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Association of endothelial nitric oxide synthase Glu298Asp gene polymorphism in psoriasis cases with hypertension

Zerrin Ogretmen,[a] Meliha Merve Hiz,[b] Fatma Silan,[c] Ahmet Uludag,[c] Ozturk Ozdemir[cd]

From the [a]Department of Dermatology, School of Medicine,; [b]Department of Biology, Faculty of Science and Arts [c]Department of Medical Genetics, School of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey [d]Department of Medical Genetics, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey

Correspondence: Dr. Meliza M. Hiz, Department of Molecular Biology and Genetics, Canakkale Onsekiz Mart University, Canakkale 17100, Burslem, Turkey T: +905332444545 mervemeliha@comu.edu.tr mervemeliha@gmail.com

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BACKGROUND AND OBJECTIVES: Psoriasis is a common autoimmune-mediated chronic, inflammatory skin disease. Although, the molecular mechanism is not completely understood, psoriasis is caused by genetic and non-genetic parameters. The current study aimed (1) to define genotype and allele frequency of endothelial nitric oxide synthase (*eNOS* Glu298Asp) gene polymorphism in hypertensive and/or non-hypertensive psoriatic patients (2) to investigate the possible relationship between the *eNOS* Glu298Asp polymorphism and the risk of hypertension among psoriatic patients in the Turkish population.

DESIGN AND SETTINGS: This cross-sectional, case-control study was performed between March 2010 and November 2012 at the University hospital in Çanakkale, Turkey

PATIENTS AND METHODS: Gene profiles of 75 psoriatic patients (21 hypertensive and 54 normotensive patients) and 55 healthy (normotensive and non-psoriatic) volunteers were compared. Peripheral blood-EDTA samples were used for total genomic DNA isolation and genotyping. Target *eNOS* gene was genotyped for patients and control groups by real-time PCR melting-curve analysis system (LightCycler 2.0,Roche, Germany, and results were compared statistically.

RESULTS: An increased T allele frequency in *eNOS* Glu298Asp single-nucleotide polymorphism (SNP) was determined in psoriatic patients when compared with normotensive non-psoriatic healthy volunteers (OR 2.3, CI 1.14-3.99), (*P*=.017). The T allele frequency was also found to be increased in hypertensive psoriatic patients when compared with healthy volunteers (4.83-fold increased, 95% CI 1.62-14.43 ([*P*=.003]) and normotensive psoriatic patients (3.03-fold increased, 95% CI 1.03-8.94 [*P*=.041]), respectively.

CONCLUSION: The current preliminary results suggested that there was a correlation between *eNOS* Glu298Asp polymorphism and hypertension among psoriatic patients in the Turkish population. The T allele frequency of *eNOS* Glu298Asp SNP was different in hypertensive psoriatic patients, and the difference was statistically significant when compared with the normotensive psoriatic patients and healthy controls. These results need to be confirmed by large-scale studies.

Psoriasis is a chronic dermatitis with unknown chronic systemic inflammatory diseases (CSID) that have same immunopathogenesis. Psoriasis is also an erythematous, scaly chronic inflammatory dermatitis with a complex immunologic basis. Psoriatic patients also suffer from comorbidities such as cancer, obesity, hypertension, insulin resistance, coronary artery diseases, metabolic syndrome, and other immune-related conditions that have similar pathogenic features. Cytokines released from these diseases cause similar clinical symptoms. It is well known that the *eNOS* gene polymorphism may cause endothelial dysfunction, which is a common finding between CSID group (e.g., hypertension, atherosclerosis, chronic inflammation, type 2 diabetes, etc.). Many different studies have been performed for investigating the correlation of cardiovascular diseases with psoriasis, and cardiovascular morbidity and mortality

were observed to be increased in psoriatic patients.[1-10]

Inflammatory processes play an important role in etiology and pathogenesis of both cardiovascular diseases and psoriasis.[3] Nitric oxide is a vasoactive substance produced from L-arginine in the vascular endothelium by endothelial nitric oxide synthase (*eNOS*) enzyme, and it is the main mediator of endothelial-dependent vasodilatation by the effect of vascular smooth muscle relaxation.[11] It plays a major role in regional blood flow regulation and inhibits thrombocyte aggregation.[12] *eNOS* gene mutations have been shown to be risk factors for coronary artery disease, myocardial infarction, and hypertension.[13-16]

Recent reports have shown that cardiovascular disease rates increase among psoriatic patients treated with systemic anti-inflammatory drugs. Therefore, determining psoriatic patients with a higher risk of hypertension is crucial to optimize required doses of systemic medications. Early diagnosis and treatment of hypertension risk in psoriatic patients will be beneficial in mortality prevention. *eNOS* Glu298Asp polymorphism may be used as a marker gene to determine the hypertension risk in psoriatic patients, thus enabling dermatologists to identify patients with hypertension risk and manage the medication plan carefully.

The current study aimed to find out the possible association between *eNOS* polymorphism and hypertension in psoriasis. The results of our study are important for assessing interactions between *eNOS* gene and hypertension treatment among psoriatic patients.

PATIENTS AND METHODS

Design and Setting

A case-control study was performed to define genotype and allelic profiles of polymorphic *eNOS* Glu298Asp gene in hypertensive psoriatic patients in the University Hospital, Çanakkale, Turkey.

Patients, Clinical Diagnosis, and Laboratory Assessment

A total of 75 psoriatic patients (21 hypertensive [28%], 54 non-hypertensive [72%], 30 male [40%], 45 female [60%], and the mean age-min-max: 44.7 [14.1] [18-80]) and 55 normotensive subjects without a prior history of psoriasis in them and their families (23 male [41.8%], 32 female [58.2%,] and the mean age-min-max: 47.6[9.9] [35-65]) were enrolled in the current study. Peripheral blood-EDTA samples were obtained during routine diagnosis from patients in Canakkale Onsekiz Mart University Training and Research Hospital by the collaboration of the Department of Medical Genetics between March 2010 and November 2012. All of the individuals in patient and control groups were informed, and written informed consent was obtained from them after the full explanation of the study.

Genotyping

Total genomic DNA was extracted from blood-EDTA samples by MagnaPure Compact (Roche, Germany) and Invitek kit extraction techniques (Invitek®; Invisorb spin blood, Berlin, Germany). Target *eNOS* gene was genotyped for patients and control groups by real-time PCR melting-curve analysis system (LightCycler 2.0, Roche, Germany). Gene amplification was performed using specific primers and probes belonging to polymorphic regions of target *eNOS* Glu298Asp single-nucleotide polymorphism (SNP) (Rs1799983). For a total volume of 20 µl polymerase chain reaction (PCR), 5 µl genomic DNA, 7.4 µl PCR-grade fluid, 1.6 µl Mg2+ solution, 4 µl of primer and probe mixture, and 2 µl Roche Fast Start Master Mix real-time PCR, Germany, were used as a reaction mixture for amplification. Briefly, the amplification conditions included pre-denaturation at 95°C for 10 minutes; 45 cycles at 95°C for 5 seconds, at 60°C for 10 seconds, and at 72°C for 15 seconds; dissolution-melting at 95°C for 20 seconds, at 40°C for 20 seconds and 0.2 continuous mode at 85°C; and cooling at 40°C for 30 seconds. Non-mutated 298G/G allele (wild) was evaluated in the 640 channel at a melting temperature (Tm) of 52°C, whereas mutated 298A/A allele was evaluated again in the same channel at a Tm of 59°C.

RESULTS

Results showed variations in the *eNOS* Glu298Asp polymorphism. A total of 75 psoriatic patients (mean age 44.7 (14.1) (18-80)] and 55 healthy controls (mean age 47.6 (9.9) (35-65)] were enrolled. In psoriatic patients, the mean diastolic blood pressure was 82.4(9.9) mm Hg and systolic blood pressure was 128.2(12) mm Hg. Twenty-one psoriatic patients (28%) were identified as hypertensive psoriatic patients and received hypertension treatment.

In this study, the association of *eNOS* polymorphism with psoriasis was analyzed by comparing psoriatic patients with normotensive non-psoriatic volunteers (**Figure 1**), (**Table 1**). The results revealed that the allele positivity of *eNOS* gene increased the psoriasis risk 2.37 times more in psoriatic patients than in normotensive and non-psoriatic volunteers (95% confidence interval [CI] 1.14-4.96, *P*=.02]. The odds ratio for the risk of psoriasis due to the substitution of G to T in the *eNOS* gene increased 2.13 times in hypertensive psoriatic patients (CI% 1.14-3.99, *P*=.017) than in

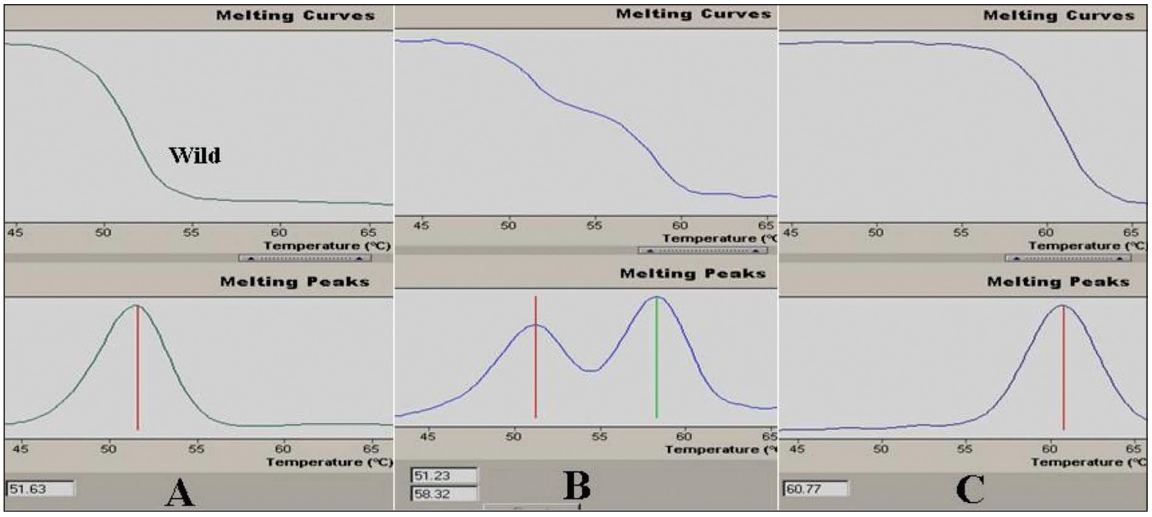

Figure 1. The melting curves and Piks Real-Time PCR profiles show wild (A), heterozygous (B) and homozygous (C) mutated eNOS Glu298Asp gene for the current results.

Table 1. Genotype prevalence and T allele frequency of *eNOS* Glu298Asp SNP in the current psoriatic patients and healthy (normotensive and non-psoriatic) volunteers from the same population.

Gene/ Genotypes	Groups				
	Patients (n:75)	Controls (n:55)	Allel Positivity [GG]vs[GT]+[TT]		
	n/%	n/%	P value	OR	CI (95%)
eNOS Glu298Asp					
G/G	38/50.7	39/70.9			
G/T	32/42.7	15/27.2	.020	2.3	1.14-4.96
T/T	5/6.6	1/1.81			
Alleles			Allel frequency difference		
			P value	OR	CI (95%)
G	108/72.0	93/ 84.5	.017	2.1	1.14-3.99
T	42/28.0[a]	27/15.4			

[a]Significant; OR: odds ratio; SNP: single-nucleotide polymorphism.

normotensive and non-psoriatic volunteers (**Figure 1**). These results suggest that *eNOS* polymorphism has a statistically significant effect to increase psoriasis risk.

The correlation of *eNOS* Glu298Asp mutation with hypertension in psoriatic patients was investigated by comparing them with normotensive, non-psoriatic volunteers (**Table 2**), aside psoriatic patients without hypertension (n:54; **Table 3**).

Hypertension accompanying psoriasis was detected in 7 out of 38 cases with normal (GG) genotypes, 13 out of 32 heterozygote (GT) genotypes, and 1 out of 5 mutant genotypes (TT) in the evaluation of 75 subjects with psoriasis.

When hypertensive psoriatic patients were compared with healthy (normotensive, non-psoriatic) individuals, an increased frequency of the *eNOS* rs1799983 T allele was found in hypertensive psoriatic patients (Odds ratio [OR]:3.04 95% CI 1.34-6.87, *P*=.0076). The results revealed that the allele positivity of *eNOS* gene increased the hypertension risk 4.88 times more among psoriatic patients than among normotensive, non-psoriatic volunteers (95% CI 1.66-14.32, *P*=.0027). When hypertensive psoriatic cases with heterozygote genotype (GT) were compared with the ones with normal genotype (GG), the conversion of single G allele into T allele increased the hypertension risk by 4.83 folds (OR 4.83, 95% CI 1.62-14.43, *P*=.0033). The overall increased risk of hypertension among psoriatic patients in the *eNOS* gene was 3.28 times (*P*=.003) more than among normotensive, non-psoriatic volunteers. It was determined in the study that T allele frequency belonging to the polymorphic *eNOS* Glu298Asp gene region was higher in hypertensive psoriatic cases than in normotensive, non-psoriatic individuals. These results also suggested that *eNOS* gene polymorphism rs1799983 G transversion to T was associated with an increased hypertension risk among psoriatic patients (**Table 2**).

The association of *eNOS* Glu298Asp (Rs1799983) polymorphism with hypertension was also performed by comparing hypertensive psoriatic patients with normotensive psoriatic cases. When cases with heterozygote genotype (GT) were compared with the ones with normal genotype (GG), the conversion of single G allele into T allele increased the hypertension risk by 3.03 folds (OR 3.03, 95% CI 1.03-8.94, *P*=.04). The poly-

morphic *eNOS* Glu298Asp gene region was higher in hypertensive psoriatic cases than in non-hypertensive psoriatic cases like healthy controls (**Table 3**).

DISCUSSION

As molecular basis and genetic susceptibility of diseases have been understood in the recent years, it was shown that basal vasodilatation deficiency, hypertension, and endothelial dysfunction developed in mice with the defect in the *eNOS* gene region.[17] The regain of vascular reactivity after *eNOS* gene transplantation in *eNOS*-defective mice[18] and rabbits with pulmonary hypertension[19] and in vivo *eNOS* injection studies in normal mice[20] indicated the significance of *eNOS* gene in preservation of healthy blood pressure levels.

Human *eNOS* gene is frequently polymorphic, and many studies have been performed on the relationship between *eNOS* gene region polymorphisms and coronary artery diseases and hypertension.[21-30] In their study performed on 226 primary hypertension patients and 200 healthy controls, Srivastava et al compared patients having GT+TT genotype with the control group to define *eNOS* Glu298Asp mutation using the PCR-restriction fragment length polymorphism (RFLP) method. They found that the primary hypertension risk increased by 2.10 folds.[28] Li et al showed that *eNOS* Glu298Asp polymorphism was related to hypertension in females.[29]

Tian et al,[22] Velloso et al,[23] Periaswamy et al[24] and Kim[25] et al reported that *eNOS* Glu298Asp poly-

Table 2. The comparison of genotype prevalence and allele frequency of target *eNOS* Glu298Asp SNP for the hypertensive psoriatic sub-group patients and normotensive non-psoriatic healthy volunteers in the presented results.

eNOS Glu298Asp Polymorphism	Hypertensive Psoriatic Patients (n:21)	Normotensive Non-Psoriatic Healthy Volunteers (n:55)		*P* value	OR (95% CI)
Genotypes					
GG	7	39	GT vs GG	.0033	4.8 (1.6-14.4)
GT	13	15	TT vs GG	.1963	5.6 (0.3-99.9)
TT	1	1	Allele Positivity	.0027	4.9 (1.7-14.3)
Alleles			**Allele frequency difference**		
G allele	27/0.643	93/0.846	T allele vs G allele	.0615	3.0 (1.3-6.9)
T allele	15/0.357[a]	17/0.154			

[a]Significant; OR: odds ratio; SNP: single-nucleotide polymorphism.

Table 3. The comparison of genotypes and allele frequency of target *eNOS* Glu298Asp SNP for the current hypertensive and normotensive psoriatic sub-group patients.

eNOS Glu298Asp Polymorphism	Hypertensive Psoriatic Group (n:21)	Normotensive Non-Psoriatic Group (n:54)		*P* value	OR (95% CI)
Genotypes					
GG	7	31	GT vs GG	.0405	3.0 (1.0-8.9)
GT	13	19	TT vs GG	.9320	1.1 (0.1-11.5)
TT	1	4	Allele Positivity	.0612	2.7 (0.9-7.7)
Alleles			**Allele frequency difference**		
G allele	27/0.643	93/0.846	T allele vs G allele	.1894	1.7 (0.8-3.6)
T allele	15/0.357	27/0.250			

OR: Odds ratio; SNP: single-nucleotide polymorphism

morphism was related to hypertension. However, Wolff et al[26] and Kishimoto et al[27] reported that *eNOS* Glu298Asp polymorphism was not related to hypertension. Actually, the effects of *eNOS* were studied in Turkish patients with essential hypertension, and the results showed that polymorphism increases the risk of hypertension in the case of high serum total cholesterol levels.[31]

This study is significant because it has been the first study that has investigated the correlation of *eNOS* Glu298Asp polymorphism and hypertension among psoriasis cases in the Turkish population. Also the study is more reliable because all the data were collected using the real-time PCR method. The previous studies performed in the Turkish population focused only on psoriasis or hypertension.[30,31]

In a study conducted on psoriasis cases from the Turkish population in which *eNOS* gene polymorphism was investigated with the MboI RFLP method, it was detected that *eNOS* polymorphism was significant for psoriasis. Yet, the MboI RFLP method has a disadvantage due to the lack of enzyme recognition specify of the target sequences at the SNP. In our present study, real-time PCR method—a new, valid, and reliable method—was used in *eNOS* genotyping in patients and controls. The findings of the present study indicated that allele positivity at the 298 G/T region in *eNOS* gene increases the psoriasis risk 2.37 times (95% CI 1.14-4.96, *P*=.020) more in psoriatic patients than in normotensive and non-psoriatic volunteers and supports the previous study findings.[30] Aside the differences in method, Senturk et al did not analyze the association of Glu298Asp with –*eNOS* gene in risk-related subgroups such as hypertensive psoriatic cases.[30]

The results in the present study indicated that polymorphism in the *eNOS* Glu298Asp gene region was a significant and prominent risk factor for hypertension in psoriatic patients. When hypertensive psoriatic patients were compared with healthy individuals (normotensive, non-psoriatic individuals) and non-hypertensive psoriatic patients, the conversion of single G allele into T allele increased the hypertension risk 3.04 folds and 1.67 folds, respectively. The current preliminary results were consistent with the literature; nevertheless, the results need to be confirmed by large-scale studies.[22-25,30]

eNOS gene polymorphism causes endothelial dysfunction, which gives birth to diseases such as hypertension, diabetes mellitus type 2, and hypercholesterolemia. All these diseases are components of metabolic syndrome. Therefore, in our opinion, endothelial dysfunction is the main cause of metabolic syndrome, and *eNOS* gene polymorphism is one of the most important reasons for its development. We think that an increased risk of hypertension in psoriatic patients is an important evidence for metabolic syndrome-psoriasis relation. According to this study, suggesting lifestyle modifications (diet, sport, weight loss, etc.), regular clinical examination, and avoiding drugs with cardiac side effects (acitretin base, TNF alpha blocker etc.) in treating psoriasis may contribute positively to psoriatic patients with *eNOS* Glu298Asp polymorphism who are suffering hypertension and diabetes. Present results showed that the T allele frequency of *eNOS* Glu298Asp SNP was different in hypertensive psoriatic patients, and the difference was statistically significant when compared with normotensive psoriatic patients and healthy controls (**Tables 1-3**).

In conclusion, the results indicated that the Glu298Asp polymorphism of the *eNOS* gene appears to be an independent risk factor for psoriasis. It should also be considered that *eNOS* Glu298Asp polymorphism in psoriatic patients may provide a genetic susceptibility for hypertension. However, further investigations are necessary for confirming the association of *eNOS* Glu298Asp polymorphism with psoriasis and its relation with increase in the risk of hypertension among psoriatic patients.

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