UNIVERSITY OF SZEGED
ALBERT SZENT-GYÖRGYI MEDICAL SCHOOL
DEPARTMENT OF DERMATOLOGY AND ALLERGOLOGY
DOCTORAL SCHOOL OF CLINICAL MEDICINE

GENETIC EXAMINATIONS IN PSORIASIS

Ph.D. thesis

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Szeged
2023.

Publications related to the subject of the thesis

I. **Képíró L**, Széll M, Kovács L, Keszthelyi P, Kemény L, Gyulai R. Genetic risk and protective factors of *TNFSF15* gene variants detected using single nucleotide polymorphisms in Hungarians with psoriasis and psoriatic arthritis. *Hum Immunol* 2014;Feb;75(2):159-62. doi: 10.1016/j.humimm.2013.11.006. Epub 2013 Nov 20. PMID: 24269700.
IF: 2.138 (Q2) (Independent citation: 22 Self citation: 2 Cumulative: 24)

II. **Képíró L**, Széll M, Kovács L, Keszthelyi P, Kemény L, Gyulai R. The association of *HLA-C* and *ERAP1* polymorphisms in early and late onset psoriasis and psoriatic arthritis patients of Hungary. *Postepy Dermatol Alergol* 2021;Feb;38(2):43-51. doi: 10.5114/ada.2021.104277. Epub 2021 Mar 10. PMID: 34408565.
IF: 1.664 (Q2) (Independent citation: 2 Self citation: 0 Cumulative: 2)

Other publications

III. Dalmády S, Kiss M, **Képíró L**, Kovács L, Sonkodi G, Kemény L, Gyulai R.Higher levels of autoantibodies targeting mutated citrullinated vimentin in patients with psoriatic arthritis than in patients with psoriasis vulgaris. *Clin Dev Immunol* 2013;2013:474028. doi: 10.1155/2013/474028. Epub 2013 Mar 18. PMID: 23573111.
IF: 2.934 (Q2) (Independent citation: 25 Self citation: 2 Cumulative: 27)

IV. Mahil SK, Twelves S, Farkas K, Setta-Kaffetzi N, Burden AD, Gach JE, Irvine AD, **Képíró L**, Mockenhaupt M, Oon HH, Pinner J, Ranki A, Seyger MMB, Soler-Palacin P, Storan ER, Tan ES, Valeyrie-Allanore L, Young HS, Trembath RC, Choon SE, Szell M, Bata-Csorgo Z, Smith CH, Di Meglio P, Barker JN, Capon F. AP1S3 Mutations Cause Skin Autoinflammation by Disrupting Keratinocyte Autophagy and Up-Regulating IL-36 Production. *J Invest Dermatol* 2016;Nov;136(11):2251-2259. doi: 10.1016/j.jid.2016.06.618. Epub 2016 Jul 5. PMID: 27388993.
IF: 6.287 (D1) (Independent citation: 77 Self citation: 7 Cumulative: 84)

V. Rózsa T, **Képíró L**, Mareczky Zs, Varga E, Korom I, Husz S, Kemény L, Bata-Csörgő Zs, Cutan polyarteritis nodosa esete. *Bőrgyógyászati és Venerológiai Szemle* 2014;90:4 pp.162-165.,4 p.
IF: 0 (Independent citation: 0 Self citation: 0 Cumulative: 0)

VI. Gubán B, Kui R, **Képíró L**, Bebes A, Groma G, Kemény L, Bata-Csörgő Zs, Abnormális STAT1 aktivitás pikkelysömörben. *Bőrgyógyászati és Venerológiai Szemle* 2016;92:1 pp.18-21.,4 p.
IF: 0 (Independent citation: 0 Self citation: 0 Cumulative: 0)

VII. **Képíró L,** Kinyó Á, Kemény L, Bata-Csörgő Zs, Zoon plazmasejtes balanitis. *Bőrgyógyászati és Venerológiai Szemle* 2017;93(2).pp.79-81.,ISSN 0006-7768.
IF: 0 (Independent citation: 0 Self citation: 0 Cumulative: 0)

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. Besides plaque psoriasis, we define flexural psoriasis, guttate psoriasis and erythroderma. Pustular psoriasis is a distinct phenotype with sterile pustules generalized or localized only on digits or palms and soles.

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. Late onset psoriasis, or type II psoriasis is characterized by an onset at or after 40 years, less severe clinical symptoms and rare family-inheritance.

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 genetic determinants. Single nucleotide polymorphisms (SNPs) of several genes have been identified as contributing to psoriasis susceptibility; *HLA-Cw*0602*, *IL23R*, *LCE3C*, *LCE3B-del*, *TNFSF15*, *ERAP1*, *TNIP1*, *RNF113*, *IFIH1*, *IL23R*, *IL12B* and *TYK2* genes, IL-23/IL-17 cytokine network and tumor necrosis factor (TNF) pathway (e.g. IL-12, IL-17, IL-23,and TNF-α). However, pustular psoriasis seems to be genetically distinct, mainly has strong associations with *IL36RN*, *AP1S3* and *CARD14* genes.

Psoriatic arthritis is a seronegative inflammatory arthritis present in nearly 25% of patients with psoriasis, and is characterized by changing degrees of oligoarthritis, polyarthritis and spondylitis and typically involves dactylitis, distal interphalangeal joint involvement, mutilating arthritis or

enthesitis classified mainly with CASPAR (classification criteria for psoriatic arthritis) criteria.

Psoriatic arthritis is strongly associated with the *HLA-B*27*, *HLA-B13*, *HLA-B57*, *HLA-B39*, *HLA-Cw6* and *HLA-Cw7* alleles, IL-23/IL-17 cytokine network and tumor necrosis factor (TNF) pathway (e.g. IL-12, IL-17, IL-23, and TNF-α).

Tumor necrosis factor alpha (TNFα) seems to be one of the most important triggers for aberrant activation of lymphocytes and keratinocytes, and is a key cytokine both in psoriasis and in psoriatic arthritis. 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. TNFSF15 inhibits endothelial-cell and endothelial-progenitor-cell differentiation and stimulates T-cell activation, Th1 cytokine production and dendritic cell maturation. TNFSF15 plays an important role in the pathogenesis of inflammatory bowel disease (IBD), atherosclerosis, rheumatoid arthritis and chronic inflammatory skin diseases.

Endoplasmic reticulum-associated amino-peptidase 1 (*ERAP1*) gene has been reported to be associated with ankylosing spondylitis (AS), psoriasis and Behcet's disease. The ERAP1 protein belongs to the M1 family of zinc metallopeptidase enzymes and 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. HLA-C*06:02 mediates an autoimmune response against melanocytes by autoantigen presentation; 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. The ERAP1 protein also contributes to the

shedding of the membrane-bound receptors of inflammatory cytokines, such as IL-1R2, TNFR1 and IL-6R.

In European population three *ERAP1* variants, rs27524 (noncoding), rs27432 (intronic) and rs30187 (Lys528ARG), were found to be genome-wide risk factors for psoriasis. Furthermore, dominant epistasis between *HLA-Cw*0602* and the *ERAP1* rs30187 SNP was identified in studies. In Han Chinese populations, *ERAP1* SNPs or gene variants in linkage disequilibrium with *ERAP1* SNPs were also found to be associated with psoriasis. A strong evidence of association for rs30187 and for CC rs30187/rs27044 haplotype has been shown in Romanian *HLA-B27* positive psoriatic arthritis population. Overall metaanalysis showed an association between rs27524 and rs30187 polymorphisms and susceptibility to psoriasis.

2. Aims

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

The study population consisted of 319 Hungarian Caucasian psoriasis vulgaris patients (designated as PsV), as well as 200 ethnically matched healthy individuals with no known multifactorial inflammatory diseases nor IDB. Of the 319 psoriasis vulgaris patients, 105 exhibited psoriatic arthritis (designated as PsA), fulfilling the Classification Criteria for Psoriatic Arthritis (CASPAR). 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.

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). Genotyping of five SNPs of the *TNFSF15* gene, five SNPs of the *ERAP1* gene and two SNPs of the *HLA-Cw*0602* gene was carried out with the PCR-based Assay-by-Design method of Applied Biosystems (Foster City, CA). 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. Genotyping of the rs10484554 SNP was used to determine the *HLA-C* status. Three SNPs of the *ERAP1* gene (rs30187, rs10050860, rs17482078) were used to construct four haplotypes. 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.

The frequencies of genotypes, 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, the age of 40 years was used for stratification of age at disease onset.

4. Results

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

The genotype distribution of the rs6478109 *TNFSF15* 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.

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

The haplotype distribution of *TNFSF15* Haplotype C (rs3810937/rs6478108/rs6478109/rs7848647/rs7869487-AGTAA), showed a significant difference ($p=0.0041$) between the group of healthy individuals

and the group of PsV patients. 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. No significant difference was found in the distribution of Haplotype A and B (rs3810937/rs6478108/rs6478109/rs7848647/rs7869487-GACGA and AGTAG) when healthy controls and patients with psoriasis and psoriatic arthritis were compared.

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

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). 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.

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). 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. 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. 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).

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. Thus, we examined whether the rs17482078/rs10050860/rs30187 haplotypes were associated with psoriasis susceptibility. We found that *ERAP1* Haplotype B (rs17482078/rs10050860/rs30187-CCT) was a risk factor only for LO-PsV (p=0.0409) and for LO-PsA (p=0.0413).

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, 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). 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). 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.

5. Discussion

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 groups of psoriatic patients with and without arthritis are analyzed separately. A significant difference was found in the distribution of *TNFSF15* Haplotype C in the groups of healthy controls and PsC 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.

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: 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.

In the second part of this study, we confirmed the previously reported genetic association of psoriasis with both *HLA-Cw6* and *ERAP1* genes. 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. Interestingly, only EO-PsV was associated with *HLA-C* in our dataset. 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.

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 rs17482078/rs10050860/rs30187-CCT haplotype was confirmed as a risk factor for ankylosing spondylitis (AS) in an AS population in Belgium, which we examined as Haplotype B (rs17482078/rs10050860/rs30187-CCT) in psoriasis susceptibility and 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* SNPs and the *HLA-C* status. 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 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. 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

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.

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.

7. Acknowledgement

I would like to express my deep sense of gratitude to my supervisors, Márta Széll Ph.D., D.Sc. and Rolland Gyulai M.D., D.Sc., who supported and encouraged me during my scientific work. I am thankful for their scientific guidance, expertise, advices and patience.

I am also grateful to Lajos Kemény M.D.,D.Sc. for providing me such a great opportunity to complete my PhD work in the Department. I express my thanks for his support and guidance.

I would like to thank to our collaborating partners, László Kovács M.D., Péter Keszthelyi M.D., Róbert Kui M.D. for providing us blood samples for genetic examinations.

I wish to thank Andrea Tanácsné Bajkán for her excellent technical assistance Éva Viharosné Dósa-Rácz, Péter Oláh, Katalin Farkas and Kornélia Tripolszki for helping me in the statistical analysis and *in silico* transcription factor binding analysis and Eszter Martinovits for the help during the preparation of the thesis.

I am indebted to my family and friends for their encouragement, patience, unconditional support and love throughout my life.

This work was supported by the OTKA NK77434, OTKA K73548, OTKA K105985 and TÁMOP-4.2.2.A-11/1/KONV, TÁMOP-4.2.2-B-10/1-2010-0012.