Assessment of the Role of NO Synthase Genes Polymorphisms in the Pathogenesis of Psoriasis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EK, AT, IK and VS designed the study. Authors EK, AS and VS managed the literature searches. Authors LS and IK supplied the patients' samples. Authors AT, EG and ZK carried out all laboratories work and performed the statistical analysis. Authors EK, AT and VS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: Psoriasis is a common (affecting 2% of the general population) chronic inflammatory skin disease characterized by impaired keratinocyte differentiation and proliferation. Nitric oxide (NO) is one of the most potent vasodilators synthesized in large quantities at the early stages of wound response, as well as in inflammation. Psoriasis has been shown to be associated with increased NO content in pathological tissues. The objective of this study was to analyze two single-nucleotide

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substitutions in the *NOS2* (rs2779249, c.-1290G>T, 5'-region) and *NOS3* (rs2070744, c.-813C>T, intron 1) genes.

Methodology: The study was performed on DNA samples obtained from 88 patients diagnosed with psoriasis and 365 control samples (population-based control). Real-time PCR with allele-specific probes for genotyping was used.

Results: No associations with the disease were observed for the evaluated substitutions (P > 0.05).

Conclusion: No associations of *NOS2* (rs2779249) and *NOS3* (rs2070744) with disease were observed. It is expected that their effect will be more noticeable in combination with other substitutions in these genes or a larger sample. It is also possible that overactive regulation of NO synthase expression in psoriasis is independent of the studied gene polymorphisms.

1. INTRODUCTION

Psoriasis is a common (affecting 2% of the general population [1]) chronic inflammatory skin disease, and, although the nature of this disease has not been fully elucidated, convincing evidence is available showing that genetic predisposition and immunological factors play an essential role in the development of this condition [2]. The development and course of the disease are also affected by infections, stress, and psychological trauma [3].

Impaired keratinocyte differentiation and proliferation characterize psoriasis as a result of an autoimmune reaction of T-lymphocytes and macrophages against skin cells. The DNA of degrading keratinocytes is an antigen that causes inflammation, angiogenesis, and proliferation of immunocompetent cells. Dermal dendritic cells present the antigen to T-helper (Th0) lymphocytes and stimulate proliferation of T-killer cells and Type 1 T-helpers, which secrete various chemical signals, cytokines: tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, interferon-γ and IL-17, which cause inflammation, and IL-22 responsible for activated proliferation of keratinocytes and impairment of their normal maturation and differentiation. Responding to these cytokines, keratinocytes produce IL-1, IL-6, and TNF-α, which are responsible for the chemotaxis of new immune cells to the site of inflammation, increased proliferation of these cells, and further development and aggravation of the inflammatory response. Immature keratinocytes that die as a result of apoptosis release more DNA, thus further stimulating the dendritic cells. It is believed that these immunopathological reactions are caused by a defect in regulatory suppressor T-cells and by abnormal secretion or function of the regulatory, anti-inflammatory cytokine IL-10 [4].

Psoriasis has an extremely complex genetic basis. In the last five years, genome-wide association study (GWAS) has revealed numerous loci, regions potentially predisposing to psoriasis [5]. Potential responsible genes can be conditionally divided into the following groups [6]:

1. those responsible for the impairment of skin barrier function;
2. genes participating in the IL-23-signaling pathway responsible for adaptive immunity;
3. genes participating in the signaling pathway of the nuclear factor NF -κB and interferon, and in the IL-17 production by cells responsible for congenital immunity;
4. genes involved in the antigen presentation.

Population- and family-based studies also demonstrate a genetic predisposition to psoriasis, as the risk of this disease is higher in relations of patients suffering from psoriasis compared with the general population and in monozygotic twins compared with dizygotic ones (20% - 73% vs. 9% - 20%, respectively, depending on the studied population) [3]. Some evidence shows an effect of epigenetic factors (DNA methylation, histone modification, and microRNA regulation) on the pathogenesis [7].

Nitric oxide (NO) is one of the most potent vasodilators. It is synthesized in large quantities at the early stages of wound response, as well as in inflammation. The function of NO is to produce local vasodilation in tissue damage and inflammation, thus promoting the attraction of cells (including those involved in the immune response) and initiation of inflammation. NO has no receptors, interacting directly with the soluble guanylate cyclase of the vascular smooth muscle

cell, thus leading to activation of the vasodilation signaling pathway. The NO synthase family consists of 3 proteins: neuronal, inducible, and endothelial NO synthases encoded by the *NOS1*, *NOS2*, and *NOS3* genes, respectively.

Psoriasis has been shown to be associated with increased NO content in pathological tissues [8, 9] as well as increased expression of NOS2 in psoriatic skin [10]. As a result, the inflammatory process in an affected skin is continuously maintained. NO is also a potent regulator of the differentiation and proliferation of keratinocytes, cells involved in the pathogenesis of psoriasis.

The objective of this study was to analyze two single-nucleotide variants (SNV) in the *NOS2* (rs2779249, c.-1290G>T, 5'-region) and *NOS3* (rs2070744, c.-813C>T, intron 1) genes using real-time PCR with allele-specific probes.

2. PATIENTS AND METHODS

2.1 Study Subjects

Blood samples were obtained from patients with psoriasis at the City Clinical Hospital No. 14 named after V. G. Korolenko (2013-2016). Patients with a diagnosis of psoriasis residing in Moscow or Moscow suburbs were selected in accordance with the International Classification of Diseases (L-40). The study sample consisted of 88 patients. DNA samples isolated from the whole blood of non-evaluated Moscow or Moscow Region patients were used as controls in this study. The control sample consisted of 365 subjects (population-based control). Blood samples were collected at a Moscow blood transfusion station. All patients enrolled in this study were familiarized with the objectives of the study and signed the Informed Consent Form. The study was conducted in accordance with the

ethical standards laid down in the Declaration of Helsinki. A qualified phlebologist collected the blood samples.

2.2 Molecular Genetic Analysis

DNA was isolated from whole blood using a Magna™ DNA Prep 200 kit (Izogen Lab, Russia). The DNA isolation procedure was conducted as prescribed by the manufacturer, using special magnetic stands. A 200 μL sample of whole blood was used for the isolation.

The fluorescent probes (TaqMan probes) and allele-specific primers for the evaluated variants were synthesized by DNA Synthesis LLC (Moscow). The fluorescent dyes VIC and FAM and the quencher BHQ1 were used in the manufacture of labeled primers for the TaqMan probes. The nucleotide sequences of the primers are presented in Table 1.

The qPCRmix-HS reagent mixture (Evrogen, Russia) was used for real-time PCR. The mixture includes all components necessary for the assay: high-fidelity Taq DNA polymerase, nucleotide triphosphate mixture, Mg^{2+}, PCR buffer. Duplicate genotyping with the use of negative control samples was performed. Each reagent mixture contained 20 pmol of each of the two (direct and reverse) primers and 20 pmol of the fluorescent probes. Real-time PCR was performed using a CFX-96 kit (Bio-Rad, USA) in accordance with the manufacturer's Instructions for Use.

2.3 Statistical Analyses

Statistical analysis of obtained data included the use of the Pearson test (chi-square, X^2). Complex genotypes associated with the disease were found with the help of the polygenic data software APSampler v.3.6.0.1 (https://sourceforge.net/projects/apsampler/).

Table 1. Sequences of the primer and probe pairs employed in the study (T_{ann} is the primer / probe annealing temperature)

Sequence of primers and probes	T_{ann},°C	Gene (SNV)	Amplicon length (bp)
F: ACCAGGGCATCAAGCTCTTC R: GCAGGTCAGCAGAGAGACTAG C: VIC-AGGGTCAGCCGGCCAG-BHQ1 T: FAM-AGGGTCAGCCAGCCAG-BHQ1	55	*NOS3* (rs2070744)	67
F: GCCTCTCAAAGTGCTAGGATTACAA R: GGGAATACTGTATTTCAGGCATTATAAGGA T: VIC-TAGCCACAATGCCCG-BHQ1 G: FAM-TAGCCACCATGCCCG-BHQ1	56	*NOS2* (rs2779249)	88

Table 2. Frequencies of the alleles and genotypes of the examined genes

NOS2	case	control	NOS3	case	control
Allele T	0.330	0.294	Allele T	0.420	0.450
Allele G	0.670	0.706	Allele C	0,580	0.550
Genotype TT	0.091	0.080	Genotype TT	0,000	0.003
Genotype TG	0.477	0.427	Genotype TC	0.841	0.894
Genotype GG	0.432	0.493	Genotype CC	0.159	0.103

3. RESULTS AND DISCUSSION

We have chosen SNVs in *NOS2* (rs2779249) and *NOS3* (rs2070744) genes as subject of our study. The T allele of the rs2779249 substitution in the *NOS2* gene results in a fivefold increase in the NOS2 transcriptional activity compared with the G allele [11]. The C allele of the rs2070744 substitution in the *NOS3* gene decreases the promoter activity of the *NOS3* gene [12]. Therefore, the combination of the T and C alleles of the *NOS2* and *NOS3* genes, respectively, should result in increased NO synthase expression. Thus, both studied SNVs affect transcriptional activity of *NOS2* and *NOS3* genes.

The molecular genetic analysis yielded frequencies of the genotypes of the evaluated variants. The obtained allele and genotype frequencies are presented in Table 2.

No associations with the disease were found for the evaluated SNV: *NOS3* (rs2070744) – X^2 = 0.487, P = 0.485 and *NOS2* (rs2779249) – X^2 = 0.859, P = 0.354. We also detected a significant deviation in the variant genotype frequencies from the Hardy-Weinberg equilibrium for the NOS3 gene: main group – X^2 = 46.32 (P = 0), control group – X^2 = 225.86 (P = 0).

The search for complex genotypes did not reveal any allele / genotype combinations associated with psoriasis.

There have been numerous reports of excessive nitric oxide production in affected skin [13,14], and the role of NO in the proliferation of keratinocytes has been described [15,16]. Expression of inducible nitric oxide synthase (NOS2) is increased in skin affected by psoriasis [17,18]. Also NOS2 and NOS3 can be drug targets for psoriasis [19,20].

The *NOS3* (rs2070744) C allele has been shown to be reliably associated with psoriasis, affecting the plasma NO concentration [1]. Other authors have reported an association between another *NOS3* gene polymorphism and psoriasis [21,22]. The *NOS2* gene (substitution rs4795067) has been found to be associated with psoriasis in a genome-wide association study [23]. No associations between these SNVs and psoriasis were found in patients with psoriasis from Moscow region.

4. CONCLUSION

We did not observe any significant associations between the evaluated variants and psoriasis. Their effects may become more pronounced when combined with other substitutions in these genes or a larger sample. Another possibility is that psoriasis is associated with signaling pathway abnormalities in affected tissues, which result in overactive regulation of NO synthase expression independently of the presence of the studied gene polymorphisms.

CONSENT

This research used de-identified administrative data obtained from the City Clinical Hospital No. 14 named after V. G. Korolenko; informed consent was not required.

ETHICAL APPROVAL

The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Coto-Segura P, Coto E, Mas-Vidal A, Morales B, Alvarez V, Díaz M, et al. Influence of endothelial nitric oxide

synthase polymorphisms in psoriasis risk. Archives of Dermatological Research. 2011;303(6):445-49.
DOI:10.1007/s00403-011-1129-9

2. Rieder E, Tausk F. Psoriasis, a model of dermatologic psychosomatic disease: Psychiatric implications and treatments. International Journal of Dermatology. 2012;51:12-26.
DOI:10.1111/j.1365-4632.2011.05071.x

3. Meglio Di, Villanova P, Nestle F, Psoriasis FO. Cold Spring Harbor Perspectives in Medicine. 2014;4(8):1-30.
DOI:10.1101/cshperspect.a015354

4. Bolevich SB, Urazalina AA. Psoriasis: Modern view at aetiopathogenesis. Vestnik rossijskoj voenno-medicinskoj akademii. 2013;2(42):202-6.

5. Ryan C, Korman NJ, Gelfand JM, Lim HW, Elmets CA, Feldman SR, et al. Research gaps in psoriasis: Opportunities for future studies. Journal of the American Academy of Dermatology. 2014;70(1):146-67.
DOI:10.1016/j.jaad.2013.08.042

6. Mineeva AA, Kozhushnaya OS, Volnukhin VA, Frigo NV, Znamenskaya LF, Kubanov AA, et al. Study of the genetic factors predisposing to the development of psoriasis. Vestnik dermatologii i venerologii. 2012;3:30-8.

7. Chandra A, Ray A, Senapati S, Chatterjee R. Genetic and epigenetic basis of psoriasis pathogenesis. Molecular Immunology. 2015;64(2):313-23.
DOI: 10.1016/j.molimm.2014.12.014

8. Li Y, Zhang G, Xiao R, Chen H, Wen H. Expression of NOS2 and HIF-1alpha with angiogenesis in affected skin biopsies from patients with psoriasis. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2010;35(9): 952-7.
DOI: 10.3969/j.issn.1672-347.2010.09.009

9. Sherratt JA, Weller R, Savill NJ. Modelling blood flow regulation by nitric oxide in psoriatic plaques. Bull Math Biol. 2002;64(4):623-41.
DOI: 10.1006/bulm.2001.0271

10. Dilek N, Dilek AR, Taskin Y, Erkinuresin T, Yalcin O, Saral Y. Contribution of myeloperoxidase and inducible nitric oxide synthase to pathogenesis of psoriasis. Postepy Dermatol Alergol. 2016;33(6): 435-439.
DOI: 10.5114/ada.2016.63882

11. Fu L, Zhao Y, Lu J, Shi J, Li C, Liu H, et al. Functional single nucleotide polymorphism-1026C/A of inducible nitric oxide synthase gene with increased YY1-binding affinity is associated with hypertension in a Chinese Han population. J Hypertens. 2009;27(5):991-1000.
DOI: 10.1097/HJH.0b013e3283294bec

12. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, et al. T-786-->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation. 1999;99(22):2864-70.
DOI:10.1161/01.CIR.99.22.2864

13. Bruch-Gerharz D, Schnorr O, Suschek C, Beck KF, Pfeilschifter J, Ruzicka T, et al. Arginase 1 overexpression in psoriasis: Limitation of inducible nitric oxide synthase activity as a molecular mechanism for keratinocyte hyperproliferation. Am J Pathol. 2003;162(1):203-11.
DOI:10.1016/S0002-9440(10)63811-4

14. Gokhale NR, Belgaumkar VA, Pandit DP, Deshpande S, Damle DK. A study of serum nitric oxide levels in psoriasis. Indian J Dermatol Venereol Leprol. 2005;71(3):175-8.
DOI: 10.4103/0378-6323.16232

15. Trepicchio WL, Ozawa M, Walters IB, Kikuchi T, Gilleaudeau P, Bliss JL, et al. Interleukin-11 therapy selectively downregulates type I cytokine proin-flammatory pathways in psoriasis lesions. J Clin Invest. 1999;104(11):1527-37.
DOI: 10.1172/JCI6910

16. Zalewska A, Wyczółkowska J, Narbutt J, Sysa-Jedrzejowska A. Nitric oxide levels in plasma and fibroblast cultures of psoriasis vulgaris patients. J Dermatol Sci. 2007; 48(3):237-40.
DOI: 10.1016/j.jdermsci.2007.06.012

17. Schwentker A, Billiar TR. Nitric oxide and wound repair. Surg Clin North Am. 2003;83(3):521-30.
DOI: 10.1016/S0039-6109(02)00207-4

18. Simonetti O, Lucarini G, Campanati A, Goteri G, Zizzi A, Marconi B, et al. VEGF, survivin and NOS overexpression in psoriatic skin: Critical role of nitric oxide synthases. J Dermatol Sci. 2009; 54(3):205-8.
DOI: 10.1016/j.jdermsci.2008.12.012

19. Gu J, Li L, Wang D, Zhu W, Han L, Zhao R, et al. Deciphering metabonomics biomarkers-targets interactions for psoriasisvulgaris by network pharmacology. Annals of Medicine. 2018;1-10.
    DOI: 10.1080/07853890.2018.1453169

20. Tampa M, Sarbu MI, Mitran MI, Mitran CI, Matei C, Georgescu SR. The pathophysiological mechanisms and the quest for biomarkers in psoriasis, a stress-related skin disease. Dis Markers. 2018;5823684.
    DOI: 10.1155/2018/5823684.

21. Senturk N, Kara N, Aydin F, Gunes S, Yuksel EP, Canturk T, et al. Association of eNOS gene polymorphism (Glu298Asp) with psoriasis. J Dermatol Sci. 2006; 44(1):52-5.
    DOI:10.1016/j.jdermsci.2006.05.011

22. Agha Z, Shaiq PA, Ahmed S, Ali L, Azam M, Ali SHB, Kaukab G, Qamar R. A study of ACE, eNOS and MTHFR association with psoriasis in Pakistani population. Meta Gene. 2018;15:65-69.
    DOI: 10.1016/j.mgene.2017.12.001

23. Stuart PE, Nair RP, Ellinghaus E, Ding J, Tejasvi T, Gudjonsson JE, et al. Genome-wide association analysis identifies three psoriasis susceptibility loci. Nat Genet. 2010;42(11):1000-4.
    DOI:10.1038/ng.693