16]; however, Jadon *et al.* also reported that there was no association with psoriatic arthritis [27]. In addition, this psoriasis-risk allele, which is common in the European population, was not associated with the disease in an East Asian population [24].

Psoriatic arthritis patients, especially in the early onset group, carrying the *ERAP1* rs27525 and rs17482078 SNPs seem to be protected from the subsequent development of the disease. Individuals carrying the rare allele of the *ERAP1* rs10050860 SNPs might be protected against psoriasis, but this effect was more prominent among patients with psoriatic arthritis, especially in the early onset group. These findings suggest that these three SNPs (rs27525, rs17482078, rs10050860) might be protective against psoriatic arthritis.

The two coding *ERAP1* SNPs (rs17482078 and rs10050860) and the rs30187 SNP are in linkage disequilibrium (Figure 1) and have been reported as a pro-

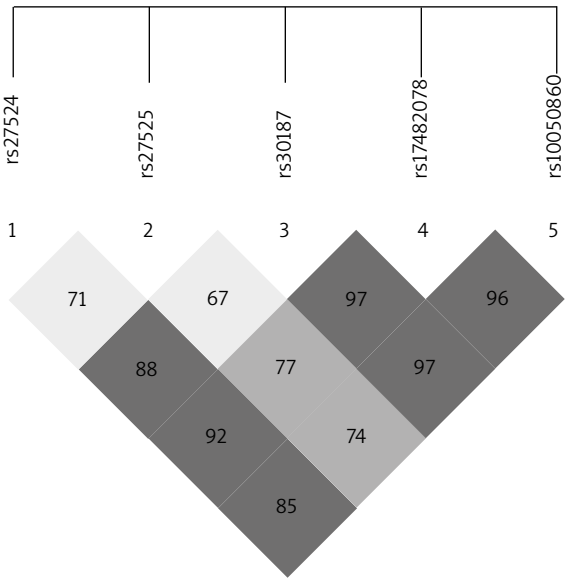

Figure 1. Linkage disequilibrium (LD) for the ERAP1 SNPs in the study population

tective haplotype (rs17482078/rs100508607rs30187/rs2287987–TTCC) in *HLA-B* positive AS patients [35]. Also the rs17482078/rs10050860/rs30187-CCT haplotype was confirmed as a risk factor for AS [42] in an AS population in Belgium, which we examined as Haplotype B (rs17482078/rs10050860/rs30187-CCT) in psoriasis susceptibility similarly to AS [35], and it was found to be a risk factor for only LO-PsV (p = 0.0409) and LO-PsA (p = 0.0413). We were unable to find any association with psoriasis or with psoriatic arthritis for Haplotype A (rs17482078/rs10050860/rs30187-CCC), Haplotype C (rs17482078/rs10050860/rs30187-TTC) and Haplotype D (rs17482078/rs10050860/rs30187-TTC). These data suggest that the Haplotype B conferring disease risk in AS also influences susceptibility to joint involvement in psoriasis.

Genome-wide association studies identified the interaction between the *ERAP1* rs27524 SNP and the *HLA-C* rs10484554 SNP; this interaction was the most prominent among individuals carrying one or two copies of the risk allele at rs10484554 [12]. In subsequent analysis, the association with *ERAP1* was not restricted to individuals carrying *HLA-Cw*0602*; however the genetic association with *ERAP1* (rs207524, rs30187, rs26653) in psoriasis was confined to individuals with an age of disease onset between 10 and 20 years [16]. In a Polish population, *ERAP1* rs27524 SNP was a susceptibility factor for *HLA-C* positive patients with late onset psoriasis [13]. In our study, the *ERAP1* rs27524 SNP in *HLA-C* positive individuals caused a 1.74-fold increased risk of PsV (p = 0.0454) and a 2.33-fold risk of PsA (p = 0.0185). Notably, the rs27524 SNP by itself was not associated either with PsV or with PsC in this dataset and was found to have only a tendency as a susceptibility factor for LO-PsA (Table 3). The *ERAP1* rs27525 SNP in *HLA-C* positive patients decreased the risk of psoriatic arthritis development (OR = 0.42, p = 0.0339); thus, the presence of these SNPs seems to protect against developing psoriatic arthritis.

Taken together, our results suggest that the genetic variants of the *ERAP1* and *HLA-C* genes contribute to the pathogenesis of psoriasis in a manner that is dependent on age of onset. Individuals with *HLA-Cw*0602* are more prone to early onset of disease (before 40 years), confirming that onset after 40 years represents a biologically valid approximation for a genetically distinct subgroup of psoriasis. The overall psoriasis group was stratified by various clinical aspects, including the age of onset and the presence or absence of psoriatic arthritis. This novel and careful stratification of patients according to the symptoms and age of onset leads to important insights for psoriasis, a heterogeneous, multifactorial disease, and might become more important for further research as well as for personalized medicine.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Boehncke WH, Schön MP. Psoriasis. Lancet 2015; 386: 983-94.
2. Gladman DD, Antoni C, Mease P, et al. Psoriatic arthritis: epidemiology, clinical features, course, and outcome. Ann Rheum Dis 2005; 64: i14-7.
3. Kepiro L, Szell M, Kovacs L, et al. Genetic risk and protective factors of TNFSF15 gene variants detected using single nucleotide polymorphisms in Hungarians with psoriasis and psoriatic arthritis. Hum Immunol 2014; 75: 159-62.
4. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. J Am Acad Dermatol 1985; 13: 450-6.
5. Nair RP, Stuart P, Henseler T, et al. Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C. Am J Hum Genet 2000; 66: 1833-44.
6. Alonso S, Tejón P, Sarasqueta C, et al. Age at disease onset may help to further characterize the disease phenotype in psoriatic arthritis. Joint Bone Spine 2016; 83: 533-7.
7. Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. Ann Rev Immunol 2014; 32: 227-55.
8. Chandran V. The genetics of psoriasis and psoriatic arthritis. Clin Rev Allergy Immunol 2013; 44: 149-56.
9. Bergboer JG, Zeeuwen PL, Schalkwijk J. Genetics of psoriasis: evidence for epistatic interaction between skin barrier abnormalities and immune deviation. J Invest Dermatol 2012; 132: 2320-31.

10. de Cid R, Riveira-Munoz E, Zeeuwen PL, et al. Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. Nat Genet 2009; 41: 211-5.
11. Cargill M, Schrodi SJ, Chang M, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet 2007; 80: 273-90.
12. Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium, Strange A, Capon F, et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. Nat Genet 2010; 42: 985-90.
13. Wiśniewski A, Matusiak L, Szczerkowska-Dobosz A, et al. 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. Hum Immunol 2017; 17: 30551-7.
14. Das A, Chandra A, Chakraborty J, et al. Associations of ERAP1 coding variants and domain specific interaction with HLA-C*06 in the early onset psoriasis patients of India. Hum Immunol 2017; 78: 724-30.
15. Queiro R, Tejón P, Alonso S, et al. Age at disease onset: a key factor for understanding psoriatic disease. Rheumatology 2014; 53: 1178-85.
16. Lysell J, Padyukov L, Kockum I, et al. Genetic association with ERAP1 in psoriasis is confined to disease onset after puberty and not dependent on HLA-C*06. J Invest Dermatol 2013; 133: 411-7.
17. Kirino Y1, Bertsias G, Ishigatsubo Y, et al. Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between HLA-B*51 and ERAP1. Nat Genet 2013; 45: 202-7.
18. Tanioka T, Hattori A, Masuda S, et al. Human leukocyte-derived arginine aminopeptidase. The third member of the oxytocinase subfamily of aminopeptidases. J Biol Chem 2003; 278: 32275-83.
19. Jensen PE. Recent advances in antigen processing and presentation. Nat Immunol 2007; 8: 1041-8.
20. Wearsch PA, Cresswell P. The quality control of MHC class I peptide loading. Curr Opin Cell Biol 2008; 20: 624-31.
21. Haroon N, Inman RD. Endoplasmic reticulum aminopeptidases: biology and pathogenic potential. Nat Rev Rheumatol 2010; 6: 461-7.
22. Guerini FR, Cagliani R, Forni D, et al. A functional variant in ERAP1 predisposes to multiple sclerosis. PLoS One 2012; 7: e29931.
23. Tsoi LC, Spain SL, Knight J, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012; 44: 1341-8.
24. Tang H, Jin X, Li Y, et al. A large-scale screen for coding variants predisposing to psoriasis. Nat Genet 2014; 46: 45-50.
25. Sun LD, Cheng H, Wang ZX, et al. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. Nat Genet 2010; 42: 1005-9.
26. Sheng Y, Jin X, Xu J, et al. Sequencing-based approach identified three new susceptibility loci for psoriasis. Nat Commun 2014; 5: 4331.
27. Jadon D, Tillett W, Wallis D, et al. Exploring ankylosing spondylitis-associated ERAP1, IL23R and IL12B gene polymorphisms in subphenotypes of psoriatic arthritis. Rheumatology 2013; 52: 261-6.
28. Helliwell PS, Taylor WJ. Classification and diagnostic criteria for psoriatic arthritis. Ann Rheum Dis 2005; 64 Suppl 2: ii3-8.
29. Evans DM, Spencer CC, Pointon JJ, et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet 2011; 43: 761-7.
30. Ombrello MJ, Kastner DL, Remmers EF. Endoplasmic reticulum-associated amino-peptidase 1 and rheumatic disease: genetics. Curr Opin Rheumatol 2015; 27: 349-56.
31. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. J Am Acad Dermatol 1985; 13: 450-6.
32. Gudjonsson JE, Karason A, Runarsdottir EH, et al. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients: an analysis of 1019 HLA-C- and HLA-B-typed patients. J Invest Dermatol 2006; 126: 740-5.
33. Zhang X, Wang H, Te-Shao H, et al. The genetic epidemiology of psoriasis vulgaris in Chinese Han. Int J Dermatol 2002; 41: 663-9.
34. Ho PY, Barton A, Worthington J, et al. HLA-Cw6 and HLA-DRB1*07 together are associated with less severe joint disease in psoriatic arthritis. Ann Rheum Dis 2007; 66: 807-11.
35. Bettencourt BF, Rocha FL, Alves H, et al. Protective effect of an ERAP1 haplotype in ankylosing spondylitis: investigating non-MHC genes in HLA-B27-positive individuals. Rheumatology 2013; 52: 2168-76.
36. Queiro R, Gonzalez S, López-Larrea C, et al. HLA-C locus alleles may modulate the clinical expression of psoriatic arthritis. Arthritis Res Ther 2006; 8: R185.
37. Winchester R, Minevich G, Steshenko V, et al. HLA associations reveal genetic heterogeneity in psoriatic arthritis and in the psoriasis phenotype. Arthritis Rheum 2012; 64: 1134-44.
38. Szczerkowska-Dobosz A, Niespodziana K, Rebala K, et al. Lack of association of HLA-C alleles with late-onset psoriasis in the northern Polish population. J Appl Genet 2007; 48: 273-5.
39. Allen MH, Ameen H, Fisher SA, et al. The major psoriasis susceptibility locus PSORS1 is not a risk factor for late-onset psoriasis. J Invest Dermatol 2005; 124: 103-6.
40. Hébert HL, Bowes J, Smith RL, et al. Identification of loci associated with late-onset psoriasis using dense genotyping of immune-related regions. Br J Dermatol 2015; 172: 933-9.
41. Bergboer JG, Oostveen AM, de Jager ME, et al. Paediatric-onset psoriasis is associated with ERAP1 and IL23R loci, LCE3C_LCE3B deletion and HLA-C*06. Br J Dermatol 2012; 167: 922-5.
42. Kadi A, Izac B, Said-Nahal R, et al. Investigating the genetic association between ERAP1 and spondyloarthritis. Ann Rheum Dis 2013; 72: 608-13.