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Evaluation of the Potential Association between NOS Gene Polymorphisms (*iNOS G-954C* and *eNOS G894T*) and Psoriasis

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Dear Editor:

Psoriasis is a common chronic skin disease that affects 0.1%～3% of the world population. The exact pathomechanism of this disease has not yet been fully elucidated. Most hypotheses assume that the disease is an immune-mediated disorder involving multigenic components and environmental factors[1].

Nitric oxide (NO) acts as an intercellular messenger contributing to the pathogenesis of various autoimmune diseases via cell proliferation, differentiation, and apoptosis[2]. Several lines of evidence indicate that NO is important in the pathogenesis of psoriasis and promotes skin microvasculature formation, keratinocyte proliferation, and keratinocyte differentiation[3].

NO is synthesized by a group of enzymes called nitric oxide synthases (NOSs). The NOS family consists of three isoforms, neuronal NOS, endothelial NOS (eNOS), and inducible NOS (iNOS). Recently, Ormerod et al.[4] have shown high levels of eNOS and iNOS expression in psoriatic lesions, suggesting their involvement together with NO in the occurrence and further development of psoriasis[4]. Considering the important role played by iNOS and eNOS in the production of NO and in the pathogenesis of psoriasis, we hypothesized that iNOS and eNOS gene polymorphisms may be associated with the risk of psoriasis. Therefore, we investigated the potential association of the *iNOS G-954C* and *eNOS G894T* polymorphisms with psoriasis and attempted to correlate the results with the clinical features in this study.

Sommer et al.[5] have shown that patients with psoriasis have a higher risk of developing hypertension than individuals without psoriasis. It has also been reported that eNOS gene polymorphisms are associated with hypertension susceptibility[6]. These findings led us to question whether any susceptibility genes are common between psoriasis and hypertension. Therefore, we studied the association of the *eNOS G894T* polymorphism and psoriasis with hypertension to investigate the mechanism underlying the relationship between psoriasis and hypertension.

The study included 212 Han Chinese patients who had psoriasis and visited the Department of Dermatology, WestChina Hospital, Sichuan University. One hundred and seventy eight age- and sex-matched Han Chinese healthy volunteers were used as the control group. For all individuals, age, sex, and history of psoriasis and hypertension were recorded. This study was approved by the organization is the Clinical Trials and Biomedical Ethics Committee of West China Hospital Sichuan University (No. 2015158).

The genes polymorphisms were determined using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. DNA was isolated from venous blood samples using a DNA extraction kit (Jingbo, Chengdu, China). PCR was used to amplify the fragments that contained the polymorphic sites. The primers for *iNOS G-954C* were as follows: 5'-ACTTGGTACTGAGGAAGGCGCTCT-3' (forward) and 5'-TAGCAAAGCCCCGTTTCAACAA-3' (reverse).

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Table 1. Clinical characteristics of the psoriatic patients and controls

Characteristic	*iNOS G-954C*		*eNOS G894T*	
	Psorisis (n=202)	Control (n=178)	Psoriasis (n=185)	Control (n=137)
Average age (yr)	41.32±10.75	42.57±11.31	42.42±9.25	42.47±9.39
Number of female/male	136 (67.3)/66 (32.7)	118 (66.3)/60 (33.7)	116 (62.7)/69 (37.3)	83 (60.6)/54 (39.4)
Age at onset (<40/≥40 yr)	152 (75.2)/50 (24.8)		137 (74.1)/48 (25.9)	
With/without family history	18 (8.9)/184 (91.1)		8 (4.3)/177 (95.7)	
Type of psoriasis (PV/other)	127 (69.3)/56 (30.7)		123 (66.5)/62 (33.5)	
With/without PsA	21 (10.4)/181 (89.6)		20 (10.8)/165 (89.2)	
With/without hypertension			21 (11.4)/164 (88.6)	11 (8.0)/126 (92.0)

Values are presented as mean±standard deviation or number (%). PV: psoriasis vulgaris, PsA: psoriatic arthritis.

Table 2. Genotype and allele frequencies (%) for the control, psoriasis and psoriatic clinical features groups

Gene	Control	Psoriasis	Family history		Age at onset (yr)		Type of psoriasis		PsA	
			Positive	Negative	<40	≥40	PV	Other	Positive	Negative
iNOS G-954C	178	202	18	184	152	50	127	75	21	181
Genotype										
GG	167 (93.8)	185 (91.6)	17 (91.3)	168 (91.3)	139 (91.4)	46 (92.0)	114 (89.8)	71 (94.7)	21 (100.0)	164 (90.6)
GC+CC	11 (6.2)	17 (8.4)	1 (8.7)	16 (8.7)	13 (8.6)	4 (8.0)	13 (10.2)	4 (5.3)	0 (0.0)	17 (9.4)
Allele										
C	12 (3.4)	22 (5.4)	1 (2.8)	21 (5.7)	17 (5.6)	5 (5.0)	17 (6.7)	5 (3.3)	0 (0.0)	22 (6.1)
G	344 (96.6)	382 (94.6)	35 (97.2)	347 (94.3)	287 (94.4)	95 (95.0)	237 (93.3)	145 (96.7)	42 (100.0)	340 (93.9)
eNOSG894T	146	176	8	177	137	48	123	62	20	165
Genotype										
GG	116 (79.5)	155 (88.0)	8 (100.0)	147 (83.0)	116 (84.7)	39 (81.3)	100 (81.3)	55 (88.7)	17 (85.0)	138 (83.6)
GT+TT	30 (20.5)	21 (12.0)	0 (0.0)	30 (17.0)	21 (15.3)	9 (18.7)	23 (18.7)	7 (11.3)	3 (15.0)	27 (16.4)
Allele										
T	21 (7.7)	31 (8.4)	0 (0.0)	31 (8.8)	22 (8.0)	9 (9.4)	24 (9.8)	7 (5.7)	3 (7.5)	28 (8.5)
G	253 (92.3)	339 (91.6)	16 (100.0)	323 (91.2)	252 (92.0)	87 (90.6)	222 (90.2)	117 (94.3)	37 (92.5)	302 (91.5)

Values are presented as number only or number (%). PV: psoriasis vulgaris, PsA: psoriatic arthritis.

eNOS G894T was amplified with the primers 5'-CATGAGGCTCAGCCCCAGAAC-3' (forward) and 5'-GTCAATCCCTTTGGTGCTCAC-3' (reverse), as previously reported[7,8]. The lengths of the amplified PCR products for *iNOS G-954C* and *eNOS G894T* polymorphisms were 680 bp and 206 bp, respectively. We used the restriction enzymes *BsaI* and *MboI* (New England Biolabs, Beverly, MA, USA) to delineate *iNOS G-954C* and *eNOS G894T* polymorphisms, respectively. Cleavage by these enzymes resulted in 490-bp and 190-bp fragments for the *iNOS G-954C* G allele and 119-bp and 87-bp fragments for the *eNOS G894T* C allele.

Statistical analyses were performed using SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA). Results are presented as mean±standard deviation. The proportions of the alleles and genotypes between patients and controls were compared using the chi-square test. A two-tailed *p*-value of 0.05 or less was considered statistically significant.

Baseline characteristics of patients and controls are summarized in Table 1. The results of the genotype and allele frequencies are presented in Table 2. No significant differences in the genotype and allele distributions were observed between patients and controls. Similarly, there were no significant differences according to the age of onset, the clinical types of psoriasis, family history, and joint involvement. In addition, patients with or without hypertension are no associations with the *eNOS G894T* polymorphism (data not shown).

To the best of our knowledge, this study is the only case-control study to date to investigate *iNOS G-954C* polymorphisms in psoriasis, although these gene polymorphisms have been already been implicated in diseases such as rheumatoid arthritis, diabetes[5]. However, our present data did not support a relationship between this gene polymorphism and susceptibility to psoriasis in the Han Chinese population. Further, our stratification analysis of clinical features showed that none of the phenotypes of psoriasis were associated with the *iNOS G-954C* polymorphism.

In 2006, Senturk et al.[8] were the first to report that the *eNOS G894T* polymorphism is implicated in Turkish patients with psoriasis However, Coto-Segura et al.[9] have shown contradictory results in Spanish patients. Considering the above differing conclusions, we aimed to assess the association between the *eNOS G894T* polymorphism and psoriasis in the Han Chinese population. Our study showed that there was no significant correlation between the gene polymorphism and psoriasis susceptibility, contrary to the reports of Senturk et al.[8], which indicated that the association may vary with the patients such as ethnicities, regions, clinical features. Moreover, in our study, none of the phenotypes of psoriasis were associated with the *eNOS G894T* polymorphism, similar to the findings for *iNOS G-954C*.

There is accumulating evidence that hypertension is common among patients with psoriasis, and the *eNOS G894T* polymorphism has been associated with hypertension[5,6]. Although our study indicated that the *eNOS G894T* polymorphism had no association with psoriasis accompanying hypertension, the possible involvement of other eNOS loci cannot be excluded.

Thus, our study may be useful in further understanding the correlation between psoriasis and NOS gene polymorphisms. However, our study has some limitations. Since the study was conducted in a single population and with a limited number of participants, it requires further confirmation in larger groups and populations.

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