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Research article

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Paraoxonase 1 (PON1) gene Q192R polymorphism in patients with vitiligo

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The approval was taken from the Kutahya Health Sciences University Ethics Committee, Turkey, March 6, 2019 (2019/03-5).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Vitiligo is a prevalent inflammatory illness that can affect the skin and mucosal surfaces and is characterized by patchy loss of skin pigmentation. Paraoxonase1 (PON1) is an esterase enzyme with antioxidant properties that binds to high-density lipoproteins. We examined whether the PON1 gene Q192R polymorphism is a risk factor for vitiligo among Turkish people.
Methods: The study included 70 controls and 60 vitiligo cases. Polymerase chain reaction and the restriction fragment length polymorphism technique were used to genotype the PON1 gene Q192R polymorphism.
Results: PON1 gene Q192R genotype distribution was 66.7% QQ, 33.3% QR, and 0% RR in the vitiligo and 81.4% QQ, 18.6% QR, and 0% RR in the control (P = 0.05). When vitiligo patients were compared with controls, the prevalence of the PON1 QR genotype was substantially higher and was linked to a 2.19-fold greater risk of developing vitiligo (odds ratio: 2.19, 95% confidence interval (CI): 0.97–4.91).
Conclusion: These findings imply that Q192R polymorphisms in the PON-1 gene may be linked to vitiligo in the Turkish population. The PON1 QR genotype may be a major genetic risk factor for vitiligo susceptibility and progression. Further studies with larger populations should more thoroughly clarify the association.

Keywords: Vitiligo, PON1 gene Q192R, Polymorphism

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Introduction

Vitiligo is a common dermatological illness characterized by skin lesions that are confined and depigmented [1, 2]. The exact etiology of vitiligo is still unknown but is thought to involve interactions between multiple genes and immunological and environmental events [3, 4]. An imbalance between the synthesis and accumulation of oxygen-reactive species (ROS) in cells and tissues causes oxidative stress. DNA damage, lipid and protein peroxidation are all caused by increased oxidative stress [1–5]. Recent research has highlighted the importance of oxidative stress in vitiligo [1–3].

Paraoxonase 1 (PON1) is an antioxidant enzyme that is linked to high density lipoprotein regulation and has been reported to lead to a reduction in oxidative stress and protect low- and high-density lipoproteins against oxidation [5, 6]. The PON1 gene is situated on chromosome 7 in the q21-q22 region. PON1 activity shows individual and ethnic differences due to genetic polymorphisms. The first polymorphism occurs when glutamine (Q genotype) is replaced with an arginine (R genotype) at locus 192 [6]. Some previous studies have reported that the PON1 Q192R gene polymorphism is associated with diseases, such as chronic obstructive pulmonary disease [6], coronary artery disease [7], and ischemic stroke [8].

To our knowledge, the PON1 Q192R gene polymorphism has not been studied in patients with vitiligo. Based on these observations, the purpose of this study was to determine the potential role of the PON1 Q192R gene polymorphism in vitiligo and its relationship to vitiligo susceptibility in a group of Turkish patients.

Materials and methods

Study design and study population

The research was conducted at Kutahya Health Sciences University's Department of Dermatology, Faculty of Medicine. In this study, 60 people with vitiligo (23 females, 37 males; mean age, 41.9 [17.1] years) and 70 ethnically matched, healthy volunteers from our healthcare workers were selected as controls (42 females, 28 males; mean age, 39.4 [16.8] years). Vitiligo was detected based on clinical symptoms and a Wood lamp examination. Additional parameters, such as demographics, family history, duration of sickness, and autoimmune diseases, were included in all patients' data. Healthy controls were selected from the same hospital if they had not been diagnosed with vitiligo based on a comprehensive physical examination by a dermatologist and had no personal or family history of autoimmune or inflammatory illnesses. Ethical approval for the research was obtained from the Ethics Committee of the Kutahya Health Sciences University, Turkey, on March 6, 2019 (2019/03-5), all procedures were performed in accordance with the Helsinki Declaration, and all patients provided informed consent.

Genomic DNA extraction

Venous blood samples were obtained from all individuals and then placed in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Samples were used to isolate genomic DNA using the phenol–chloroform method. Following extraction, the concentration and purity of DNA were evaluated spectrophotometrically. DNA quality was also evaluated on a 0.7% agarose gel electrophoresis, and the DNA was stored at −20°C until further analyses.

Genotyping of PON1 Q192R polymorphism

PON1 Q192R polymorphism was genotyped using polymerase chain reaction (PCR) and fragment length polymorphism (PCR–RFLP) based on the protocol described previously by Öktem et al. [9]. The primers used for PCR are mentioned in Table 1. In a total volume of 20 mL, the reaction mixture contained 3 mL (100 ng) of genomic DNA, 10.7 mL nuclease-free H_2O, 2 mL 10X PCR buffer, 1 μL $MgCl_2$ 25 mM, 2 mL 2 mM dNTPs (abm, Canada), 1 mL (each) of 10 pM corresponding forward and reverse primers (GenScript, USA), and 0.3 mL (3U/mL) Taq Polymerase (abm, Canada). With the aid of a thermal master cycler gradient (Thermo Scientific, EU Lithuania), PCR products were amplified and then digested with BspI (New England Biolabs, Ipswich, MA, USA) after which they were electrophoresed on 4% agarose gel and stained with ethidium bromide (Table 1).

Statistical analysis

The frequency distributions of each allele and genotype of the PON1 Q192R polymorphism were compared between the cases and controls using the $\chi 2$ and Fisher's exact tests for calculating odds ratios (OR) with 95% confidence intervals (CI). The association between PON1 genotypes and the clinical characteristics of the vitiligo patients were assessed using a $\chi 2$ test for categorical variables and an analysis of variance (ANOVA) for continuous variables. Statistical significance was considered a P-value ≤ 0.05. The SPSS software was used to conduct the statistical analysis (version 24.0).

Results

Characteristics of vitiligo patients and controls

Among the patients who visited the dermatology clinics of the Medicine Faculty, Kutahya Health Sciences University, a total sample of 60 unrelated patients with generalized vitiligo and 70 unrelated controls without a history of autoimmune and/or inflammatory illnesses were enrolled in the study. Age and gender variations between the case and control groups were insignificant (P = 0.34 and P = 0.65, respectively). Table 2 shows the demographic and clinical characteristics of the vitiligo cases.

Table 1: Summary of conditions for the PON1 Q192R genetic analyses

SNP	Prime sequence (5'-3')	Tm (°C)	PCR product size	Restriction Enzyme	Genotyping
PON1 Q192R	F-TATTGTTGCTGTGGGACCTGAG R-GACATACTTGCCATCGGGTGAA	60 °C	199 bp	BspI	QQ: 199 bp QR: 135 bp - 199 bp RR: 135 bp

PON 1: Paraoxonase 1, SNP: single nucleotide polymorphism, Tm: primer annealing temperature, bp: base pairs

Table 2: Demographic and clinical characteristics of vitiligo and control subjects

Parameter	Vitiligo (n = 60)	Controls (n=70)
Gender n (%)		
Female / Male	23 (38.3) / 37 (61.7)	42 (60) / 28 (40)
Age, years	41.9 (17.1)	39.4 (16.8)
Mean duration of the disease, months	87.5 (145.6)	-
Age of vitiligo onset, years	34.7 (18.1)	-
Age of vitiligo onset, n (%)		
Early-onset, (< 30 years)	15 (25)	-
Late-onset, (≥ 30 years)	45 (75)	-
Type of vitiligo, n (%)		
Generalized	46 (76.7)	-
Localized	10 (16.7)	-
Acrofacial	4 (6.7)	-
Skin type, n (%)		
2	7 (11.7)	-
3	47 (78.3)	-
4	6 (10)	-
Initial location, n (%)		
Head neck	17 (28.3)	-
Upper extremity	2 (3.3)	-
Body	20 (33.3)	-
Lower extremity	6 (10)	-
Hands	15 (25)	-
With/without Koebner phenomenon, n (%)	5 (8.3) / 55 (91.7)	-
With/without Halo nevi, n (%)	5 (8.3) / 55 (91.7)	-
With/without leukotrichia, n (%)	17 (28.3)/43 (71.7)	-
With/without repigmentation, n (%)	17 (28.3)/43 (71.7)	-
With/without Family history of vitiligo, n (%)	18 (30.5)/41 (69.5)	-
With/without other skin disease, n (%)	5 (8.3)/55 (91.7)	-
With/without other autoimmune disease, n (%)	10 (16.7)/50 (83.3)	-
With/without stress, n (%)	46 (76.7)/14 (23.3)	-

Association between PON1 Q192R genotypes and alleles with the risk of developing vitiligo

Table 3 shows the allele and genotype frequencies for the distribution of the PON1 Q192R gene polymorphism. The genotype frequencies of the vitiligo and control groups were in Hardy–Weinberg Equilibrium (P = 0.12 and P = 0.39, respectively).

The frequencies of each PON1 gene Q192R genotype were revealed as 66.7% for QQ (n = 40), 33.3% for QR (n = 20), and 0% for RR (n = 0) in the vitiligo patient; 81.4% for QQ (n = 57), 18.6% for QR (n = 13) and 0% for RR (n = 0) in the control. The distribution of the PON1 gene Q192R genotypes was found to be significantly different between groups ($\chi2 = 3.71$; df = 1; P = 0.05). A positive association between the risk of developing vitiligo and the QR genotype of PON1 was also noted (OR = 2.19, 95% CI 0.97–4.91; P = 0.05) as shown in Table 3.

The Q allele was reported in 83.3% (n = 100) of the vitiligo and 89.3% (n = 125) of the controls. The R allele was observed in 16.7% (n = 20) of the vitiligo and 10.7% (n = 15) of the controls. No discernible variations in the PON1 gene Q192R allele distributions between patients and controls were found ($\chi2 = 1.96$; df = 1; P = 0.16) as shown in Table 3.

Table 3: Distributions of PON1 Q192R polymorphism genotype and allele frequencies in the study populations and risk of vitiligo

PON1 Q192R	Genotype/Allele	Vitiligo (n = 60) n (%)	Controls (n = 70) n (%)	OR	95% CI	*P*-value
	QQ	40 (66.7)	57 (81.4)	0.45	0.20 - 1.02	0.05
	QR	20 (33.3)	13 (18.6)	2.19	0.97 - 4.91	0.05
	RR	-	-	-	-	-
	$\chi2 = 3.71$, df = 1, P = 0.05					
	Q	100 (83.3)	125 (89.3)	0.60	0.29 - 1.23	0.22
	R	20 (16.7)	15 (10.7)	1.66	0.81 - 3.42	0.22
	$\chi2 = 1.96$, df = 1, P = 0.16					

OR: odds ratio, CI: confidence interval

Association between PON1 Q192R genotypes and clinical characteristics of the vitiligo patients

Patients who had a history of vitiligo in their families had more QR genotypes than QQ genotypes on average (47.4% versus 22.5%; P = 0.05) as shown in Table 4.

Table 4: Baseline clinical and demographical features of the study patients with vitiligo stratified according to PON1 Q192R gene polymorphisms

Characteristic	QQ	QR	RR	*P*-value
Gender, male/female, n (%)	17/23 (42.5/57.5)	6/14 (30/70)	-	0.975
Age (years)	39.3 (17.0)	47.1 (16.7)	-	0.08
Disease duration (months)	79.6 (144.6)	103.2 (150.0)	-	0.604
Age of vitiligo onset (years)	32.7 (18.7)	38.7 (16.6)	-	0.224
Age of vitiligo onset, n (%)				
Early-onset, (< 30 years)	12 (30)	3 (15)	-	0.206
Late-onset, (≥ 30 years)	28 (70)	17 (85)	-	
Type of vitiligo, n (%)				
Generalized	30 (75)	16 (80)	-	0.511
Localized	8 (20)	2 (10)	-	
Acrofacial	2 (5)	2 (10)	-	
Skin type, n (%)				
2	6 (15)	1 (5)	-	0.123
3	32 (80)	15 (75)	-	
4	2 (5)	4 (20)	-	
Family history of vitiligo, n (%)				
Yes	9 (22.5)	9 (47.4)	-	0.05*
No	31 (77.5)	10 (52.6)	-	

Data were analyzed by analysis of variance and $\chi2$ test. Mean plus standard deviation of the mean values are presented for age, disease duration, age of vitiligo onset. * P value ≤ 0.05 was considered significant.

Discussion

Vitiligo is a multifactorial polygenic skin disorder with a complicated pathogenesis that is caused by both genetic and non-genetic factors. This disorder may involve genes related to melanin manufacturing, the antioxidant system, and the control of autoimmune diseases [10]. PON1 is involved in both inflammation and lipid metabolism. This enzyme leads to a reduction in low-density lipoprotein (LDL) oxidation and has been shown to be deficient in many disease states. PON1 gene polymorphisms are linked to the metabolic syndrome [11]. The full etiology and pathogenesis of vitiligo is yet unknown. Consequently, in light of these findings and those in the literature, PON1 may play a significant role in the pathogenesis of vitiligo by protecting cells from oxidative stress. For understanding the etiopathogenesis of vitiligo and creating viable treatment options, genetic research is crucial.

To the best of our knowledge, only a few studies concerning the PON1 Q192R gene polymorphism in vitiligo have been published. As a result, this study is the first to use the PCR–RFLP method to assess the link between the PON1 Q192R gene polymorphism and vitiligo. The current study sought to determine the relationship between PON1 Q192R gene polymorphism and the risk of developing vitiligo. An independent case-control study was conducted using a sample of 60 vitiligo patients and 70 matched controls. Seçkin et al. [12] found no significant differences between vitiligo and PON1 Q192R gene polymorphism using LightCycler PCR technology [12]. Although no discernible variation in the allele frequencies of the PON1 gene Q192R polymorphism in this investigation was found, the vitiligo and control groups were found to have significantly different genotype distributions. The PON1 Q192R genotype frequency was statistically and substantially higher in the vitiligo patient when compared with the controls. In individuals with the QR genotype, we found a 2-fold relative risk increase for the onset of vitiligo. In addition, patients with vitiligo in their families had a higher prevalence of the QR genotype than the QQ genotype. The PON1 QR genotype was thus found to be strongly related to the chance of developing vitiligo and may therefore contribute to vitiligo according to our findings.

PON1 is an antioxidant enzyme related to high-density lipoprotein (HDL) regulation in the blood and is a very important ester hydrolase that protects lipoproteins from oxidation [13].

Serum PON1 activity has been examined in various dermatological conditions, including Behcet disease [14] and psoriasis [15]. Age, dyslipidemia, diabetes mellitus, smoking, hypertension, and increased oxidative stress have all been linked to low PON1 activity [16]. Ramadan et al. [17] demonstrated that both tissue and serum levels of PON1 were significantly reduced in the vitiligo patients when compared with the controls [17]. In this study, it was not possible to examine serum PON1 levels. Pola et al. [18] suggested that serum levels of the PON1 protein are influenced by the 192 Q/R polymorphism of the PON1 gene [18]. No studies in the literature addressing serum PON1 levels and the PON1 gene Q192R polymorphism in vitiligo are available.

Limitations

The fact that we were unable to precisely gauge the serum levels of the PON1 gene was one of the study's drawbacks. Highlighting the consequences of the Q192R variation in the PON1 gene and correlating the genotyping results would have been helpful. Very little evidence in the literature describing the relationship of vitiligo to the PON1 gene variation exists. As a result, comparing the findings of this study to vitiligo and PON1 gene polymorphism is not conceivable. Therefore, this study needs to be supported by further, research including a larger sample size of patients.

Conclusion

Consequently, a significant difference between the vitiligo and PON1 gene Q192R polymorphism in the Turkish population was found. The QQ genotype in controls and the QR genotype in vitiligo were found to be abundant. Additionally, it was discovered that patients with a family history of vitiligo had a much higher frequency of the PON1 QR genotype. The relative risk of developing vitiligo was shown to have increased 2-fold in those with the QR genotype. Interestingly, it has been discovered that the PON1 gene Q192R polymorphism is linked to vitiligo, and more research in this area will shed light on the function of PON1 gene polymorphism in vitiligo pathogenesis, treatment, and prevention.

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