TLR2 and TLR4 Polymorphisms Are Not Associated with Acne Vulgaris

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Key Words

Acne vulgaris · Toll-like receptors · Single-nucleotide polymorphism

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit affecting most teenagers and numerous adults. For most patients, acne is limited to occasional flares of comedones and pustules; however in severe cases, host inflammatory response can result in painful nodules and disfiguring scars. Acne is a multifactorial disease; traditional etiological factors include increased sebum production, ductal hyperkeratosis, colonization with *Propionibacterium acnes* and production of inflammatory mediators, but, according to twin studies, genetic factors also contribute to the development of the symptoms [1–4].

In the case of multifactorial diseases, the presence of several predisposing gene alterations assists the environmental factors in eliciting the symptoms. Up to now only a few suspected genes have been investigated in connection with acne susceptibility [5–8]. Toll-like receptors (TLRs) are expressed on the surface of several immune cells, and they are responsible for the recognition of mi-

crobial molecular patterns. Since TLR2 and TLR4 affect immune processes, they may play a role in the pathogenesis of acne. TLR2 and TLR4 expression was found to be increased in the epidermis of acne lesions in vivo [9]; furthermore, TLR2 was expressed on the cell surface of macrophages surrounding pilosebaceous follicles, and *P. acnes* induced the cytokine production of monocytes through a TLR2-dependent pathway [10].

Here we have investigated two mutations of the *TLR2* gene (C2179T in the NM_003264.2 sequence and rs5743708 causing the amino acid changes Arg677Trp and Arg753Gln, respectively) as well as two polymorphisms of the *TLR4* gene (rs4986790 and rs4986791 causing the amino acid changes Asp299Gly and Thr399Ile, respectively) in 101 Caucasian subjects, 63 with acne vulgaris and 38 healthy controls. These polymorphisms have been suggested to be associated with several infectious diseases [11]. Acne vulgaris diagnosis was defined as a physician's diagnosis. Acne patients were subclassified into three groups, as having severe symptoms (acne conglobata group, n = 17), medium symptoms (acne papulopustulosa group, n = 39) and mild symptoms (acne comedonica group, n = 7). The control group comprised patients showing no symptoms of acne during their life.

We isolated genomic DNA from whole blood obtained from the study subjects, using the Perfect gDNA Blood Mini Isolation Kit (Eppendorf AG, Hamburg, Germany)

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Table 1. Allele frequencies of the observed polymorphisms (rs4986790 and rs4986791) in the *TLR4* gene causing the amino acid changes Asp299Gly and Thr399Ile, respectively

	Acne vulgaris (n = 63)	Control (n = 38)	USA ¹ (n = 47)
<i>TLR4</i> Asp299Gly			
A	0.952	0.934	0.904
G	0.048	0.066	0.096
<i>TLR4</i> Thr399Ile			
C	0.952	0.934	0.947
T	0.048	0.066	0.053

¹ From the database of the IIPGA, www.innateimmunity.net.

and the Generation Capture Column Kit (Gentra Systems Inc., Minneapolis, Minn., USA) according to the manufacturer's instructions. Informed consent was obtained from each individual before sample collection, and the study was conducted according to the Helsinki Declarations. Primer sets were designed by Primer 3 software at <http://frodo.wi.mit.edu/cgi-bin/primer3> such that products did not exceed 300 bp and synthesized by the Oligonucleotide Laboratory, Biological Research Center of the Hungarian Academy of Sciences (Szeged, Hungary). PCR was performed in a total volume of 50 µl containing 200 ng of template DNA, 1–1 µl of 10 pmol/µl primers and 25 µl REDTaq ReadyMix PCR Reaction Mix (Sigma-Aldrich Co., St. Louis, Mo., USA). The cycling profile involved denaturation at 95°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 1 min for 40 cycles. Final extension was continued at 72°C for 10 min. The amplification was carried out in a MyCycler Thermal System (Bio-Rad, Hercules, Calif., USA). PCR products were purified by Quantum Prep PCR Kleen Spin Columns (Bio-Rad) according to the manufacturer's instructions. The alleles of TLRs were confirmed by sequencing (Agricultural Biotechnology Center, Godollo, Hungary) and analyzed by the Bio Edit program (Ibis Therapeutics, Carlsbad, Calif., USA). Statistical analysis was performed at the statistical homepage faculty.vassar.edu/lowry/VassarStats.html. Allele frequency data were compared using Fisher's exact probability test; a p value less than 0.05 was considered significant.

Allele frequencies of the studied single-nucleotide polymorphisms of the *TLR4* gene causing the amino acid changes Asp299Gly and Thr399Ile in the acne vulgaris and control group are shown in table 1. The two studied polymorphisms of the *TLR4* gene occurred simultane-

Table 2. Allele frequencies of the observed mutations (C2179T in the NM_003264.2 sequence and rs5743708) in the *TLR2* gene causing the amino acid changes Arg677Trp and Arg753Gln, respectively

	Acne vulgaris (n = 63)	Control (n = 38)	USA ¹ (n = 89)
<i>TLR2</i> Arg677Trp			
	SNP not detected	SNP not detected	
<i>TLR2</i> Arg753Gln			
G	SNP not detected	SNP not detected	0.977
A	SNP not detected	SNP not detected	0.023

¹ From the database of the IIPGA, www.innateimmunity.net.

ously (n = 11) composing a haplotype. Furthermore, the individuals carrying these alleles were all heterozygotes for both *TLR4* single-nucleotide polymorphisms in our study population. These findings correspond with the data in the literature [12, 13]. There were no statistical differences in the prevalence of these two polymorphisms between the control and acne vulgaris groups, even if the acne vulgaris group was divided into subclasses according to the severity of symptoms. The frequencies of the observed alleles were in good agreement with data from the database of the National Heart, Lung and Blood Institute, Programs for Genomic Applications (IIPGA, www.innateimmunity.net).

The studied mutations of the *TLR2* gene were not detected in our study population (table 2). The mutation causing the Arg753Gln amino acid change has a low prevalence according to the databases as well. Furthermore, at the time we had completed the sequencing of our samples, Malhotra et al. [14] demonstrated that the mutation causing the Arg677Trp change, which had been published in 2001 [15], was not a true polymorphism, but a sequencing error.

Our recent data have shown that certain *P. acnes* strains are capable of activating the innate immune responses in keratinocytes, and this induction is TLR2 and TLR4 dependent [16]. Beside keratinocytes, sebocytes and professional immune cells, e.g. macrophages, also express TLRs, through which *P. acnes* can contribute to inflammation at the site of acne lesions [10, 17]. Although the polymorphisms and mutations of *TLR2* and *TLR4* genes could be hypothesized to contribute to the pathogenesis of acne, the single-nucleotide polymorphisms studied here were not proved to assist in the development of acne. The fact that this study could not find any asso-

ciations between acne and the studied *TLR2* and *TLR4* polymorphisms does not exclude the possibility that other polymorphisms of these genes would contribute to acne pathogenesis. Further studies are needed to decide whether additional *TLR2* and *TLR4* polymorphisms are associated with acne and/or the reported upregulation of these genes in acne [9] is the result of upstream regulatory events.

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References

- 1 Zouboulis CC, Eady A, Philpott M, Goldsmith LA, Orfanos C, Cunliffe WC, Rosenfield R: What is the pathogenesis of acne? *Exp Dermatol* 2005;14:143–152.
- 2 Koreck A, Pivarcsi A, Dobozy A, Kemeny L: The role of innate immunity in the pathogenesis of acne. *Dermatology* 2003;206:96–105.
- 3 Oberemok SS, Shalita AR: Acne vulgaris. I. Pathogenesis and diagnosis. *Cutis* 2002;70:101–105.
- 4 Evans DM, Kirk KM, Nyholt DR, Novac C, Martin NG: Teenage acne is influenced by genetic factors. *Br J Dermatol* 2005;152:579–581.
- 5 Ostlere LS, Rumsby G, Holownia P, Jacobs HS, Rustin MH, Honour JW: Carrier status for steroid 21-hydroxylase deficiency is only one factor in the variable phenotype of acne. *Clin Endocrinol (Oxf)* 1998;48:209–215.
- 6 Sawaya ME, Shalita AR: Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne. *J Cutan Med Surg* 1998;3:9–15.
- 7 Paraskevaidis A, Drakoulis N, Roots I, Orfanos CE, Zouboulis CC: Polymorphisms in the human cytochrome P-450 1A1 gene (CYP1A1) as a factor for developing acne. *Dermatology* 1998;196:171–175.
- 8 Ando I, Kukita A, Soma G, Hino H: A large number of tandem repeats in the polymorphic epithelial mucin gene is associated with severe acne. *J Dermatol* 1998;25:150–152.
- 9 Jugeau S, Tenaud I, Knol AC, Jarrousse V, Quereux G, Khammari A, Dreno B: Induction of toll-like receptors by *Propionibacterium acnes*. *Br J Dermatol* 2005;153:1105–1113.
- 10 Kim J, Ochoa MT, Krutzik SR, Takeuchi O, Uematsu S, Legaspi AJ, Brightbill HD, Holland D, Cunliffe WJ, Akira S, Sieling PA, Gdowski PJ, Modlin RL: Activation of toll-like receptor 2 in acne triggers inflammatory cytokine responses. *J Immunol* 2002;169:1535–1541.
- 11 Schroder NW, Schumann RR: Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis* 2005;5:156–164.
- 12 Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA: *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;25:187–191.
- 13 Schippers EF, van't Veer C, van Voorden S, Martina CA, le Cessie S, van Dissel JT: TNF-alpha promoter, Nod2 and toll-like receptor-4 polymorphisms and the in vivo and ex vivo response to endotoxin. *Cytokine* 2004;26:16–24.
- 14 Malhotra D, Relhan V, Reddy BS, Bamezai R: *TLR2* Arg677Trp polymorphism in leprosy: revisited. *Hum Genet* 2005;116:413–415.
- 15 Kang TJ, Chae GT: Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol* 2001;31:53–58.
- 16 Nagy I, Pivarcsi A, Koreck A, Szell M, Urban E, Kemeny L: Distinct strains of *Propionibacterium acnes* induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. *J Invest Dermatol* 2005;124:931–938.
- 17 Kim J: Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. *Dermatology* 2005;211:193–198.