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Article

Polymorphisms of the *GCLC* Gene Are Novel Genetic Markers for Susceptibility to Psoriasis Associated with Alcohol Abuse and Cigarette Smoking

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Abstract: The aim of this pilot study was to investigate whether single nucleotide polymorphisms (SNP) in the gene encoding the catalytic subunit of glutamate cysteine ligase (*GCLC*) are associated with the risk and clinical features of psoriasis. A total of 944 unrelated individuals, including 474 patients with a diagnosis of psoriasis and 470 healthy controls, were recruited for the study. Six common SNPs in the *GCLC* gene were genotyped using the MassArray-4 system. Polymorphisms rs648595 (OR=0.56 95%CI 0.35-0.90, P_{perm}=0.017) and rs2397147 (OR=0.54 95%CI 0.30-0.98, P_{perm}=0.05) were associated with susceptibility to psoriasis in males. In the male group, diplotype rs2397147-C/C×rs17883901-G/G was associated with decreased risk of psoriasis (FDR-adjusted P=0.014), whereas diplotype rs6933870-G/G×rs17883901-G/G (FDR-adjusted P=0.045) showed association with increased disease risk in females. Joint effects of SNPs with tobacco smoking (rs648595 and rs17883901) and alcohol abuse (rs648595 and rs542914) on the risk of psoriasis were observed (P_{perm}≤0.05). Furthermore, we found multiple sex-independent associations between *GCLC* gene polymorphisms and various clinical features such as earlier disease onset, the psoriatic triad, and specific localizations of skin lesions. The present study is the first to show that polymorphisms of the *GCLC* gene are significantly associated with the risk of psoriasis and related to its clinical features.

Keywords: Psoriasis; genetic susceptibility; oxidative stress; glutathione; glutamate cysteine ligase; *GCLC*; single nucleotide polymorphism; cigarette smoking; alcohol abuse; gene–environment interactions

1. Introduction

Psoriasis is a chronic immune-inflammatory-mediated dermatosis characterized by thickened, scaly erythema or plaques [1,2]. Psoriasis is recognized by the World Health Organization as a serious non-communicable disease [3]. Clinical variants of the disease include psoriasis vulgaris, arthritis, pustular, and erythrodermic types; however, psoriasis vulgaris is the most common form, accounting

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for about 90% of cases and affecting 3% of Caucasians [4]. A study by Kubanov and co-workers demonstrated a substantial disease burden on psoriasis patients in Russia [5].

The etiology and pathogenesis of psoriasis remain mysteries, making the disease's management more challenging [6]. Psoriasis is characterized by sustained inflammation, which results in uncontrolled keratinocyte proliferation and defective differentiation [7]. Psoriatic inflammation is caused and maintained by disruptions in the innate and adaptive cutaneous immune responses [6,8], which coexist with autoinflammatory perpetuation or T cell-driven autoimmune reactions [7]. The overlap of autoimmune and autoinflammatory mechanisms in the pathogenesis of psoriasis has led to the development of biological therapy for the disease. However, despite the fact that targeted therapies focusing on the inhibition of cytokines such as IL-23 and IL-17 showed high clinical efficacy, psoriasis remains so far not curable disease [7].

Psoriasis is known as a complex multifactorial disease whose development is determined by the interaction between genetic and environmental factors [9–11]. Linkage analysis, an effective method to identify the chromosomal location of disease genes, has discovered nine separate genomic regions known as Psoriasis Susceptibility regions (PSORS1-9), comprising many genetic variants, a part of which has been fine-mapped as disease-linked loci [11,12]. Progress in the development of high-throughput genotyping technologies enabled the implementation of genome-wide association studies (GWAS), a research approach where large case-control cohorts were genotyped for tens of thousands of single nucleotide polymorphisms (SNPs) across the genome [11]. According to the GWAS catalog (https://www.ebi.ac.uk/gwas/home, accessed on April 29, 2023), 57 GWASs have been conducted so far to unravel the genetic background of psoriasis in different populations around the world, and 946 SNPs have been identified as loci associated with disease susceptibility or severity and those influencing the efficacy of anti-psoriatic therapy. Nevertheless, despite considerable genetic research and achievements, the etiology of psoriasis and its primary molecular mechanisms remain elusive.

It has been argued that increased production of reactive oxygen species (ROS) and a decreased antioxidant defense leading to the activation of oxidative stress are involved in the pathogenesis of psoriasis and influence disease duration and severity [13–16]. Despite the fact that the important role of oxidative stress in the etiopathogenesis of psoriasis remains undisputable after decades of research, a limited number of studies have been undertaken so far to assess whether genetic variation of antioxidant defense enzymes contributes to psoriasis susceptibility. A larger portion of the studies looked for the link between psoriasis risk and genetic polymorphisms of glutathione-S-transferases [17–20], enzymes catalyzing the conjugation of reduced glutathione (GSH) to xenobiotic compounds for their detoxification.

Glutathione is a low-molecular-weight thiol, a tripeptide consisting of glutamate, cysteine, and glycine, which plays a major role in maintaining intracellular redox balance and antioxidant defense [21]. It is involved in many crucial biological functions, such as xenobiotic detoxification, maintaining mitochondrial function, modulation of cell proliferation, wound healing, and inhibition of apoptosis [21,22]. Furthermore, glutathione is utilized as a cofactor by glutathione peroxidases and glutathione S-transferases for glutathionylation of selected proteins and toxic substance conjugation. GSH is also required for the maturation of cytosolic iron-sulfur proteins, which are essential for cell viability and involved in the maintenance of DNA metabolism, genome integrity, protein translation, and other critical biological functions [22,23]. It is important to note that glutathione is involved in the skin metabolic clearance system [24], protects DNA and mitochondria from oxidative damage, and ensures the survival of keratinocytes in normal and wounded skin [25]. Glutathione deficiency is well known to be associated with increased susceptibility to oxidative stress, a pathological condition implicated in the pathogenesis of psoriasis [26], and therefore we can suggest that oxidative stress may be responsible for the modulation of inflammatory and autoimmune mechanisms underlying the diseases [27,28]. Despite the obvious importance of glutathione in skin metabolism, existing research data in psoriasis on the roles of genes encoding enzymes involved in glutathione metabolism, primarily glutamate cysteine ligase, an enzyme catalyzing the initial rate-limiting step of GSH biosynthesis [29], is surprisingly absent. We propose that genetic polymorphisms of

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glutamate cysteine ligase may explain inter-individual differences in glutathione biosynthesis and influence the risk of psoriasis, making SNPs attractive markers for testing disease susceptibility. Therefore, the purpose of our pilot study was to investigate whether common polymorphisms at the gene encoding the catalytic subunit of glutamate cysteine ligase (*GCLC*) are associated with the risk and clinical features of psoriasis.

2. Materials and Methods

2.1. Study Participants and Clinical Examination

Informed consent was signed by all subjects involved in this study. The protocol of the present study was approved by the Ethical Review Committee of Kursk State Medical University (protocol No. 8, 13.11.2017). A total of 944 unrelated individuals of European descent (predominantly Russians), including 474 patients with a diagnosis of psoriasis and 470 healthy controls, were used for this study. The enrollment of patients with psoriasis was done in Medvenka Central District Hospital (Kursk region), the Center for Medical Examinations and Prevention (Kursk), and Kursk Regional Multidisciplinary Clinical Hospital in a period between September 2018 and December 2021. The control group of subjects without chronic diseases was recruited from our previous studies [30–32]. The diagnosis of psoriasis was verified by qualified dermatologists based on the typical clinical picture of skin rashes and their localization [6]. The study included patients with classic plaque psoriasis, palmoplantar, seborrheic, and scalp psoriasis, von Zumbusch type of generalized pustular psoriasis, inverse psoriasis, guttate psoriasis, and erythrodermic psoriasis, as well as psoriasis comorbidities such as psoriatic arthritis and onychodystrophy [33]. The Psoriasis Area and Severity Index (PASI) was used for the clinical assessment of the severity of the course of psoriasis [34]. Enrolled patients did not suffer from chronic infectious diseases, including HIV and hepatitis, and did not have severe chronic conditions that manifested before psoriasis. Patients who were receiving biologic therapy at the time of the recruitment and pregnant women were not included in the study. Study participants completed a validated doctor-administered questionnaire [35] to assess risk factors for psoriasis, such as cigarette smoking [36] and alcohol consumption [37]. Information on smoking status (ever/never) was available from all psoriatic patients and healthy subjects. Data on alcohol intake were available from all patients with psoriasis and only 220 individuals from the control group. Alcohol intake habits were assessed through the number of drinks consumed per week, as described previously [38,39]. Briefly, according to the reported frequency of alcohol intake, study individuals were categorized into two groups: (1) subjects who consumed alcohol 1 to 2 days a month or less often and (2) those drinking alcohol 1 or more days a week. The second group was considered as alcohol abusers.

2.2. Selection of single nucleotide polymorphisms (SNPs)

GCLC is a catalytic subunit of glutamate-cysteine ligase and is the first rate-limiting enzyme of glutathione synthesis [29]. Six common (minor allele frequency ≥ 5%) SNPs such as rs524553, rs542914, rs648595, rs6933870, rs2397147 and rs17883901 of the *GCLC* gene were selected for the study according to the functional properties of the polymorphisms (the presence of eQTL, Expression Quantitative Trait Loci, in the skin from GTEx portal, https://gtexportal.org) and linkage disequilibrium ($r2 \geq 0.8$) between them (HapMap data, European population). Candidate Gene SNP Selection (GenePipe) at the SNPinfo Web Server (https://snpinfo.niehs.nih.gov/snpinfo/selegene.html (accessed on April 25, 2021)) was used for SNP selection.

2.3. Genetic Analysis

Venous blood samples were collected from the cubital vein of study subjects into EDTA-coated tubes and immediately frozen and stored at −20°C until processed. Total DNA was purified by the standard phenol/chloroform extraction and ethanol precipitation. Genotyping of the SNPs was performed with the MALDI-TOF mass spectrometry iPLEX platform on the MassArray-4 system (Agena Bioscience, Inc., San Diego, CA, USA). Primer sequences used for genotyping are available

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upon request. To guarantee quality control, 5% of DNA samples were genotyped in duplicates while being blind to the case-control status. The concordance rate of the control genotyping was >99%. Genetic investigations were carried out at the Research Institute for Genetic and Molecular Epidemiology of Kursk State Medical University (Kursk, Russia).

2.4. Statistical Analysis

Statistical power was estimated using the GAS power calculator (https://csg.sph.umich.edu/abecasis/gas_power_calculator/, accessed on May 21, 2022). It has been estimated that we could detect a genotype relative risk (GRR) of 1.30–1.45 with 82–98% power in the overall analysis (474 cases and 470 controls) and a GRR of 1.40–1.5 with 76–83% power in the analysis of groups stratified by sex/risk factors at $\alpha = 0.05$. Fisher's exact test was used to assess the distribution of genotype frequencies according to the Hardy-Weinberg equilibrium (HWE). Allele and genotype frequencies in the study groups and their associations with the risk of psoriasis were analyzed using the PLINK software v.1.9 [40]. Logistic regression analysis was used to evaluate the associations of *GCLC* gene polymorphisms with the risk of psoriasis and binary clinical phenotypes. The crude odds ratio (OR) and 95 percent confidence intervals (95% CI) were calculated to assess SNP-binary phenotype associations. Associations of SNPs with continuous phenotypes were evaluated with linear regression analysis, with estimation of differences in mean between genotypes and 95% CI using the SNPstats software (https://www.snpstats.net/start.htm, accessed on April 12, 2023). For SNP-disease associations, allelic, recessive, dominant, and log-additive genetic models were evaluated. Haplotype analysis and visualization of the haplotypic structure of the *GCLC* gene were performed by the Haploview software, v.4.2 [41]. P-values (P_{perm}) for allele/genotype/haplotype associations were estimated through adaptive permutations using PLINK and Haploview. Gene-environment interactions were analyzed in groups stratified by risk factors such as cigarette smoking and alcohol abuse. Replication for associations between *GCLC* gene polymorphisms and psoriasis was performed using the Gene ATLAS database of the UK Biobank (http://geneatlas.roslin.ed.ac.uk (accessed on January 17, 2023)). Associations of pairwise genotype combinations (diplotypes) with the risk of psoriasis were estimated by the chi-squared test and adjusted for multiple comparisons by the false discovery rate (FDR) procedure (False Discovery Rate Online Calculator, https://tools.carbocation.com/FDR, accessed on April 9, 2023).

3. Results

3.1. Baseline and clinical characteristics of the study patients

Baseline and clinical characteristics of the study patients are listed in Table 1. The group of patients with psoriasis was matched to the control group for sex (P = 0.30). The psoriasis patients were more than ten years younger than the healthy subjects. The duration of psoriasis was 10 (4–21) years. The mean age of disease onset was 27 (18–40) years old. The number of smokers in each group was about equal. However, the number of subjects abusing alcohol in the patient group was seven times higher than in the control group (P<0.0001). The psoriatic triad was diagnosed in 54.4% of patients. Most often, psoriatic rashes in patients were observed in the upper (80.0%) and lower (57.4%) extremities, head (47.9%), and trunk (33.08%), which is typical for psoriasis. The most prevalent comorbidities among psoriasis patients were hypertension (22.6%), chronic renal (6.4%), and gastrointestinal (7.0%) diseases.

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Table 1. Baseline and clinical characteristics of the study patients.

Characteristics	Patients with psoriasis n=474	Healthy controls n=470	*P*-value*
Baseline characteristics			
Age, mean ± standard deviation	44.3 ± 13.6	55.3 ± 6.7	**<0.0001**
Males, n (%)	252 (53.2)	234 (49.8)	0.30
Females, n (%)	222 (46.8)	236 (50.2)	
Risk factors			
Smokers, (ever/never), n (%)	168 (35.4)	148 (31.5)	0.20
Alcohol abusers[1], n (%)	105 (21.2)	7 (3.2)	**<0.0001**
Locations of psoriatic lesions			
Psoriatic triad	256 (54.0)	-	-
Scalp	227 (47.9)	-	
Trunk	160 (33.08)	-	-
Hands	379 (80.0)	-	-
Legs	272 (57.4)	-	-
Joints	128 (27.0)	-	-
Low back	24 (5.1)	-	-
Knees	59 (12.4)	-	-
Hips	21 (4.4)	-	-
Elbows	33 (7.0)	-	-
Fingers	60 (12.6)	-	-
Ankles	24 (5.1)	-	-
Feet/toes	23 (4.9)	-	-
Thumbs	18 (3.8)	-	-
Shoulders	11 (2.3)	-	-
Wrists	33 (7.0)	-	-
Nails	123 (25.9)	-	-
Comorbidities			
Type 2 diabetes, n (%)	15 (3.2)	-	-
Arterial hypertension, n (%)	106 (22.6)	-	-
Coronary artery disease, n (%)	27 (5.7)	-	-
Cerebral stroke, n (%)	9 (1.9)	-	-
Chronic thyroid disease, n (%)	7 (1.5)	-	-
Chronic renal disease, n (%)	30 (6.4)	-	-
Chronic gastric disease, n (%)	33 (7.0)	-	-
Chronic pulmonary disease, n (%)	7 (1.5)	-	-
Oncological disease, n (%)	8 (1.7)	-	-

[1] Data on alcohol intake were available from 220 subjects of the control group.
*Bold is statistically significant P-value.

3.2. Association of GCLC Gene Polymorphisms with the Risk of Psoriasis

Genotype frequencies for five polymorphisms were *GCLC* in Hardy-Weinberg equilibrium in both cases and controls. Only one SNP, rs17883901, showed a deviation from HWE in both groups (P=0.001). We analyzed associations between the *GCLC* gene polymorphisms and the risk of psoriasis in entire groups and groups stratified by sex. Table 2 shows a summary of associations between *GCLC* gene polymorphisms and psoriasis risk in the entire and sex-stratified groups. Allelic, additive, dominant, and recessive genetic models of SNP-disease associations were evaluated, and P-values (P_{perm}) were assessed through adaptive permutation tests. The most significant Pperm was considered to be the selected genetic model of SNP-disease association. Genotype and allele frequencies of the

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GCLC gene in healthy controls and patients with psoriasis, along with the most significant Pperm of SNP-disease associations, are reported in Table 3. As can be seen from Table 3, none of the polymorphisms showed an association with the risk of psoriasis as analyzed in the entire group of patients. However, the sex-stratified analysis allowed detecting that SNPs rs648595 (OR=0.56 95%CI 0.35-0.90, P_{perm}=0.017, recessive model) and rs2397147 (OR=0.54 95%CI 0.30-0.98, P_{perm}=0.05, recessive model) of the *GCLC* gene were associated with susceptibility to psoriasis in males.

Table 2. A summary of associations between *GCLC* gene polymorphisms and psoriasis risk in the entire and sex-stratified groups.

SNP ID	Minor allele	N	Permutation *P*-values (P_{perm}) estimated for genetic models of SNP-disease associations*			
			Allelic	Additive	Dominant	Recessive
			Entire groups			
rs524553	T	939	0.36	0.28	0.42	0.20
rs542914	A	941	0.18	0.23	0.67	0.11
rs648595	G	941	0.21	0.58	1.00	0.13
rs6933870	G	942	1.00	1.00	1.00	0.86
rs2397147	C	940	0.48	0.29	0.86	0.40
rs17883901	A	810	0.63	0.78	1.00	0.15
			Males			
rs524553	T	485	0.38	0.43	0.50	0.20
rs542914	A	485	0.55	0.41	1.00	0.28
rs648595	G	484	**0.048**	0.23	0.86	**0.017**
rs6933870	G	485	0.25	0.13	0.32	0.09
rs2397147	C	484	0.11	0.11	0.31	**0.05**
rs17883901	A	418	1.00	1.00	1.00	0.33
			Females			
rs524553	T	454	0.78	0.67	0.58	0.78
rs542914	A	456	0.59	0.32	0.59	0.48
rs648595	G	457	1.00	0.64	0.52	0.78
rs6933870	G	457	0.32	0.45	0.55	0.22
rs2397147	C	456	1.00	1.00	0.59	0.43
rs17883901	A	392	0.58	0.78	0.67	0.06

Significance of SNP-disease associations was assessed by adaptive permutations using the PLINK software, v.1.9.

Table 3. Genotype and allele frequencies of the *GCLC* gene in healthy controls and patients with psoriasis*.

SNP	Genotype/ allele	Healthy Controls n (%)[1]	Patients with psoriasis n (%)[1]	OR[2] (95% CI)	P_{perm}[3]
		Entire groups			
rs524553	C/C	273 (58.3)	285 (60.5)		
	C/T	173 (37.0)	171 (36.3)	0.67 (0.34-1.30)	0.20[R]
	T/T	22 (4.7)	15 (3.2)		
	T	217 (23.2)	201 (21.3)	0.90 (0.72-1.12)	0.36
rs542914	C/C	168 (35.8)	174 (36.9)		
	C/A	227 (48.4)	240 (50.9)	0.75 (0.52-1.08)	0.11[R]
	A/A	74 (15.8)	58 (12.3)		
	A	375 (40.0)	356 (37.7)	0.91 (0.76-1.09)	0.18
rs648595	T/T	147 (31.4)	144 (30.4)	0.75 (0.54-1.05)	0.13[R]

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	T/G	225 (48.1)	252 (53.3)		
	G/G	96 (20.5)	77 (16.3)		
	G	417 (44.6)	406 (42.9)	0.94 (0.78-1.12)	0.21
rs6933870	C/C	160 (34.0)	163 (34.5)		
	C/G	237 (50.4)	240 (50.9)	0.93 (0.65-1.33)	0.86[R]
	G/G	73 (15.5)	69 (14.6)		
	G	383 (40.7)	378 (40.0)	0.97 (0.81-1.17)	0.99
rs2397147	T/T	183 (39.2)	198 (41.9)		
	T/C	231 (49.5)	230 (48.6)	0.90 (0.74-1.09)	0.29[A]
	C/C	53 (11.3)	45 (9.5)		
	C	337 (36.1)	320 (33.8)	0.91 (0.75-1.09)	0.48
rs17883901	G/G	334 (89.1)	388 (89.2)		
	G/A	35 (9.3)	44 (10.1)	0.43 (0.11-1.72)	0.15[R]
	A/A	6 (1.6)	3 (0.7)		
	A	47 (6.3)	50 (5.7)	0.91 (0.60-1.38)	0.63
Males					
rs524553	C/C	137 (58.5)	152 (60.6)		
	C/T	84 (35.9)	91 (36.2)	0.56 (0.23-1.38)	0.20[R]
	T/T	13 (5.6)	8 (3.2)		
	T	110 (23.5)	107 (21.3)	0.88 (0.65-1.19)	0.38
rs542914	C/C	81 (34.6)	87 (34.7)		
	C/A	117 (50.0)	134 (53.4)	0.75 (0.44-1.26)	0.28[R]
	A/A	36 (15.4)	30 (11.9)		
	A	189 (40.4)	194 (38.6)	0.93 (0.72-1.20)	0.55
rs648595	T/T	71 (30.5)	78 (31.1)		
	T/G	110 (47.2)	138 (55.0)	**0.56 (0.35-0.90)**	**0.017[R]**
	G/G	52 (22.3)	35 (13.9)		
	G	214 (45.9)	208 (41.4)	0.83 (0.65-1.07)	**0.048**
rs6933870	C/C	73 (31.2)	87 (34.7)		
	C/G	120 (51.3)	134 (53.4)	0.64 (0.38-1.06)	0.09[R]
	G/G	41 (17.5)	30 (11.9)		
	G	202 (43.2)	194 (38.6)	0.83 (0.64-1.07)	0.25
rs2397147	T/T	85 (36.5)	101 (40.2)		
	T/C	116 (49.8)	130 (51.8)	**0.54 (0.30-0.98)**	**0.05[R]**
	C/C	32 (13.7)	20 (8.0)		
	C	180 (38.6)	170 (33.9)	0.81 (0.63-1.06)	0.11
rs17883901	G/G	167 (89.3)	204 (88.3)		
	G/A	17 (9.1)	25 (10.8)	0.54 (0.09-3.24)	0.33[R]
	A/A	3 (1.6)	2 (0.9)		
	A	23 (6.1)	29 (6.3)	1.02 (0.58-1.80	0.99
Females					
rs524553	C/C	136 (58.1)	133 (60.5)		
	C/T	89 (38.0)	80 (36.4)	0.91 (0.62-1.32)	0.58[D]
	T/T	9 (3.8)	7 (3.2)		
	T	107 (22.9)	94 (21.4)	0.92 (0.67-1.25)	0.78
rs542914	C/C	87 (37.0)	87 (39.4)		
	C/A	110 (46.8)	106 (48)	0.88 (0.68-1.15)	0.32[A]
	A/A	38 (16.2)	28 (12.7)		
	A	186 (39.6)	162 (36.7)	0.88 (0.68-1.15)	0.59
rs648595	T/T	76 (32.3)	66 (29.7)	1.13 (0.76-1.68)	0.52[D]
	T/G	115 (48.9)	114 (51.4)		

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	G/G	44 (18.7)	42 (18.9)		
	G	203 (43.2)	198 (44.6)	1.06 (0.82-1.37)	0.99
rs6933870	C/C	87 (36.9)	76 (34.4)		
	C/G	117 (49.6)	106 (48.0)	1.37 (0.82-2.27)	0.22[R]
	G/G	32 (13.6)	39 (17.6)		
	G	181 (38.3)	184 (41.6)	1.15 (0.88-1.49)	0.32
rs2397147	T/T	98 (41.9)	97 (43.7)		
	T/C	115 (49.1)	100 (45)	1.29 (0.70-2.37)	0.43[R]
	C/C	21 (9.0)	25 (11.3)		
	C	157 (33.5)	150 (33.8)	1.01 (0.77-1.33)	0.99
rs17883901	G/G	167 (88.8)	184 (90.2)		
	G/A	18 (9.6)	19 (9.3)	0.30 (0.03-2.95)	0.06[R]
	A/A	3 (1.6)	1 (0.5)		
	A	24 (6.4)	21 (5.1)	0.80 (0.44-1.45)	0.58

* The table shows the best genetic models for SNP-disease associations.
[1] Absolute number and percentage of individuals/chromosomes with a particular genotype/allele.
[2] Odds ratio with 95% confidence intervals (crude analysis) estimated for the best association model.
[3] *P*-value estimated for the best association model through adaptive permutations. Superscripts denote SNP association models: R, recessive; D, dominant; A, additive.
Bold depicts statistically significant P-values and odds ratios.

None of the polymorphisms was significantly associated with the risk of psoriasis in females.

3.3. Joint Effects of GCLC Gene Polymorphisms on the Risk of Psoriasis

The joint effects of *GCLC* gene polymorphisms on psoriasis risk were evaluated through haplotype and diplotype analyses. The *GCLC* haplotypes and their association with psoriasis risk in the entire and sex-stratified groups are shown in Table 4. Four common haplotypes of *GCLC* (H1–H4) with a frequency of more than 5% were identified in the study groups. The rare haplotype H12, with a frequency of 1%, was detected only in females. Figure 1 shows the linkage disequilibrium plot of the *GCLC* gene generated by the Haploview software. The polymorphism rs17883901 was not linked to any of the other studied SNPs in the *GCLC* gene. As can be seen from Table 4, none of the haplotypes was meaningfully associated with the risk of psoriasis, both in the entire and sex-stratified groups (P_{perm}>0.05).

Table 4. Haplotypes of the *GCLC* gene and their association with psoriasis risk in the entire and sex-stratified groups

Haplotypes	SNP						Patients with psoriasis	Healthy Controls	Chi Square	*P*-value
	rs524553	rs542914	rs648595	rs6933870	rs2397147	rs17883901				
Entire groups										
H1	C	C	T	C	T	G	0.482	0.463	0.635	0.426
H2	T	A	G	G	C	G	0.154	0.162	0.192	0.661
H3	C	A	G	G	C	G	0.121	0.128	0.186	0.666
H4	C	C	G	G	T	G	0.056	0.043	1.681	0.195
H5	C	A	T	C	T	G	0.043	0.042	0.017	0.898
H6	C	C	G	C	T	G	0.032	0.027	0.422	0.516
H7	C	C	T	C	T	A	0.019	0.026	0.940	0.332

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H8	T	A	G	C	T	G	0.017	0.027	2.247	0.134
H9	T	A	G	G	C	A	0.023	0.020	0.133	0.715
H10	C	C	T	G	C	G	0.018	0.018	0.016	0.900
H11	C	A	G	G	C	A	0.010	0.013	0.301	0.583
H12	-	-	-	-	-	-	-	-	-	-
						Males				
H1	C	C	T	C	T	G	0.495	0.457	1.407	0.236
H2	T	A	G	G	C	G	0.160	0.175	0.357	0.550
H3	C	A	G	G	C	G	0.115	0.136	0.989	0.320
H4	C	C	G	G	T	G	0.045	0.043	0.017	0.896
H5	C	A	T	C	T	G	0.048	0.030	2.040	0.153
H6	C	C	G	C	T	G	0.026	0.024	0.040	0.842
H7	C	C	T	C	T	A	0.020	0.024	0.223	0.637
H8	T	A	G	C	T	G	0.016	0.022	0.456	0.499
H9	T	A	G	G	C	A	0.025	0.018	0.604	0.437
H10	C	C	T	G	C	G	0.015	0.023	0.839	0.359
H11	C	A	G	G	C	A	0.013	0.014	0.030	0.863
H12	-	-	-	-	-	-	-	-	-	-
						Females				
H1	C	C	T	C	T	G	0.463	0.464	0.001	0.981
H2	T	A	G	G	C	G	0.150	0.158	0.109	0.741
H3	C	A	G	G	C	G	0.130	0.119	0.243	0.622
H4	C	C	G	G	T	G	0.069	0.045	2.445	0.118
H5	C	A	T	C	T	G	0.037	0.052	1.155	0.283
H6	C	C	G	C	T	G	0.034	0.027	0.418	0.518
H7	C	C	T	C	T	A	0.023	0.028	0.321	0.571
H8	T	A	G	C	T	G	0.018	0.031	1.511	0.219
H9	T	A	G	G	C	A	0.017	0.017	0.010	0.919
H10	C	C	T	G	C	G	0.021	0.013	0.761	0.383
H11	C	A	G	G	C	A	-	-	-	-
H12	T	C	G	G	C	G	0.010	0.010	0.001	0.983

Estimation of haplotype frequencies and significance of haplotype-disease associations was done using the Haploview software, v.4.2.

The results of the diplotype analysis are shown in Table 5. In the entire group, genotype combinations such as rs542914-C/C × rs648595-G/T (FDR-adjusted P=0.03) and rs648595-G/G × rs6933870-C/G (FDR-adjusted P=0.016) of *GCLC* showed associations with increased and decreased risk of psoriasis, respectively. In the male group, diplotype rs2397147-C/C ×rs17883901-G/G was associated with decreased risk of psoriasis (FDR-adjusted P=0.014), whereas diplotype rs6933870-G/G × rs17883901-G/G (FDR-adjusted P=0.045) showed an association with increased disease risk in females. The remaining six diplotypes associated with disease risk in males did not reach statistical significance after adjusting for multiple tests.

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Table 5. *GCLC* genotype combinations showed associations with psoriasis risk.

Genotype combination	Patients		Controls		*P*-value	OR (95% CI)[3]
	n[1]	%[2]	n[1]	%[2]		
Entire groups						
rs542914-C/C × rs648595-G/T	55	11.7	35	7.5	**0.03**	1.63 (1.04-2.54)
rs648595-G/G × rs6933870-C/G	13	2.8	28	6.0	**0.016**	0.45 (0.23-0.87)
Males						
rs524553-C/C ×rs648595-G/G	7	2.8	18	7.7	0.025	0.36 (0.15-0.85)
rs524553-C/C× rs6933870-G/G	6	2.4	15	6.4	0.05	0.37 (0.15-0.95)
rs542914-A/A × rs648595-G/G	19	7.6	31	13.3	0.038	0.54 (0.30-0.98)
rs648595-G/G × rs17883901-G/G	21	9.1	33	17.7	0.009	0.47 (0.26-0.84)
rs6933870-G/G × rs2397147-C/C	20	8.0	32	13.7	0.042	0.55 (0.30-0.99)
rs6933870-G/G × rs17883901-G/G	19	8.2	27	14.4	0.044	0.53 (0.29-0.99)
rs2397147-C/C ×rs17883901-G/G	11	4.8	21	11.2	**0.014**	0.40 (0.19-0.85)
Females						
rs6933870-G/G × rs17883901-G/G	32	15.8	17	9.0	**0.045**	1.88 (1.01-3.52)

[1] Absolute number of individuals with particular genotype combination (minor alleles in genotypes are underlined).

[2] Percentage of individuals with particular genotype combination.

[3] OR, odds ratio; CI, confidence interval. Bold is statistically significant P-value after an adjustment for FDR of 0.05 (https://tools.carbocation.com/FDR).

3.4. Gene-Environment Interactions and Psoriasis risk

Since psoriasis is a multifactorial disease, it appears important to investigate the joint influence of environmental risk factors and gene polymorphisms on disease development. Two risk factors, such as cigarette smoking and alcohol abuse, were used for the analysis of gene-environment interactions in psoriasis. Table 6 shows a summary of associations between *GCLC* gene polymorphisms and psoriasis risk in groups stratified by cigarette smoking and alcohol abuse habits. We found that SNP rs648595 is associated with the risk of psoriasis in cigarette smokers (OR=0.55, 95%CI 0.31-0.99, P_{perm}=0.049, recessive model), whereas no association of this polymorphism was seen in non-smokers (OR=0.88, 95%CI 0.59-1.31, P_{perm}=0.52, recessive model). In contrast, SNP rs17883901 showed association with the risk of psoriasis in non-smokers (OR=0.22, 95%CI 0.02-1.97, P=0.14, P_{perm}=0.002, recessive model), whereas no association with this variant was observed in smoker subjects (OR=0.89, 95%CI 0.11-5.90, P=0.84, P_{perm}=0.99, recessive model). Notably, polymorphisms rs542914 (OR=0.57, 95%CI 0.36-0.90, P_{perm}=0.015, recessive model) and rs648595 (OR=0.60, 95%CI 0.39-0.92, P_{perm}=0.03, recessive model) of *GCLC* were associated with decreased risk of psoriasis in non-drinkers of alcohol. However, no protective effects of these SNPs against the risk of psoriasis were identified in alcohol abusers (P>0.05).

Table 6. A summary of associations between *GCLC* gene polymorphisms and psoriasis risk in cigarette smoking- and alcohol abuse-stratified groups.

SNP ID	Minor allele	Permutation *P*-values (P_{perm}) estimated for genetic models of SNP-disease associations								
		N	Genetic models				N	Genetic models			
			Allelic	Additive	Dominant	Recessive		Allelic	Additive	Dominant	Recessive
		Smokers					Non-smokers				
rs524553	T	315	1.00	0.52	0.63	0.64	624	0.46	0.43	0.86	0.34
rs542914	A	315	0.86	0.55	0.67	0.67	626	0.21	0.59	0.86	0.10
rs648595	G	316	0.12	0.44	0.52	**0.049**	625	0.86	0.78	1.00	0.52
rs6933870	G	315	0.65	0.52	0.86	0.59	627	1.00	1.00	0.86	0.67
rs2397147	C	315	0.67	0.33	0.48	0.25	625	0.86	0.46	0.39	0.73
rs17883901	A	275	0.24	0.16	0.09	1.00	535	0.18	0.11	0.18	**0.002**
		Alcohol abusers					Non-drinkers				
rs524553	T	110	0.26	0.09	0.10	NA	580	0.24	0.08	0.20	0.15
rs542914	A	112	0.11	0.053	0.06	NA	579	**0.034**	**0.026**	0.16	**0.015**
rs648595	G	112	0.33	0.19	0.58	NA	580	**0.05**	**0.04**	0.26	**0.03**
rs6933870	G	111	0.18	0.11	0.23	NA	581	0.29	0.14	0.18	0.27
rs2397147	C	112	0.19	0.22	0.14	NA	579	0.20	0.09	0.14	0.25
rs17883901	A	98	0.79	NA	NA	NA	498	0.55	0.48	0.67	0.09

Significance of SNP-disease associations was assessed by adaptive permutations using the PLINK software, v.1.9. NA, not available.

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3.5. Replication of Associations between GCLC Gene Polymorphisms Psoriasis Risk in a population of UK Biobank

It is stated that replication helps ensure that a genotype-phenotype relationship discovered in an original study represents a credible association and is not a chance finding or an artifact due to uncontrolled biases [42,43]. Therefore, we performed a replication analysis of associations between the studied *GCLC* gene polymorphisms and psoriasis susceptibility in two large populations from the UK Biobank.

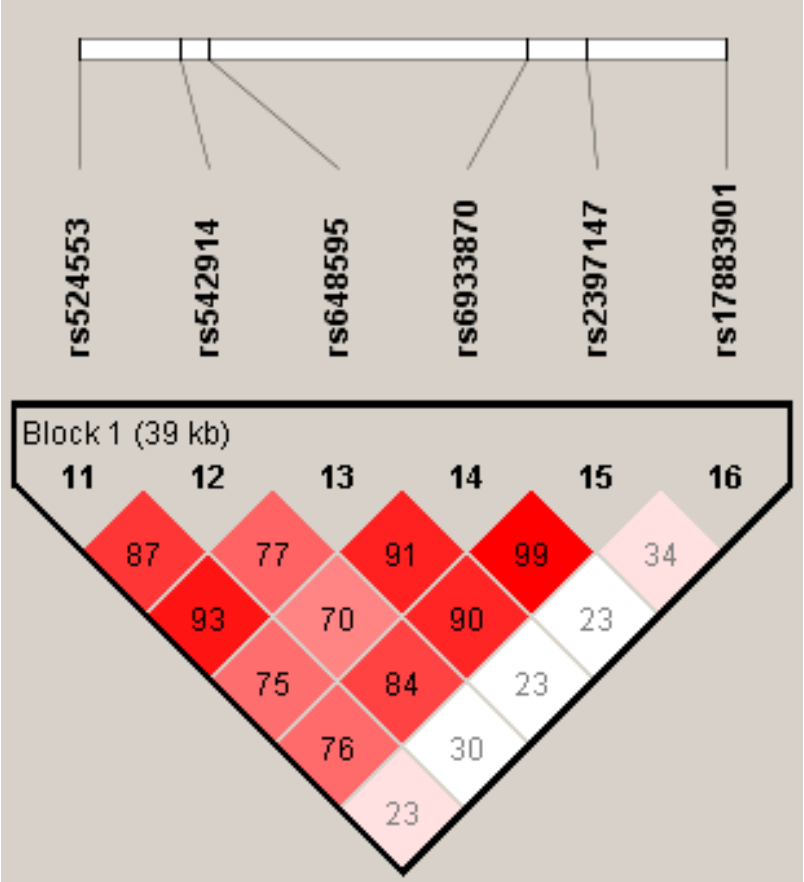

Figure 1. Linkage disequilibrium (LD) plot of the *GCLC* gene generated by the Haploview software, v.4.2. Lewontin's standardized coefficient D' values serve as a means to represent LD. The magnitude and significance of pairwise LD are shown by shading, with a red-to-white gradient showing higher-to-lower LD values.

Table 7 shows the results of replication analysis to confirm associations between the studied polymorphisms of the *GCLC* gene and psoriasis risk in a population of the UK Biobank. It has been revealed that two SNPs, such as rs6933870 (P=0.063) and rs2397147 (P=0.057) of *GCLC*, showed a clear tendency in their association with the risk of psoriasis in one of the UK cohorts. Formally, we cannot conclude that the *GCLC* gene polymorphisms we studied are replicated in an independent population. Non-replication of SNP-disease associations might be in part explained by inter-population genetic differences, and this issue has been proposed to be readily resolved by the use of a gene-based approach rather than either an SNP-based or a haplotype-based approach [42,44]. Pursuing this proposal, we performed an association analysis of psoriasis with all SNPs of the *GCLC* gene genotyped in the UK Biobank cohorts. As a result (Table 8), 75 and 21 SNPs of the *GCLC* gene in the first and second UK Biobank cohorts, respectively, have been found to be associated with the risk of psoriasis at a P-value ≤ 0.05. Two polymorphisms of *GCLC*, such as rs547541077 (P=0.004) and rs7764361 (P=0.039) were associated with psoriasis risk in both cohorts.

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Table 7. Replication of associations between the studied polymorphisms of the *GCLC* gene and psoriasis risk in a population of the UK Biobank[1].

Psoriasis phenotype[2]	Variant	Eff, allele	beta	OR beta	P-value	MAF	HWE
psoriasis	rs524553	T	0.00030445	1.03	0.24054	0.248703	0.8257
L40 Psoriasis	rs524553	T	0.00014144	1.03	0.43139	0.248703	0.8257
psoriasis	rs542914	A	0.00031437	1.03	0.16739	0.409665	0.7591
L40 Psoriasis	rs542914	A	0.00018358	1.03	0.24466	0.409665	0.7591
psoriasis	rs648595	G	0.00034186	1.03	0.12131	0.485677	0.2804
L40 Psoriasis	rs648595	G	0.00019125	1.04	0.21101	0.485677	0.2804
psoriasis	rs6933870	G	0.00041555	1.04	0.062535	0.478105	0.1793
L40 Psoriasis	rs6933870	G	0.00015391	1.03	0.3195	0.478105	0.1793
psoriasis	rs2397147	C	0.00043391	1.04	0.057101	0.407803	0.6808
L40 Psoriasis	rs2397147	C	0.000164	1.03	0.29943	0.407803	0.6808
psoriasis	rs17883901	G	-0.0001631	0.986	0.68281	0.0837	0.05018
L40 Psoriasis	rs17883901	G	-0.0002265	0.959	0.4129	0.0837	0.05018

[1] The calculations were obtained from the Gene ATLAS web site (http://geneatlas.roslin.ed.ac.uk/). accessed by 28.04.2023

[2] "Psoriasis" phenotype investigated in a cohort of 5175 cases and 447089 controls).: "L40 Psoriasis" phenotype investigated in a cohort of 2437 cases and 449827 controls;

MAF. minor allele frequency; HWE. Hardy-Weinberg equilibrium P-value.

Table 8. Polymorphisms the ***GCLC*** gene showed significant associations ($P \leq 0.05$) with the risk of psoriasis in a population of the UK Biobank.

N	Variant	Position	Eff, Allele	Trait	Beta	*P*-value	MAF
Psoriasis phenotype: "psoriasis" (5175 cases and 447089 controls)							
1	rs183555084	53463377	A	psoriasis	0.0054328	0.00048311	0.005415
2	rs536001584	53491157	A	psoriasis	0.0069889	0.0037087	0.002296
3	rs78863400	53507843	G	psoriasis	0.0020045	0.0049101	0.0245
4	rs114919458	53478492	A	psoriasis	0.0020638	0.0077221	0.020874
5	rs77162334	53473387	A	psoriasis	0.0015616	0.0084049	0.036438
6	rs547541077	53524639	A	psoriasis	0.0083841	0.011327	0.001201
7	rs55661362	53463674	G	psoriasis	0.0026909	0.012278	0.011041

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8	rs78331008	53489705	G	psoriasis	0.0014586	0.014341	0.036042
9	rs115558853	53325654	C	psoriasis	-0.0019831	0.016188	0.018765
10	rs6902510	53493460	T	psoriasis	-0.00052994	0.019054	0.405458
11	rs62398116	53405203	G	psoriasis	-0.0008575	0.019471	0.110262
12	rs189491343	53341496	G	psoriasis	-0.0019418	0.020189	0.01847
13	rs7762921	53319569	T	psoriasis	-0.00065758	0.021591	0.1828
14	rs62398159	53490625	A	psoriasis	-0.00051684	0.022213	0.406839
15	rs56013020	53390696	A	psoriasis	0.00082793	0.022789	0.103296
16	rs7739121	53510423	C	psoriasis	-0.00049679	0.024997	0.467815
17	rs72944719	53358473	G	psoriasis	-0.0010805	0.025673	0.05524
18	rs7761225	53315323	C	psoriasis	-0.00064261	0.025687	0.179565
19	rs6458936	53314296	G	psoriasis	-0.00064298	0.025697	0.179334
20	rs1914707	53311047	G	psoriasis	-0.00063643	0.026486	0.181629
21	rs563831	53327107	G	psoriasis	0.00063512	0.026486	0.183707
22	rs4715409	53511015	T	psoriasis	-0.00049168	0.02667	0.467022
23	rs1518511	53313237	C	psoriasis	-0.00063702	0.027149	0.179343
24	rs6908614	53501678	T	psoriasis	-0.00048943	0.027196	0.462606
25	rs642103	53323152	G	psoriasis	-0.00062689	0.028507	0.18174
26	rs1914706	53311463	T	psoriasis	-0.00062752	0.028627	0.181766
27	rs72943672	53399516	T	psoriasis	-0.00074715	0.028945	0.1182
28	rs6933919	53313748	G	psoriasis	-0.00062901	0.029059	0.179555
29	rs4712030	53317469	A	psoriasis	-0.00062437	0.029102	0.181758
30	rs1467408	53351289	A	psoriasis	-0.00052426	0.029222	0.361091
31	rs9382209	53311804	G	psoriasis	-0.00062389	0.02952	0.18191
32	rs149644917	53519358	A	psoriasis	-0.010751	0.029585	0.000499
33	rs1401155	53312629	C	psoriasis	-0.00062709	0.029593	0.17955
34	rs9357769	53508264	C	psoriasis	0.00048131	0.029829	0.4664
35	rs6908786	53494357	A	psoriasis	-0.00047818	0.03092	0.466556
36	rs587178	53325255	T	psoriasis	0.00061535	0.031491	0.182191
37	rs6901352	53500138	C	psoriasis	-0.0004754	0.031615	0.466514
38	rs6908860	53494615	T	psoriasis	-0.00047638	0.031652	0.464814
39	rs681682	53440021	C	psoriasis	-0.0072738	0.032871	0.001361
40	rs543473	53439524	T	psoriasis	-0.0072796	0.032941	0.001359
41	rs681585	53439958	G	psoriasis	-0.0072742	0.033023	0.00136
42	rs9474608	53505134	A	psoriasis	-0.00047139	0.033072	0.466612
43	rs681635	53439987	A	psoriasis	-0.0072632	0.033272	0.001359
44	rs2397146	53360119	A	psoriasis	-0.00053256	0.033642	0.273716
45	rs607285	53326491	T	psoriasis	0.00060766	0.033745	0.182155
46	rs62416866	53398370	A	psoriasis	-0.00077679	0.033936	0.100838
47	rs742528	53360191	A	psoriasis	-0.00052981	0.034548	0.273993
48	rs623928	53335695	T	psoriasis	0.00061135	0.034551	0.180506
49	rs629162	53326283	G	psoriasis	0.00060422	0.034685	0.182369

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50	rs676637	53335353	C	psoriasis	0.00061072	0.03473	0.180538
51	rs624432	53335555	G	psoriasis	0.00061046	0.034804	0.180555
52	rs642625	53333732	T	psoriasis	0.00061027	0.034833	0.180511
53	rs618033	53339289	T	psoriasis	0.00061046	0.034957	0.180357
54	rs600722	53332887	T	psoriasis	0.00060973	0.034961	0.180513
55	rs631783	53338531	A	psoriasis	0.00060876	0.035396	0.180454
56	rs619955	53338845	T	psoriasis	0.00060877	0.035396	0.180457
57	rs485371	53341627	T	psoriasis	0.00060874	0.035527	0.180356
58	rs12196344	53457292	A	psoriasis	-0.00048763	0.036061	0.404087
59	rs9367538	53506487	G	psoriasis	-0.00046273	0.036479	0.466245
60	rs7764361	53492467	C	psoriasis	0.00046427	0.037421	0.456163
61	rs663087	53342704	T	psoriasis	0.00060223	0.037659	0.180217
62	rs646403	53347484	T	psoriasis	0.00059431	0.040381	0.180136
63	rs12194171	53464937	C	psoriasis	0.00046011	0.041523	0.3968
64	rs11756739	53316777	A	psoriasis	0.0029885	0.04429	0.006094
65	rs4712031	53320273	G	psoriasis	-0.00056517	0.04448	0.190022
66	rs2092421	53473076	A	psoriasis	-0.00045072	0.045589	0.398208
67	rs4269374	53461179	G	psoriasis	-0.00044872	0.04647	0.397012
68	rs9349679	53470507	A	psoriasis	-0.00044669	0.047497	0.39642
69	rs34997452	53518439	T	psoriasis	-0.0027868	0.047543	0.006643
70	rs10807461	53472150	T	psoriasis	-0.00044608	0.047762	0.398057
71	rs738472	53477038	C	psoriasis	-0.00045789	0.048043	0.353182
72	rs6458946	53472830	T	psoriasis	-0.00044442	0.048672	0.397982
73	rs114749455	53489865	G	psoriasis	0.0022206	0.048751	0.0103
74	rs2143399	53461749	A	psoriasis	-0.00044292	0.049341	0.397029
75	rs74357476	53476523	T	psoriasis	0.0014009	0.050596	0.025311
Psoriasis phenotype: “L40 Psoriasis” (2437 cases and 449827 controls)							
1	rs185956124	53496212	C	L40 Psoriasis	0.0026856	0.0036274	0.00747649
2	rs547541077	53524639	A	L40 Psoriasis	0.0065265	0.0044446	0.00120121
3	rs189622943	53509408	T	L40 Psoriasis	0.0035101	0.0095446	0.00341133
4	rs183043870	53509634	G	L40 Psoriasis	0.0035128	0.0095673	0.00341141
5	rs78735978	53360036	C	L40 Psoriasis	0.0012576	0.015913	0.0231714
6	rs41271287	53370147	T	L40 Psoriasis	0.0011902	0.018795	0.0236652
7	rs17215384	53510321	T	L40 Psoriasis	0.00039365	0.02118	0.28084
8	rs77516417	53373662	A	L40 Psoriasis	-0.001175	0.021204	0.02313
9	rs574202	53481989	G	L40 Psoriasis	0.00035417	0.021427	0.489829
10	rs12661112	53486714	A	L40 Psoriasis	0.00037194	0.021838	0.343991
11	rs563699	53479410	C	L40 Psoriasis	0.00035124	0.022359	0.490659
12	rs558026	53478773	A	L40 Psoriasis	0.00035803	0.022979	0.392597
13	rs583513	53477688	T	L40 Psoriasis	0.00034605	0.024098	0.491525
14	rs7759126	53484485	C	L40 Psoriasis	0.00035645	0.028105	0.343339
15	rs12665537	53509452	G	L40 Psoriasis	0.00035343	0.030008	0.33107

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16	rs67228890	53511814	G	L40 Psoriasis	0.00034794	0.034841	0.327456
17	rs74449072	53521238	G	L40 Psoriasis	0.00061918	0.039019	0.0749875
18	rs7764361	53492467	C	L40 Psoriasis	0.00031881	0.039179	0.456163
19	rs9382225	53511696	T	L40 Psoriasis	-0.00033714	0.039962	0.328914
20	rs5020412	53349885	C	L40 Psoriasis	0.00084548	0.041197	0.0354
21	rs4715412	53511836	T	L40 Psoriasis	-0.00033138	0.044253	0.328611

3.6. Association of GCLC Gene Polymorphisms with Clinical Features of Psoriasis

Associations of *GCLC* gene polymorphisms with clinical manifestations of psoriasis were analyzed and adjusted for sex. It has been revealed that a carriage of genotypes rs542914CA and AA of *GCLC* was positively associated with the psoriatic triad (OR=1.72, 95%CI 1.18-2.51, P=0.005). Earlier onset of psoriasis was associated with the effects of SNPs rs648595 (difference -2.04, 95%CI -3.67 - -0.40, P=0.015) and rs6933870 (difference -1.73, 95%CI -3.36 - -0.10, P=0.038). Carriage of genotype rs524553TT of *GCLC* was found to be associated with more frequent flare-ups of psoriasis (difference 0.67, 95%CI 0.01 - 1.33, P=0.047). Polymorphisms have been found to be associated with psoriasis localization features. Figure 2 summarizes the findings of the analysis. SNP rs648595 showed association with scalp psoriasis (OR=1.32, 95%CI 1.01-1.74, P=0.04, log-additive genetic model). Polymorphisms rs648595 (difference 0.17, 95%CI 0.00 - 0.35, P=0.048, additive genetic model) and rs2397147 (difference 0.27, 95%CI 0.03 - 0.50, P=0.025, overdominant genetic model) of *GCLC* were associated with an increased area of skin lesions on the scalp. In addition, genotypes rs2397147TC and C/C were associated with increased infiltration (difference 0.23, 95%CI 0.03 - 0.43, P=0.023) and peeling (difference 0.22, 95%CI 0.03 - 0.42, P=0.026) of psoriatic lesions on the trunk. Genotype rs524553CT was also associated with increased infiltration (difference 0.22, 95%CI 0.01 - 0.42, P=0.037) and peeling (difference 0.22, 95%CI 0.02 - 0.43, P=0.029) of psoriatic lesions on the trunk. The polymorphism rs17883901 of *GCLC* was found to be associated with psoriasis on the knees (OR=2.34, 95%CI 1.20-4.58, P=0.019, additive genetic model). Moreover, genotype rs17883901AA was associated with psoriasis on the wrist (OR=31.25, 95%CI 2.68-364.40, P=0.007) and fingers (OR=13.99, 95%CI 1.25-157.15, P=0.03, recessive model). Interestingly, genotypes rs648595 GT and GG were also found to be associated with type 2 diabetes in patients with psoriasis (OR=2.80, 95%CI 1.06-7.37, P=0.021). Notably, all the observed associations with clinical features occurred regardless of sex.

4. Discussion

Since the skin is frequently exposed to environmental insults such as ultraviolet irradiation, exposure to toxic chemicals, or mechanical injury causing oxidative or chemical stress, one of the principal physiologic roles of the skin is as a robust barrier against xenobiotics and free radicals for their metabolic elimination and detoxification [25,45,46]. For promoting these functions, human skin possesses a significant potential for phase II metabolism through reactions of glucuronidation, sulfation, N-acetylation, and glutathione conjugation [45] and therefore the cytoprotective effects of GSH are likely to be of importance in this tissue. Experimental studies by Telorack and co-workers [25] have revealed that knockout mice with keratinocyte-specific deficiency in glutamate cysteine ligase showed a strong reduction in the viability of cell culture in vitro and in the skin in vivo. Furthermore, the authors observed that keratinocytes in glutathione-deficient mice died by apoptosis, ferroptosis, and necroptosis, and the increased cell death was attributed to increased levels of reactive oxygen and nitrogen species, causing DNA and mitochondrial damage [25]. This important research demonstrates the epidermis's exceptional antioxidant (glutathione) capability, which ensures skin integrity and effective wound healing. A deficiency of skin glutathione may contribute to psoriasis development. Genetic polymorphisms of glutamate cysteine ligase that are correlated with a decrease in *GCLC* mRNA and protein expression, enzyme activity, and GSH content [47–51] represent attractive markers for studying the molecular mechanisms of psoriasis. It is known from the literature, polymorphisms of the *GCLC* gene have been found to be associated with the risk

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of cardiometabolic diseases such as coronary artery disease [52,53], ischemic stroke [54], type 1 [55,56] and type 2 [51] diabetes mellitus, polycystic ovary syndrome [57], nonalcoholic fatty liver disease [58] as well as other multifactorial disorders such as bronchial asthma [59], pulmonary tuberculosis [60] and colorectal cancer [61]. However, no studies have been designed so far to investigate the role of *GCLC* gene polymorphisms in psoriasis susceptibility.

The present study is the first to show that polymorphisms of the *GCLC* gene are significantly associated with the risk of psoriasis and related to its clinical features. Despite the fact that none of the studied polymorphisms showed an association with the risk of psoriasis in the overall group, two SNPs, such as rs648595 and rs2397147, were found to be associated with a decreased risk of psoriasis in males, suggesting sexual dimorphism in the relationship between the gene variation and susceptibility to psoriasis. Sexual dimorphism was also seen in associations between *GCLC* diplotypes and disease risk: rs2397147-C/C × rs17883901-G/G was associated with decreased risk of psoriasis in males, whereas diplotype rs6933870-G/G × rs17883901-G/G showed an association with increased disease risk in females. These findings were not surprising because gender differences in psoriasis risk and severity have become a discussable issue among dermatologists in the last few years [62,63].

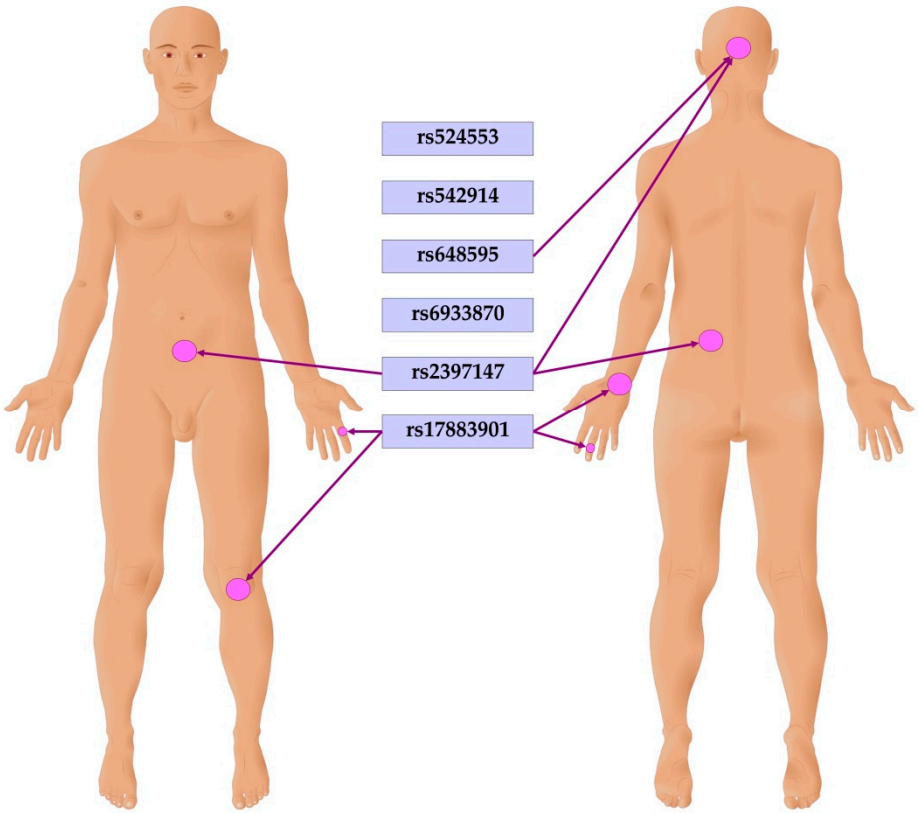

Figure 2. Associations of *GCLC* gene polymorphisms with psoriatic lesions on the body.

Notably, sexual dimorphism has also been demonstrated in some genetic association studies on skin disease such as atopic dermatitis [64]. Environmental risk factors such as cigarette smoking and alcohol abuse may explain the mechanisms by which sexual dimorphism determines susceptibility to psoriasis [37,36,65]. We investigated the effect of *GCLC* gene polymorphisms on psoriasis risk depending on these environmental risk factors in the studied population. We found that polymorphism rs648595 is associated with the risk of psoriasis in exclusively cigarette smokers. Another SNP, rs17883901 of *GCLC*, was associated with the risk of psoriasis only in non-smokers. Furthermore, polymorphisms rs542914 and rs648595 were found to be associated with decreased risk

of psoriasis in non-drinkers of alcohol, whereas no protective effects of these SNPs against the disease risk were seen in subjects who were alcohol abusers.

In addition, the present study revealed multiple sex-independent associations between *GCLC* gene polymorphisms and various clinical features in patients. In particular, SNP rs542914 was associated with the psoriatic triad, whereas rs648595 and rs6933870 polymorphisms were associated with an earlier onset of psoriasis. Furthermore, SNP rs524553TT of *GCLC* was found to be associated with more frequent flare-ups of psoriasis. The polymorphisms rs648595, rs2397147, rs524553, and rs17883901 have been specifically associated with psoriatic lesions of various localizations on the body. These findings suggest area-specific genetic effects of the studied polymorphisms of the *GCLC* gene that may be attributed to inter-individual differences in gene expression and therefore rates in glutathione biosynthesis by the skin from different body areas, as was demonstrated with regard to the rate of glutathione conjugation in different organs [66]. It is also known that the levels of glutathione may vary in sun-exposed and sun-protected areas [67], suggesting that UV exposure may impact glutathione biosynthesis in the skin.

A replication analysis of associations between the studied *GCLC* gene polymorphisms and psoriasis susceptibility in two large populations from the UK Biobank allowed the identification of two SNPs, such as rs6933870 and rs2397147, that showed a clear tendency in the association with the risk of psoriasis. We hypothesize that differences in genetic architecture between the studied populations, including changes in minor allele frequencies and linkage disequilibrium between polymorphisms, may explain the non-replication of SNP-disease relationships. In this context, if the *GCLC* gene is important for psoriasis pathogenesis, this means that other SNPs of this gene may contribute to disease risk in the UK Biobank populations. Pursuing this assumption, we have analyzed all SNPs of the *GCLC* gene genotyped in the UK Biobank cohorts with regard to their association with psoriasis risk and found that more than 70 of *GCLC* gene polymorphisms are associated with disease risk, at least at a P-value ≤ 0.05.

The functional implications of *GCLC* polymorphisms associated with the development of psoriasis were particularly important for the pathophysiological interpretation of the observed SNP-phenotype relationships. Functional annotation of some polymorphisms of the *GCLC* gene was performed in our previous study [54]. In particular, we found that allele rs648595G (this SNP showed the most significant associations with psoriasis) is associated with decreased expression of *GCLC* in blood, non-sun-exposed suprapubic skin, and sun-exposed lower leg skin. Meanwhile, this allele is associated with increased levels of *GCLC* mRNA in the pancreas, where its expression is two-fold lower than in the skin (GTEx portal, https://gtexportal.org, accessed on May 1, 2023). Similar associations were seen for the rs2397147C allele. Polymorphism rs648595 of *GCLC* has regulatory potential and is located in transcription factor (TF) binding or DNase hypersensitivity sites [54]. Data from the mQTL Database (http://www.mqtldb.org, accessed on May 1, 2023) shows that, at the pregnancy time point, SNP rs648595 is associated with trans-mQTL - hypomethylation of CpG cg06093712 (position 4:13546145) and trans-mQTL - hypomethylation of CpG cg16209652 (position 2: 142888957). Annotation of the rs648595 polymorphism with the bioinformatics resource HaploReg v4.2 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php, accessed on May 1, 2023) showed that the SNP is predicted to be located within the TF-binding site for transcription factor AP-1 (activator protein 1) that is known to control gene expression in response to various stimuli such as cytokines, growth hormones, stress, and infections [68]. In the liver, SNP rs648595 is enriched with enhancer (H3K4me1 and H3K27ac) and promotor (H3K4me3 and H3K9ac) histone marks that may regulate the transcriptional activity of genes. In particular, H3K4me1 (monomethylation on lysine 4 of histone H3) is a dynamic modification that was specifically found to mark both active and primed enhancers [69]. Enhancers bearing the H3K4me1 mark were found to be poised for activation in response to external stimuli [70]. A work by Lauberth and co-workers [71] provided the mechanism by which H3K4me3 promotes rapid gene activation. Finally, H3K9ac co-occurs highly with histone marks such as H3K14ac and H3K4me3 and these three marks are known as the hallmark of active gene promoters [72]. Taken together, the above data from epigenetic studies clearly show that polymorphism rs648595 of the *GCLC* gene represents an important genetic variant that allows for the

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activation of gene expression in the liver. Thus, it can be assumed that in the liver, the main organ that synthesizes most of the body's glutathione to provide organs and tissues, this polymorphism, located in the enhancer region, is apparently involved in the mechanisms of activation of the *GCLC* gene, thereby increasing the rate of glutathione biosynthesis.

Our findings of gene-environment interactions indicate that risk factors such as cigarette smoking and alcohol abuse can modify the associations between *GCLC* gene polymorphisms and the risk of psoriasis (Table 6). It is known that reduced glutathione plays an important role in ethanol detoxification, and acute ethanol administration was found to deplete GSH in the liver and other organs [73]. The leveling of the protective effects of the rs648595 and rs542914 polymorphisms in chronic alcohol abusers appears to be explained by the fact that persistent ethanol intake may diminish the endogenous pool of glutathione [74,75]. Meanwhile, in vitro study by Kimura and co-workers [76] has revealed that primary human hepatocytes treated with 100 and 200 mM ethanol were found to induce transcriptional activity of the *GCLC* gene through the activation of the NF-κB pathway. Tobacco smoking is also well known to deplete glutathione [77–79]. Thus, our study supports the causative roles of tobacco smoking and alcohol abuse in the development of psoriasis, and the negative effects of these environmental factors eliminate the protective role of polymorphisms of the *GCLC* gene against disease risk. Sexual dimorphism in the discovered associations of *GCLC* gene polymorphisms with the risk of psoriasis is apparently associated with differences in environmental risk factors such as smoking and alcohol abuse between males and females. Considering the well-recognized role of oxidative stress in the pathogenesis of psoriasis [13–16], the mechanisms by which glutathione exerts protective effects against disease development are associated with the key role of glutathione in detoxifying ROS and environmental toxicants, penetrating and generating in the skin. We think that the role of glutathione in the pathogenesis of psoriasis is not limited by the protection of cells from oxidative damage. The involvement of glutathione in the molecular mechanisms of psoriasis may also be explained by the role of GSH in the regulation of cell proliferation and wound healing as well as in the inhibition of apoptotic pathways [21,22]. Furthermore, glutathione play an important role in the regulation of the immune system and inflammation, two faces of the same biological coin [80]. Glutathione has been shown to have a wide range of effects on the immune system, either activating or suppressing the immune response to control inflammation. In particular, reduced glutathione is required for the control of innate and adaptive immunological processes such as T-lymphocyte proliferation, phagocytic activity of polymorphonuclear neutrophils, and dendritic cell functions, as well as antigen presentation by antigen-presenting cells [80–82]. Changes in glutathione concentrations may be critical in many autoimmune disease disorders, including psoriasis, that are commonly induced, detrimented, and perpetuated by inflammatory or immune responses mediated by ROS [83]. For instance, glutathione was found to suppress the immune reaction in mice with allergic contact dermatitis [84]. GSH is known to inhibit the production of most inflammatory cytokines and maintain adequate production of interferon-gamma by dendritic cells, a process that is essential for the host defense against intracellular pathogens [80].

Our study has several limitations. Since our study was the first to investigate the contribution of *GCLC* gene polymorphisms to psoriasis risk in relatively small groups of patients, further studies in independent populations with a larger sample size are required to replicate the observed associations. However, such studies are recommended to follow the gene-based approach to look for associations between psoriasis and a wider spectrum of polymorphisms in the *GCLC* gene. Following this approach, nevertheless, it should be taken into account that the studied polymorphisms might be characterized by weak or moderate phenotypic effects that cannot be reproduced in independent populations given their genetic heterogeneity in minor allele frequencies and linkage disequilibrium between the loci [85,86]. Some studies have recently reported that genetic differences between races or ethnicities may account for inter-population differences in glutathione metabolism [47,87]. A relatively small number of study subjects in the case and control groups did not allow analyzing the joint effects of *GCLC* gene polymorphisms and environmental risk factors (smoking and alcohol abuse) separately in males and females in order to obtain estimates of sex-specific gene-

environmental interactions contributing to psoriasis susceptibility. Since the studied polymorphisms of the *GCLC* gene are located in noncoding regions, their phenotypic effects should be interpreted with caution because no investigations were done to assess gene expression in skin biopsies of study patients.

5. Conclusions

The present study demonstrated for the first time that polymorphisms in the gene encoding the catalytic subunit of glutamate cysteine ligase represent novel genetic markers for susceptibility to psoriasis. The phenotypic effects of *GCLC* gene polymorphisms on the development of psoriasis are modified by tobacco smoking and alcohol abuse, known environmental factors increasing disease risk. We suppose that the *GCLC* gene may contribute to the risk of psoriasis through a diminished biosynthesis of glutathione in the skin, where GSH regulates a plethora of cellular processes such as redox homeostasis, detoxification of xenobiotics, innate and adaptive immune functions, inflammation, cell proliferation and differentiation, and apoptosis that have been implicated in disease pathogenesis. Better understanding the relation between the *GCLC* gene polymorphisms and glutathione biosynthesis in the skin and the molecular mechanisms by which this gene contributes to psoriasis will open new scientifically based options for disease therapy and prevention targeted on glutathione metabolism. The use of pharmacogenetics and precision medicine approaches [88,89] will make it possible to subclassify patient groups based on environmental risk factors and clinically significant genetic variants that affect glutathione metabolism and thus personalize treatment and prevention of psoriasis.

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Data Availability Statement: Data supporting reported results are available upon request.

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