SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of the genetic background of alcohol consumption and obesity in psoriatic patients

by Zita Szentkereszty-Kovács

Supervisor: Dániel Törőcsik, PhD

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF HEALTH SCIENCES

DEBRECEN, 2022

Investigation of the genetic background of alcohol consumption and obesity in psoriatic patients

By Zita Szentkereszty-Kovács

Supervisor: Daniel Törőcsik, PhD

Doctoral School of Health Sciences, University of Debrecen

Head of the **Examination Committee***:	Margit Balázs, DSc
Members of the Examination Committee:	Eszter Szlávicz, PhD
	Zsuzsa Rákosy, PhD

The examinatition is held online and starts at 10:00 a.m., on the 24th of May, 2022.

Head of the **Defense Committee**:	Margit Balázs, DSc
Reviewers:	Mariann Harangi, DSc
	Judit Danis, PhD
Members of the Defense Committee:	Eszter Szlávicz, PhD
	Zsuzsa Rákosy, PhD

The PhD Defense is held online and starts at 2:00 p.m., on the 24th of May, 2022.

Publicity is provided online. If you wish to participate, please indicate it in a message sent to the email adress: jenei.tibor@med.unideb.hu by 2:00 p.m., on the 23rd of May, 2022 latest. After the deadline there is no longer possible to connect to the PhD Defense due to technical issues.

INTRODUCTION

Psoriasis was already known in ancient Rome, although at that time its nomenclature merged into other exfoliating skin conditions. The term ‘psoriasis’ has been introduced only in 1841 by Ferdinand Hebra, who also provided the clinical description of the disease.

Understanding its pathology has been a central issue of numerous studies over the past half century. Due to the results of the emerging molecular biological research methods, our knowledge about this chronic systemic disease increased and the concept that an epithelial cell defect is in the background changed. Besides positing the immune system and several inflammatory pathways into the focus, more and more details were revealed also of its multifactorial origin. Initiating factors are now determined not only by environmental ones but also by genetic predisposition, as part of which more than 60 psoriasis susceptibility loci was mapped in the human genome in the last decade.

The related comorbidities are diverse: almost all organ systems could be affected, including the musculoskeletal (psoriatic arthritis) and the cardiovascular system or even the central nervous system (psychiatric disorders such as depression and dependencies). Although, in terms of therapy, biological agents mean a reassuring solution for the treatment of skin and possible joint symptoms in almost all patients with psoriasis, our results confirm the need for incorporating additional knowledge and individualized treatment strategies to the management due to the different comorbidities. In the course of our work, we aimed to explore the relationship between obesity, - being a part of the metabolic syndrome -, and increased alcohol consumption with psoriasis at the level of genetic factors more extensively.

Development of psoriasis at the cellular level

Besides genetic predisposition, different so-called “trigger factors” such as environmental stressors and pathogens (especially Streptococcus), trauma, all together with smoking and alcohol consumption increase the production of antimicrobial peptides by keratinocytes. One such antimicrobial peptide is cathelicidin (LL37), which forms a complex with the DNA and RNA released from damaged keratinocytes to activate both conventional (cDCs) and plasmacytoid dendritic cells (pDCs), induce the production of interferon alpha (IFN-α), tumor necrosis factor-α (TNF-α) and of nitric oxide (NO), which then further promote inflammation. By migrating to the local lymph node, pDCs activate T-helper (Th)1 and Th17 cells by producing interleukin (IL)-23 and IL-17. Recirculate to the skin Th1 an Th17 cells produce IL-17A/F and IL-22 cytokines, leading to increased proliferation of keratinocytes. As a result, the 28-day maturation cycle changes to a few days, which leads to the formation of a thickened epidermis. At the same time, they also induce the production of additional proinflammatory mediators (antimicrobial peptides, cytokines, chemokines), which attract neutrophil granulocytes and macrophages and more DCs to the skin, amplifying the inflammatory processes.

Genetic background of psoriasis

Cumulative incidence of psoriasis in families raised the possibility of genetic predisposition relatively early. To the best of our knowledge, if both parents have psoriasis, their child has a 50% chance of inheriting it, while if only one parent is affected, then the chance is reduced to 16%. According to twin studies, the concordance is relatively high - between 35% and 72% - in the case of monozygotic twins, while in case of dizygotic twins this rate is only between 15% and 23%. However, the fact that in monozygotic, psoriasis-concordant twins, the time of onset,

severity, and localization of symptoms showed many similarities, but were not 100% also suggested a role for environmental factors.

SNP studies

A single nucleotide polymorphism (SNP) is a change in the DNA sequence that affects a single nucleotide with frequency of at least 1% in the population. The Hapmap (map of haplotypes) is a catalog, which can be interpreted as a collection of the most common SNPs. So far more than 11.5 million SNPs have been described in the human genome, most of which have no effect on the phenotype, either because it is either in the non-coding region or because it is a synonymous SNP, which encodes the same amino acid. However, SNPs affecting the coding regions may influence the structure and functions of the protein they encode, thus playing a role in the development of pathological processes.

GWAS studies

The first GWAS study involving psoriasis patients was completed in 2007, genotyping 25,125 SNPs in 466 cases and 500 controls, however by now studies involving 20,000 cases and control groups of 280,000 are also available. If the difference between the study and control groups is significant for a given SNP, an association is likely to exist between the SNP and the disease. As a result of the methodological advances, by now more than 60 psoriasis susceptibility regions have been identified in the human genome, with genes encoding proteins that may play a prominent role in the pathogenesis of the disease.

Genetic associations in psoriasis

Numerous genes have been identified so far to be associated with psoriasis, of which HLA-C is the most important one. Its variant, HLA-Cw6, shows the strongest association with the early-onset psoriasis, affecting the immune response and antigen presentation to CD8+ T lymphocytes.. Although due to the multifactorial background, only 10% of allele carriers develop the disease, this does not call into question the magnitude of its discovery. Further associated genes encode proteins involved in Th17 activation, such as *IL12B* and *IL23A*, encode the interleukin 23 receptor (*IL23R*) or nuclear factor kappa-B (NF-κB) inhibitor zeta (*NFκBIZ*), which is a target for IL-17 signaling in keratinocytes.

Linkage analyzes also led to the identification of nine different chromosomal regions, the so-called Psoriasis Susceptibility (PSORS 1-9) regions, that are also predisposing to psoriasis.

PSORS1 is in the major histocompatibility complex (MHC) region, located on the short arm of chromosome 6 (6p21.3), containing several genes associated with psoriasis, such as *HLA-C*, the coiled-coil alpha-helical rod protein (*CHCR1*) and corneodesmosin (*CDSN*) encoding ones. While the function of CHCR1 is still poorly understood, corneodesmosin is involved in skin desquamation, a process that is known to be altered in psoriasis.

Although in the case of PSORS2 and PSORS4, weaker connectivity signals were observed, they were confirmed by several independent studies, while other PSORS regions (PSORS-3, -5, -6, -7, -8, -9) were not reproducible in all populations.

In the PSORS2 region a mutation in the gene encoding the caspase recruitment domain family member 14 (*CARD14*) needs to be highlighted, leading to increased production of pro-inflammatory cytokines through enhancement of NF-κB pathway activity.

In the PSORS4 region, the Epidermal Differentiation Cluster (*EDC*) is an evolutionarily conserved genomic segment containing more than 60 genes involved in the terminal

differentiation of keratinocytes. Out of these, genes encoding late cornified envelope 3B (*LCE3B*) and 3C (*LCE3C*) are the most outstanding ones, which show a strong association with psoriasis, confirming the importance of the impairment of skin barrier function in the pathogenesis of psoriasis.

Role of alcohol in psoriasis

As reviewed and reported in many studies, significant alcohol intake is an independent risk factor in the onset of psoriasis, presuming and partly revealing numerous mechanisms in the background of the disease. Alcohol use is also able to worsen the already-existing disease via increased TNF-α production and triggers alcohol-related disorders (e.g., alcoholic liver disease); furthermore, the treatment response in psoriasis is less favorable in cases of considerable alcohol consumption as these data have also been explored in a meta-analysis. In alcoholics, the activity of pro-inflammatory cytokines was found to be elevated, which might also contribute to the inflammatory process in psoriatic patients.

Ingested alcohol (i.e., ethanol) can be metabolized primarily to acetaldehyde by the alcohol dehydrogenase (ADH) enzyme family, catalase and cytochrome P450 isoform 2E1 (CYP2E1) and further oxidized into acetate and acetyl coenzyme A (acetyl-CoA) by the enzyme aldehyde dehydrogenase (ALDH). Acetyl-CoA serves as a precursor for the generation of acetone, which is formed by the decarboxylation of acetoacetic acid. During ethanol metabolism, the formation of reduced nicotinamide adenine dinucleotide (NADH) is increased which delays the degradation of acetyl-CoA and thus elevates acetone blood levels. Ethanol and its metabolites (acetaldehyde and acetone) can be both initiating and exacerbating factors at the cellular level in the inflammatory processes of psoriasis.

The effect of alcohol on keratinocytes

It is known that from consumed and digested ethanol a measurable amount finds its way to and within the human skin, being secreted by the eccrine glands (primarily sweat glands) or during passive diffusion. The potential effects of transdermal alcohol have been investigated *in vitro* and found that ethanol and its metabolite acetone increase the proliferation and mRNA

expression of proliferation-associated genes (α5 integrin, cyclin D1 and keratinocyte growth factor receptor [KGFR]) in HaCaT keratinocytes and may thus increase the permeability of the skin, disrupting its barrier function. In addition, alcohol also has an effect on lipid metabolism: the risk of high triglycerides is increased with increasing alcohol consumption, and thus it may also affect the lipid composition of the skin barrier.

Reactive oxygen species (ROS) generated in the skin of alcohol consumers during ethanol metabolism and acetaldehyde formation are able to regulate the mitogen-activated protein kinase/activator protein 1 (MAPK/AP1), the NF-κB and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signal transduction cascades. This oxidative stress, together with TNF-α, one of the major cytokines in psoriasis, results in a positive feedback loop, leading to additional ROS formation and IL-1, IL-6 and IL-8 inflammatory cytokine production in primary human keratinocytes, thus contributing to the pathogenesis of psoriasis.

The family of protein kinase C (PKC) isoenzymes regulates important cellular functions including cell growth, differentiation and cytokine production. Characteristic PKC isoenzyme patterns were described in HaCaT cells and it is generally accepted that the down-regulated PKC subspecies in psoriasis may be involved in aberrant cell growth and differentiation. The interaction of ethanol with PKC is complex and the results are generally contradictory due to the various species/tissues and different alcohol exposures that were used in the experiments; however, it is worth noting that through the regulation of PKCs, ethanol might negatively affect the cell cycle and tissue growth in psoriasis.

The effect of alcohol on the immune system

Ethanol and its previously listed metabolites also affect both innate and adaptive immunity and may have a direct or indirect impact on immune cells. Ethanol was found to augment the uric

acid-induced NLR family pyrin domain containing 3 (NLRP3) inflammasome activation and IL-1β production through the repression of the aryl hydrocarbon receptor (AhR) in macrophages, increase the proliferation and induce the release of interferon gamma (IFN-γ) in lymphocytes and elevate the production of TNF-α from peripheral blood monocytes and macrophages. The soluble form of TNF-α is released by the TNF-α-converting enzyme (TACE), the overexpression of which was described in psoriatic lesions. Furthermore, excessive alcohol consumption may contribute to its increased expression in peripheral blood mononuclear cells (PBMCs) and, in consequence, to elevated plasma soluble TNF-receptor 1 (sTNF-R1) concentrations in patients with psoriasis.

The relationship between keratinocytes and immune cells is well known also in the pathomechanism of psoriasis. When supernatants of keratinocytes isolated from the skin of psoriatic patients were co-cultured with HUT78 lymphocytes, findings showed increased protein levels of IFNγ, transformed growth factor-α (TGFα) and IL-6 after ethanol treatment, and suggested that ethanol also affects the epidermal and immune cell communication by triggering the Th1 response.

Murine models also support a role for ethanol in skin inflammation. *In vitro* studies showed that the expression of C-C motif chemokine ligand 20 (CCL20) (an essential chemoattractant molecule in psoriasis) was induced in normal murine epidermal keratinocytes when the medium was supplemented with ethanol. Moreover, the skin of ethanol-administrated hairless mice is characterized by attenuated skin hydration with significantly increased transepidermal water loss, up-regulated expression of TNF receptor 2 (TNFR2), decreased production of ceramide and type I collagen and increased plasma TNF-α concentrations. In addition, anti-TNF-α antibody treatment ameliorated the impaired skin barrier function in these mice. Chronic ethanol feeding also negatively affected both the number and the function of murine epidermal and dermal T cell subsets.

Altogether, these data suggest that alcohol consumption can adversely affect psoriasis symptoms at the cellular level by influencing the proliferation, differentiation and skin barrier function of keratinocytes and inducing immune dysfunction via altering the production of inflammatory cytokines by immune cells.

Alcohol consumption and addiction in psoriatic patients

Susceptibility to different addictions seems to be increased in the psoriatic population. Conducting a study based on a self-reported screening test by a validated questionnaire of the six most common addictions in Germany with 102 psoriatic patients, Eyerich et al. found that the risk of alcohol abuse, nicotine abuse and gambling were significantly higher in the study group compared to the general population.

The impact of alcohol consumption on the severity of the disease was investigated by many researchers, while McAleer et al. did not find significant correlation between excessive alcohol intake and disease severity by applying validated questionnaires (Michigan Alcohol Screening Test, Alcohol Use Disorders Identification Test - AUDIT, CAGE) and biomarkers, such as γ glutamyl-transferase (γGT) and carbohydrate-deficient transferrin (CDT), on a study cohort of 135 patients with moderate to severe chronic plaque psoriasis, Kirby et al did. Meanwhile, in another small study population using whole-blood phosphatidylethanol (PEth) to verify alcohol intake, the level of alcohol consumption correlated with the extent of psoriasis.

Applying the AUDIT and CAGE questionnaires to facilitate the recognition of potential alcohol problems, we involved 214 psoriasis patients in our study to assess alcohol use in our psoriasis population; however, only 27 confirmed regular alcohol use, while 34 denied drinking alcohol at any time. As the prevalence of daily alcohol consumption was found to be over 40% and alcohol abuse is estimated to be around 10% in the Hungarian population, it is very unlikely

that the results from our psoriasis population were indeed valid, suggesting that integrating and dealing with the drinking habits of psoriasis patients faces great limitations. Indeed, the power of stigmatization is strong as society considers alcoholism as a taboo, in spite of the estimated 40-70% inheritance predisposition in its development based on twin and adoption studies. Interestingly, most genes or SNPs that can be linked to alcohol dependence in a predisposing or protective manner, could be associated to the process of alcohol metabolism or neurotransmission.

Obesity in psoriasis

Epidemiological studies revealed that metabolic syndrome (MetS), which includes the clustering of insulin resistance, dyslipidaemia, hypertension, and obesity, has a pooled odds ratio (OR) of 2.14 in psoriatic patients in comparison to the general population.

Importantly, obesity is not only involved in sustaining a low-grade inflammation through the production of cytokines such as TNF-α and IL-6, but may also modify the effect of therapies, especially of TNF-α inhibitors. Despite these findings, although patients are routinely screened and treated for psoriatic arthritis, for other common comorbidities such as cardiovascular disorders and MetS, they are still not properly screened for and managed, which may be partially explained by the fact that the question of whether MetS is indeed associated with psoriasis at the level of pathogenesis or is perhaps more a result of impaired life quality of patients leading to behavior changes is yet to be answered in full. A cross-sectional, population-based twin study of 37,481 Danish twins including psoriasis-discordant (dizygotic) twin pairs, which found obesity more common in the twin with psoriasis compared with the one without psoriasis, while in monozygotic twin pairs, less correlation was found between obesity and psoriasis. However, to define the exact role for genetic components in a disease that is mostly multifactorial, such as obesity, is a great challenge. Out of 870 SNPs identified so far as being associated with obesity, only 5% showed a direct link with the increase in the body mass index (BMI) score. Most of these SNPs are in genes related to the regulation of appetite and satiety at the CNS level (e.g., BDNF, LEPR, MC4R, NEGR1, NPY, TMEM18), insulin secretion and action (e.g., ADIPOQ), adipogenesis (e.g., PPARG), and energy and lipid metabolism (e.g., FTO, UPC2).

OBJECTIVES

In our research, we aimed to better understand the relationship between psoriasis and alcohol consumption and obesity at the level of genetic factors.

1. We aimed to reveal if genetic predisposition of alcohol consumption exists in the Hungarian psoriasis population, using the Hungarian average population as a control. By examining the relationship between 23 alleles associated with alcohol consumption and psoriasis selected on the basis of the latest literature data, we sought to answer the question whether genetic factors may also be responsible, in addition to environmental factors, for alcohol consumption in patients with psosriasis.

2. A previous study at our Clinic, which examined the clinical and epidemiological characteristics and the comorbities of the psoriasis patient population (n = 377), found that obesity was more common in patients with late onset psoriasis, especially in the eldery group of patients. Although this result suggested a potential role for the environmental factors in linking obesity and psoriasis, still the effect of the genetic background couldn't be ruled out completely. Therefore, we aimed to investigate the possible role of genetic factors in obesity in patients with psoriasis.

MATERIALS AND METHODS

Study population and patient characteristics

Besides the 2,967 individuals from the Hungarian general population (HG), for the studies on the SNPs associated with alcohol consumption a total of 776 patients (580 patients from Department of Dermatology, University of Debrecen and 196 patients from Department of Dermatology, Semmelweis University, Budapest) diagnosed with psoriasis vulgaris, while for the studies on the SNPs associated with obesity a total of 574 patients from University of Debrecen, Department of Dermatology diagnosed with psoriasis vulgaris were enrolled.

The diagnosis of psoriasis was approved by at least two dermatologists. To assess the severity of psoriasis, Psoriasis Area and Severity Index (PASI) score was used. Symptoms were dichotomized as follows: in case of patients receiving only topical treatment and/or PASI <10 without therapy was specified as mild psoriasis, ≥10 as severe psoriasis. Patients receiving systemic therapy were all included in the severe psoriasis group independent of the recent PASI score. Data including family history (familial aggregation was positive if psoriasis existed in at least one more case among first degree relatives extended with grandparents in familial anamnestic data), age at onset (early onset: ≤40 years, late onset: >40 years), alcohol consumption habits and BMI scores (categories were divided into three categories: normal weight: BMI < 25, overweight: 25 ≤ BMI < 30, and obese: BMI ≥ 30) were collected.

Sample representative of the HG population in terms of geographic, age, and sex distributions were obtained from a population-based disease registry, the General Practitioners' Morbidity Sentinel Stations Program (GPMSSP).

SNP selection

Selection of SNPs associated with alcohol consumption

Our study based on previous GWAS studies and relevant systemic literature reviews, utilized SNPs of candidate genes most likely to be associated with the characteristics of alcohol consumption by encoding enzymes involved in alcohol metabolism and in pathways related to dependence: alcohol dehydrogenase *(ADH1B, ADH1C)*, aldehyde dehydrogenase *(ALDH1A1, ALDH2)* and neurotransmitters in the dopaminergic *(SLC6A3, DDC)*, GABAergic *(GABRA2, GABRG1)*, serotonergic *(HTR1B, MAOA, TPH2)*, cholinergic *(CHRM2)*, glutamatergic *(GRIN2A)* and opioidergic *(POMC, OPRM1, OPRK1)* pathways, as well as one SNP in the gene encoding a protein involved in neural development and dendritic growth neurogenesis *(BDNF)*.

Selection of SNPs associated with obesity

Our study utilized the same collection set of 20 SNPs in candidate genes likely to be associated with the development of obesity that was previously assessed in the HG population. SNP selection, with an emphasis on GWAS (PubMed) data, was based on a systematic literature review that revealed which SNPs showed significant associations with obesity-related features and had a minor-allele frequency >5% in the HapMap dataset for a European ancestry population sample (CEU) (www.hapmap.org).

DNA Preparation

DNA isolation was performed from ethylenediaminetetraacetic acid (EDTA) - anticoagulated blood samples using the MagNA Pure LC DNA Isolation Kit-Large Volume (Roche

Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. The extracted DNA samples were stored at −30 ˚C until measurements were carried out.

Genotype assessment

Genotyping of the selected SNPs was performed by Mutation Analysis Core Facility (MAF) of Clinical Research Center, Karolinska University Hospital (Stockholm, Sweden) using the Mass Array platform with iPLEX Gold Chemistry (Sequenom). The validation, concordance analysis and quality control were conducted by MAF according to their protocol, resulting in a successful genotyping outcome. HLACw*0602 typing was carried out as described previously in the literature.

Statistical analyses

The data were analyzed using STATA 12.0 Statistical software (StataCorp LP, College Station, TX, USA) and by SPSS 25 (SPSS package for Windows, Release 25; SPSS Inc., Chicago, IL, USA). The Mann–Whitney U and χ 2 tests were used to compare the mean age distribution of the two study groups. The existence of the Hardy–Weinberg equilibrium (HWE) and significant differences in the allele and genotype frequencies between the two populations were examined with the χ 2 test. To decrease the proportion of false positive results, a p threshold of 0.002 was applied (Bonferroni correction); otherwise, the threshold for significance was 0.05. To take account of confounding effects of gender and age on differences between study populations, linear regression models were constructed. Psoriatic samples were divided into several subgroups defined by clinical parameters such as familial aggregation, age at onset, and severity as described previously.

In case of SNPs associated with alcohol consumption association analyses were done according to additive, dominant and recessive models. To further confirm the findings two psoriatic populations from Hungary (Budapest vs. Debrecen) were also compared.

In case of SNPs associated with obesity association analyses were performed according to an additive model using age and sex as covariates by using PLINK v1.07 software (Center of Human Genetic Research (CHGR), Boston, MA, USA). For the power calculation of the association analyses, the Quanto version 1.2 software (Department of Population and Public Health Sciences, Keck School of Medicine of University Southern California, Los Angeles, CA, USA) was used.

RESULTS I.

Comparison of the psoriasis group to the Hungarian general population

The mean age was 49.15 years±16.82 in the case of psoriatic patients and 45.53 years±14.62 in the case of the HG population. The mean age of the study groups was significantly different according to the Mann–Whitney U test ($p<0.001$). The proportion of male individuals in psoriatic sample was significantly higher (psoriatic: 60.6% vs. HG: 46.8%, $p<0.001$). Allele frequencies in the study populations were calculated on the basis of the obtained genotype distributions. ALDH2 rs671 and SLC6A3 rs6530 were monomorphic in both groups, and were consequently excluded from further analyses. Differences between the psoriatic and HG population remained significant only for one SNP, rs1229984 in *ADH1B*, after multiple test correction. Comparing the allele frequency distribution of SNP rs1229984, significantly higher prevalence of effect allele C was found in the psoriatic population compared to the HG population (94.46% vs. 92.04%, $p<0.001$). Significant differences were observed between study groups in the association analysis according to the additive (ORadditive=1.58, 95% CI 1.23–2.03, $p<0.001$) and the recessive model (ORrecessive=1.58, 95% CI 1.22–2.04, $p=0.001$) but not the dominant model (ORdominant=4.29, 95% CI 0.55–33.08, $p=0.163$).

Stratified analysis of the psoriasis group

By comparing the psoriasis subgroups several strata of psoriatic patients were defined, such as familial aggregation vs. sporadic case, early-onset of disease (≤40 years) vs. late-onset (>40 years) and mild (PASI score <10) vs. severe psoriasis symptoms (PASI score ≥10, or receiving systemic therapy). The number of psoriatic subjects in the subgroup analysis were

sufficient to attain power of 80% and to detect an OR of 1.9 (assuming at least case–control ratio of 1.8 and alfa=0.05). The statistical power was calculated using Epiinfo 7.2 StatCalc calculator. Significant results were found only in case of one SNP, rs1799971 (μ-opioid receptor gene, OPRM1) when familial cases were compared to sporadic cases. The effect allele G increased the risk of familial psoriasis by twofold compared to sporadic cases both in additive and in dominant models (ORadditive= 1.99, 95% CI 1.36–2.91, $p < 0.001$, ORdominant=2.01, 95% CI 1.35–3.01, $p<0.001$).

When creating an additional subgroup from psoriatic patients with history of early-onset (≤40 years) and familial aggregation and comparing it to the HG population, in case of the rs1229984 (ADH1B) and rs1799971 (OPRM1) significant associations were found. The effect allele 'C' (rs1229984) both in the additive and recessive models, increased the risk of psoriasis, however, in the subgroup analysis the OR was much larger (ORadditive=2.41, 95% CI 1.26–4.61, $p < 0.01$, ORrecessive = 2.42, 95% CI 1.26–4.68, p1.26–4.68, $p<0.01$). Furthermore, the G allele of the rs1799971 (OPRM1) was also significantly associated with increased psoriasis risk in the additive and dominant models (ORadditive= 1.75, 95% CI 1.27–2.43, $p = 0.001$, ORdominant=1.82, 95% CI 1.26–2.63, $p=0.001$).

Familial aggregation and early-onset of psoriasis inevitably suggest the involvement of genetic factors and is highly associated with HLA-Cw*0602. In our cohort, psoriatic patients who had a history of familial aggregation, the risk allele of HLA-Cw*0602 gene occurred more frequently compared to patients having sporadic disease (53.80% vs. 34.78%, $p<0.001$). However, neither rs1229984 (ADH1B) nor rs1799971 (OPRM1) showed a significant association when subgroups formed on the presence of at least one HLA-Cw*0602 allele were compared. Assessing a possible gene–gene interaction between HLA-Cw*0602 and the ADH1B and OPRM1 genes in the early-onset vs. late-onset and familial aggregation vs. sporadic subgroups of patients no evidence on epistasis was found in any of the subgroups.

Furthermore, there were no differences in the risk allele distribution among HLA-Cw*0602 positive men and women in case of any SNPs.

RESULTS II.

Characteristics of study populations

When compared to the HG population, the proportion of male individuals in the psoriatic group was significantly higher (psoriatic: 61.8% vs. HG: 46.8%, $p < 0.001$), while the mean age was 50.28 years ± 15.55 in the case of patients with psoriasis and 45.53 years ± 14.62 in the case of the HG population. The BMI distribution was not different in the study populations (psoriatic: 30.0 kg/m2 (SD ± 6.5) vs. HG: 29.3 kg/m2 (SD ± 6.9)).

Stratifying psoriatic patients to early-onset (≤40 years) and late-onset (>40 years) groups, the mean age among the early-onset was significantly lower than in the late-onset group according to the Mann–Whitney U test ($p < 0.001$). Comparing familial aggregation and sporadic occurrence between early- and late-onset groups showed that the sporadic occurrence was significantly higher among the late-onset group, while the familial aggregation showed significantly greater incidence in the early-onset group using $\chi 2$ statistics ($p < 0.001$). With regard to BMI, in accordance with our previous study, the proportions of obese patients were significantly higher in the late-onset psoriasis group. In respect of gender and severity, there were no differences between the two subgroups.

Frequency and impact of the selected SNPs in the study populations

All analyzed obesity-predisposing SNPs were in Hardy–Weinberg equilibrium in the two study groups of HG and psoriasis. Risk allele frequencies showed no significant differences between the two study groups, even when further subgroups of psoriatic patients were created (early- vs. late-onset psoriasis). Although the sample size showed rather low power for all the SNPs (6–

46%), suggesting that they have a weak contribution to the development of obesity, statistically significant associations were still found in the psoriasis populations. Both the LEPR gene variant rs1137101 and the BDNF gene variant rs925946 showed strong association with obesity in the association analysis, as indicated by the beta values (1.068 (0.360; 1.777I), p = 0.003; and 1.237 (0.414; 2.059), p = 0.003, respectively). A further association signal was found for SNPs in the FTO gene (rs1558902, ß = 0.407, 95%CI (0.137; 0.677), p = 0.0032; rs1121980, ß = 0.446 (0.176; 0.715), p = 0.0012; rs9939609, ß= 0.410, 95% (0.139; 0.681); p = 0.003; rs9941349, ß = 0.434 95% (0.163; 0.706), p = 0.0017) and for the MC4R gene (rs17782313; ß = 0.457 95% CI (0.136; 0.779), p = 0.0053; rs12970134, ß = 0.463 95% CI (0.150; 0.775), p = 0.0037) in the general population, which is in contrast to previous studies that found an association of rs9939609 (FTO) and rs17782313 (MC4R) with obesity and psoriasis, although in a genetically different cohort of psoriatic patients.

LEPR rs1137101 is associated with obesity in the early- but not in the late-onset psoriasis group

LEPR rs1137101 was further analyzed in psoriasis sub-populations based on BMI values, such as normal weight (reference), overweight, and obese. In the case of the GG genotype, a significant association with obesity was found when comparing the obese population to the normal weight subgroup (OR: 2.67 (1.34; 5.31), p = 0.005).

Next, we aimed to assess if the association of rs1137101 with obesity also showed a correlation with the onset of psoriasis; therefore, we stratified and compared the early-onset (≤40 years) and late-onset (>40 years) psoriasis groups. We found that the GG genotype distribution remained significant in the early- but not in the late-onset obese subgroup. Interestingly, just as in the whole psoriasis population group, the association between rs1137101 and obesity was independent of gender or disease severity.

Investigating the distribution of BMI categories in respect of rs1137101 genotypes AA, AG, and GG, there were significant differences within the group of early-onset psoriatic patients (p = 0.049). While the proportion of patients with normal weight was significantly less in subjects with two risk alleles (GG genotype) than in the group of patients with two wild alleles (AA genotype (*)), the proportion of obesity was higher in the case of the GG genotype compared to the AA genotype (based on 95% confidence intervals, the difference was borderline significance (#)). In the case of the group of late-onset psoriatic patients, there were no significant differences between BMI categories and the genotypes (p = 0.122).

In line with the literature, in our cohort a familial predisposition for psoriasis was more frequent in the early- than in the late-onset subgroup (37.5% vs. 16.7%); therefore, we aimed to exclude the possibility of a familial accumulation for the GG genotype. In our analyses, no differences were found between the frequency of genotypes when subgroups of patients with sporadic and familial history were compared either in the early- or in the late-onset psoriatic groups.

BDNF rs925946 is associated with obesity in the early- but not in the late-onset psoriasis group

In case of rs925946 in the BDNF gene, the TG genotype (risk allele is T) showed significant association with obesity among psoriatic patients. A significantly higher prevalence of the TG subjects was found in the obese and in the overweight populations compared to the normal weight population (OR: 2.02 (1.21; 3.35), p = 0.007, OR: 1.91 (1.14; 3.19), p = 0.013). Assessing the differences between the early- and the late-onset subgroups, the occurrence of the TG genotype (but not the TT) was significantly higher in the early-onset subgroup both in the overweight and obese categories compared to normal weight (OR: 2.08 (1.12; 3.84), p = 0.020; OR:2.26 (1.24; 4.14), p = 0.008). The association between rs925946 and obesity was also independent of gender or disease severity. Defining the BMI categories according to

rs925946 genotypes of TT, TG, and GG, a significant difference was found in the group of early-onset psoriatic patients ($p = 0.047$). The proportion of normal weight was significantly higher in the case of GG than in that of subjects with the TG genotype. In the group of late-onset psoriatic patients, there was no significant difference between the different BMI categories and genotypes ($p = 0.455$).

DISCUSSION

The association between psoriasis and increased alcohol consumption has been reported in several studies, delivering the conclusion that alcohol consumption or abuse is an independent risk factor for the development of the disease, however whether this association is driven by genetic factors has not been answered yet. Therefore we evaluated the relationship between 23 SNPs related to increased alcohol intake and dependence in a Hungarian psoriasis group. We found that the frequency of the genetic variant rs1229984 (ADH1B) increased in the whole psoriasis group, while genetic variant rs1799971 (OPRM1) showed higher prevalence in the familial form of psoriasis patients. Importantly, the risk of psoriasis related to these variants increased further in the subgroup of psoriatic patients with history of early onset and familial aggregation, but with no association to the HLA-Cw*0602 allele. A limitation of this study is that although both SNPs were confirmed to be significant in our two independent Hungarian study subgroups, their prevalence did not reach significance values in a psoriasis cohort from Sweden. Therefore, these findings call for further studies to map these SNPs in a geographic distribution.

ADH1B is a key enzyme in the metabolism of ethanol to acetaldehyde and subsequent oxidation to acetate. The allele C which was increased in the psoriasis population leads to an increased enzyme activity, thus to decreased levels of the harmful acetaldehyde following alcohol ingestion. In contrast, the protective allele is linked to a high blood acetaldehyde concentration, which is accounted to make drinking unpleasant, and is behind the “Oriental flushing response” characterized by facial flushing, headache, tachycardia, and nausea These symptoms altogether are considered to be a genetic deterrent to heavy drinking and alcoholism among East-Asians, where the predisposing alleles in rs1229984 of ADH1B is around 10% in contrast to the 90%

found in the Hungarian and European populations. Importantly, ADH1B is expressed not just in the liver where the majority of the alcohol metabolism takes place, but in the skin as well. Moreover, its metabolite acetaldehyde, was found to induce the proliferation and the production of pro-infammatory cytokines in keratinocytes under *in vitro* conditions, that could provide a missing piece in the puzzle of how alcohol could increase the severity of psoriasis. Another interesting aspect for further investigations is that ADH1B is most likely capable of metabolizing retinoic acid as well which could account for an altered response of psoriatic patients to retinoic acid based therapies.

OPRM1 encodes the μ-opioid receptor, which upon activation by its ligands, such as opioids and analgesic agents such as beta-endorphin, modulates the dopamine system. It is implicated in complex behavior patterns such as alcohol dependence in Caucasian, native American tribes as well as in a study group of Asian descends in alcohol dependence associated impulsivity. Out of the alleles, 118G has the major susceptibility effect. In individuals carrying 118G stimulation, sedation, and positive mood levels after alcohol intake were significantly higher than in controls. These effects are confirmed in great by studies on its antagonist, naltrexone, which is prescribed to alcohol dependent people to help them reduce cravings, control or abstain from drinking. However, there are also studies that did not find a higher risk for alcohol dependence among OPRM1 118G-allele carriers, just as there are alcohol dependent patients who do not observe the beneficial effects of naltrexone. Such different outcomes may have numerous reasons in the background, ranging from the diversity of the patient populations enrolled to the anamnestic difficulties presented in almost all psychiatric disorders and therapies.

Regarding psoriasis, there is a significant amount of data on the impact of psoriasis on health behavior by causing psychological stress and psychosocial disability. However, how neurotransmitters are involved in the observed changes and could integrate into the psoriasis

skin-brain axis has been addressed only in limited details. Importantly, opioid receptors, besides the CNS, are also expressed in the epidermis (μ and κ isoforms) with a possible role in transmitting the sensation of itch. While activation of μ-opioid receptors induces pruritus, activation of κ-opioid receptors is suggested to have a suppressive effect. Moreover, the κ-opioid, but not the μ-opioid receptor was down-regulated in psoriasis skin. These results altogether put forward the question how itch is affecting the alcohol use of psoriasis patients and how the detected risk allele of OPRM1 could contribute. Our results, therefore, open new perspectives to stratify psoriasis subgroups also based on itch and alcohol use. Moreover, the association between psoriasis and the opiate signaling pathway, provides excellent start points for further studies on understanding not just psoriasis associated stress and related risk behavior but also to identify psychiatric disorders that may be linked to psoriasis or at least to subsets of psoriatic patients on the level of genetic association.

The results of our studies also suggest that although obesity is more frequent in the late-onset group of psoriatic patients, for an obese patient belonging to the early-onset group, there is a higher chance to have risk alterations in the LEPR rs1137101 and in the BDNF rs925946 polymorphisms compared to an obese patient from the late-onset or from the general Hungarian population. Therefore, in the case of patients with psoriasis, there may be prominent differences in the background of obesity, which could influence not only further patient characteristics but could also have an impact on the manifestation of psoriasis. However, the limitations of our study should be kept in mind, as the group for the related studies comes from a single geographic area. Nevertheless, our results could be starting points for further studies, enhancing the role of adipokines is the development of psoriasis.

Based on the principal biological properties of the various bioactive proteins secreted by adipocytes, the so called adipokines play a key role in the immune-metabolic dialog with respect to the regulation of immune responses locally and systemically. Although the family of

adipokines includes members with pro- as well as anti-inflammatory properties, the increased amount of the white adipose tissue (WAT) altogether leads to a strengthened pro-inflammatory state, which is pathognomonic in the manifestation of psoriasis.

Leptin, an inflammatory adipokine, has a primary role to regulate weight by acting on its receptors in the hypothalamus; however, it is also known to link lipid metabolism with inflammation. Of the LEPR, multiple splice variants have been identified, but only the long isoform (Ob-Rb) can induce pathways that result in the activation of NF-κB, which is the major transcription regulator of inflammatory mediators that are also active in psoriasis. Such pathways include JAK2/STAT3 signaling, and the MAPK family (p38 MAPK), as well as the stress activated c-Jun N-terminal kinase (JNK) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathways. Beyond the results of a cellular level, murine studies have also described an increased inflammation in the imiquimod-induced psoriasis-like skin in leptin deficient (ob/ob) mice. In human skin samples LEPR together with leptin was found to have significantly higher expression levels in severe psoriatic patients, even with normal BMI, compared to patients with mild–moderate psoriasis and controls in histological studies. Moreover, leptin mRNA expression in the subcutaneous adipose tissue also positively correlated with the severity of psoriasis and the BMI in obese psoriatic patients, suggesting that leptin-induced signaling mediates both local as well as systemic changes. Still, while in the case of the gene encoding leptin, rs7799039 was associated with the plasma leptin levels and the metabolic syndrome with psoriasis, at least to our knowledge, our study was the first to link a polymorphism in the gene encoding LEPR to psoriasis. Further findings, that in certain populations, besides obesity, rs1137101 has been linked to type 2 diabetes mellitus, various types of cancer such as endometrial, prostate, colorectal and renal cell carcinoma as well as to keloid formation, multiple sclerosis severity score and to the outcome of renal transplantation,

suggests that sub-stratification and follow up of the involved individuals may also be a promising basis for prevention strategies.

Neurotrophins (e.g., brain-derived neurotrophic factor (BDNF]) are also associated with inflammatory diseases, as well as MetS. BDNF activity is mediated through the TrkB receptor and the neurotrophin receptor p75 (p75NTR), also known as the low-affinity nerve growth factor receptor, which belongs to the TNF-receptor superfamily. In psoriasis, BDNF was found to be crucial in the maintenance of normal keratinocyte apoptosis and epidermal homeostasis, while p75NTR protein was found to be absent in lesional psoriasis skin. Regarding its serum levels as well as its staining intensities, significantly lower levels were detected in both the epidermis and the dermis of psoriatic patients compared to the control group. Interestingly, in assessing another polymorphism, the rs6265 in the BDNF gene, its combined effect with higher BMI was found to increase the risk and clinical severity of psoriasis in the Chinese Han population; nevertheless, as shown in both this and our previous studies, we found no significant results in the case of rs6265 in our psoriasis cohort.

Further findings, that the blood levels of BDNF were found to be reduced not only in psoriasis but also in depression, and thus may link major depression with psoriasis, call for studies to also integrate BDNF into psycho-dermatology. However, the stigma of depression, which is similar to what we experience in regard to alcohol consumption, greatly limits the data collection for such studies. Still, it is tempting to challenge BDNF as a possible target that could modify obesity, psoriasis severity, and the related depression at once.

SUMMARY

Excessive alcohol consumption and obesity are two of the comorbidities associated with psoriasis, where the pivotal role of the disease related lifestyle is assumed.

Although the relationship between alcohol consumption and psoriasis has been examined from several perspectives, our research group was the first to provide evidence for a possible role of genetic determinants in it. Our results suggest, that among the psoriasis population a higher proportion of individuals may carry genetic alterations predisposing for increased alcohol consumption compared to the Hungarian general population. Integrating our results into the understanding of the psoriasis-psychological stress-alcohol consumption vicious circle, set the basis for further studies to deliver novel approaches into psoriasis management.

Investigating the role of genetic factors in the development of obesity, which is also part of the metabolic syndrome, we found that although obesity is more common in patients with late-onset psoriasis, an obese patient with early-onset psoriasis is more likely to carry effect alleles in the SNPs of *LEPR* rs1137101 and *BDNF* rs925946 than the one belonging to the late-onset group or to the Hungarian general population. Therefore, our results raise the necessity also to consider genetic predisposition in the obesity of psoriatic patients, and emphasize the need for a holistic treatment strategy in patient care with a special focus on the individual characteristics of a given psoriatic patient.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/530/2021.PL
Subject: PhD Publication List

Candidate: Zita Szentkereszty-Kovács
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. **Szentkereszty-Kovács, Z.**, Gáspár, K., Szegedi, A., Kemény, L., Kovács, D., Töröcsik, D.: Alcohol in Psoriasis-From Bench to Bedside.
Int. J. Mol. Sci. 22 (9), 1-14, 2021.
DOI: http://dx.doi.org/10.3390/ijms22094987
IF: 5.923 (2020)

2. **Szentkereszty-Kovács, Z.**, Fiatal, S., Janka, E. A., Kovács, D., Szegedi, A., Remenyik, É., Töröcsik, D.: Leptin Receptor (rs1137101) and Brain-Derived Neurotrophic Factor (rs925946) Gene Variants Are Associated with Obesity in the Early- but Not in the Late-Onset Population of Hungarian Psoriatic Patients.
Life (Basel). 11 (10), 1-14, 2021.
DOI: http://dx.doi.org/10.3390/life11101086
IF: 3.817 (2020)

3. **Szentkereszty-Kovács, Z.**, Fiatal, S., Szegedi, A., Kovács, D., Janka, E. A., Herszényi, K., Holló, P., Nikamo, P., Ståhle, M., Remenyik, É., Töröcsik, D.: The prevalence of ADH1B and OPRM1 alleles predisposing for alcohol consumption are increased in the Hungarian psoriasis population.
Arch. Dermatol. Res. 311 (6), 435-442, 2019.
DOI: https://doi.org/10.1007/s00403-019-01915-y
IF: 2.339

List of other publications

4. Töröcsik, D., Kovács, D., Póliska, S., **Szentkereszty-Kovács, Z.**, Lovászi, M., Dull, K., Szegedi, A., Zouboulis, C. C., Ståhle, M.: Genome wide analysis of TLR1/2- and TLR4-activated SZ95 sebocytes reveals a complex immune-competence and identifies serum amyloid A as a marker for activated sebaceous glands.
PLoS One. 13 (6), 1-20, 2018.
DOI: http://dx.doi.org/10.1371/journal.pone.0198323
IF: 2.776

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

5. **Szentkereszty-Kovács, Z.**, Lovászi, M., Zatik, Z., Duró, E., Kovács, D., Töröcsik, D.: Perspektívák a faggyúmirigy kutatásban.
Bőrgyógyász. Venerol. Szle. 93 (3), 126-135, 2017.
DOI: http://dx.doi.org/10.7188/bvsz.2017.93.3.7

6. Paragh, L., **Szentkereszty-Kovács, Z.**, Töröcsik, D., Remenyik, É.: Acne szisztémás kezelése.
Bőrgyógyász. Venerol. Szle. 92 (3), 145-154, 2016.
DOI: http://dx.doi.org/DOI 10.7188/bvsz.2016.92.3.6

Total IF of journals (all publications): 14,855
Total IF of journals (publications related to the dissertation): 12,079

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

16 December, 2021

DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

AKNOWLEDGEMENTS

First of all, I would like to thank my supervisor, Dr. Dániel Törőcsik that with his commitment to scientific research, he taught me a new approach beside the „clinician" one, and in addition to his valuable and high-quality professional guidance, he stood by me as a true mentor with his optimistic character and always encouraging attitude.

I am grateful to Prof. Dr. Andrea Szegedi and Prof. Dr. Éva Remenyik for providing a supportive background as the head of the institute and for allowing me to turn to them at any time for professional help.

I would like to thank Dr. Szilvia Fiatal and Dr. Eszter Janka for their tremendous help in the statistical work.

I am also very grateful to Dr. Dóra Kovács and Dr. Marianna Lovászi for helping me with many other things within our research group in addition to the laboratory work.

Thank you to Dr. Imre Veres for providing the histological specimens and to József Oláh for providing the imaging material.

I would also like to thank Prof. Dr. Péter Holló, Dr. Krisztina Herszényi, Prof. Mona Ståhle and Pernilla Nikamo for providing the collaboration samples and data.

Also, I am thankful to all my colleagues who have ever added anything to make this work happen.

Last but not least, I would like to thank my true friends for their encouragement, but most of all I would like to thank my family: my little daughter, Lea , my husband, Gábor, and my parents for loving and supporting me on this long journey.