Acne Vulgaris is Associated with the Human β-Defensin 1-Gene Polymorphisms in Han Chinese Ethnic Group Patients

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Objective: To study the relationship between the single nucleotide polymorphisms (SNPs) of the human β-defensin 1-gene (DEFB1) and the genetic susceptibility of acne vulgaris in the Han Chinese ethnic group.

Methods: A total of 104 patients with acne vulgaris and 126 healthy participants were included in our study. We analyzed the association between acne vulgaris and the polymorphisms in the DEFB1 G-52A, C-44G, and G-20A gene. We then analyzed the relationship between the different genotypes and the susceptibility to acne vulgaris.

Results: The frequency of DEFB1 C-44G genetic polymorphisms between the acne vulgaris group and the control group was significantly different (P < 0.05). The frequency of DEFB1 G-20A genetic polymorphisms between the acne vulgaris group and the control group was also significantly different (P < 0.05).

Conclusion: The −44G or −20A allele showed a low expression in acne vulgaris, which has already been shown to correlate with the low risk of acne vulgaris among Chinese Han patients. This further supports the contribution of the DEFB1 gene to the pathogenesis of acne.

Keywords: human β-defensin 1, genetic polymorphisms, acne vulgaris, Cutibacterium acnes, Han Chinese

Introduction

Acne is a painful and disfiguring disease that leaves some individuals with permanent physical and psychological scars.1,2 The pathogenesis of acne vulgaris is linked to multiple factors. One of the factors that contribute to the pathogenesis of acne is Cutibacterium acnes.3 Inflammatory acne results from the action of Cutibacterium acnes, which metabolizes sebaceous triglycerides, consumes glycerol, and releases free fatty acids, neutrophil, and complement attractants.4 Cutibacterium acnes contributes to the inflammatory nature of acne by inducing monocytes to secrete pro-inflammatory cytokines.5 Moreover, Cutibacterium acnes trigger antimicrobial peptide and cytokine secretion of keratinocytes in vitro.

Antimicrobial peptides could protect interfaces from infection with pathogenic microorganisms. In human skin, antimicrobial peptides are produced mainly by keratinocytes, neutrophils, sebocytes, or sweat glands. In some skin diseases, there is an inverse correlation between the severity of the disease and the level of antimicrobial peptide production.6

The human β-defensins (hBDs) are found primarily in epithelial cells and numerous sites throughout the body.7,8 DEFB1 is generally transcribed at a constitutive low level in
epithelial cells. However, this transcription is induced by a variety of factors, including microbes and cytokines. There is increasing evidence that pro-inflammatory cytokines (such as interleukin-1β and tumor necrosis factor-α) and bacterial lipopolysaccharides can increase regulation in the human β-defensins. The DEFB1 gene is one of the main antimicrobial peptides that play a central role in the pathogenesis of acne. Therefore, the many factors that induce human β-defensins transcription play a complex role for these peptides in inflammatory responses and innate immunity, such as acne.

By genotype analysis of the single nucleotide polymorphisms and controls in the DEFB1 promoter region of Chinese Han patients with acne, this paper aims to explore the correlation between mononucleotide polymorphism in the DEFB1 promoter region and susceptibility and prognosis of these patients to provide an early warning mechanism for acne. At the same time, it provides the basis for the formulation of early individualized treatment plans and the development of defense-related drugs for patients with acne. This study aims to assess whether the three single nucleotide polymorphisms (SNPs), located in the 5′-untranslated region (UTR) of DEFB1 G-52A, C-44G, and G-20A (rs1799946, rs1800972, and rs11362, respectively) are related to acne vulgaris in a sample of patients in the Han Chinese ethnic group.

Materials and Methods

Subjects

Patients belonging to the Han Chinese ethnic group were recruited between April 2017 and May 2019 at the Wuhan No.1 Hospital (China). A total of 104 Han Chinese patients (41 males and 63 females) with acne vulgaris served as the acne vulgaris group, and 126 healthy subjects (63 males and 63 females) served as the control group (Figure 1). These patients had an average age of 24.96 ± 5.78 and a course of 1 ~ 23 years, with an average treatment time of 8.37 ± 5.3 years. The healthy control group of 126 Han Chinese subjects was randomly selected. They were intern students and medical staff who had never suffered from acne in our hospital, aged 20 ~ 38 years, with an average age of 23.56 ± 3.22. There was no statistically significant difference in age composition between the patient group and the healthy control group (P > 0.05).

According to the clinical classification standard for acne, 104 patients with acne were divided into three types, namely 32 cases of mild acne, 42 cases of moderate acne, and 30 cases of severe acne. The ethics committee of our hospital approved this study. All participants signed written informed consent.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) patients with acne vulgaris; (2) patients older than 18; (3) patients who were Han Chinese. Exclusion criteria: (1) patients with polycystic ovary syndrome, diabetes, hyperthyroidism, or thyropernia; (2) patients with androgen-related diseases; (3) patients with infectious diseases; (4) patients with occupational acne or pharmacy acne tetter; (7) patients who used tretinoin or hormone within two months before enrollment into this study.

Genomic DNA Isolation

Genomic DNA was extracted from the peripheral blood leukocytes collected from the patients using the improved NaI method. The DNA was stored at −20 °C.

Figure 1 Detection of human β-defensin-1 gene variations in the 5′-UTR (G-52A, C-44G, and G-20A) by restriction enzyme digestion with NaIV, Hgal, ScrFI, respectively, followed by 3% agarose gel electrophoresis. Lanes 1 AA genotype, lanes 2 GA genotype, lanes 3 GG genotype of SNP G-52A; lanes 4 CC genotype, lanes 5 CG genotype, lanes 6 GG genotype of SNP C-44G; and lanes 7 AA genotype, lanes 8 GA genotype, lanes 9 GG genotype of SNP G-20A. B is the blank comparison.

Abbreviation: M, maker.
**Table 1** Allele Distribution at the DEFBI Locus in Acne Vulgaris and Control Groups (Numbers with Percentages in Parentheses)

| Group                  | C-44G |                  | G-20A                  |
|------------------------|-------|------------------|------------------------|
|                        | C     | G                | A                      |
| Acne vulgaris group    |       |                  |                        |
| Mild-acne subgroup     | 189   | (90.87)          | 19 (9.13)              |
| Moderate-acne subgroup | 54    | (84.37)          | 10 (15.63)             |
| Severe-acne subgroup   | 78    | (92.86)          | 6 (7.14)               |
| Control group          | 57    | (95)             | 3 (5)                  |

**PCR Amplification**

The primer sequence was as follows: forward primer 5'-CTT GAC TGG GGC ACC TCC CTT CAG-3' and reverse primer 5'-CCC CCC TGG GGA TGG GAA ACT C-3'.

PCR was performed in a reaction mixture consisting of 60 ng of genomic DNA, 0.4 mM of each primer, 2.5 mM MgCl2, 200 μM of each dNTP, 3 μL of 10×PCR Gold buffer, and 1 μL of AmpliTaq Gold DNA polymerase adjusted to 30 μL with water. This PCR program consisted of denaturing at 95 °C for ten minutes followed by 30 cycles of denaturing at 95°C for 60 seconds, annealing at 66 °C for 60 seconds and extension at 72 °C for 60 seconds, and a final extension for ten minutes at 72 °C.

**Statistical Method**

This study used the software program SPSS 13.0. Continuous variables were expressed as mean ± SD. Hardy–Weinberg equilibrium analyses were performed to compare observed and expected genotype frequencies by using the X² test. Discontinuous variables were expressed as a percentage (%). For two comparisons, each value was compared by a t-test when each datum conformed to a normal distribution, while the non-normally distributed continuous data were compared using non-parametric tests. The counting data were tested by Chi-square tests. A value of P < 0.05 was considered statistically significant.

**Results**

**The General Data**

The acne vulgaris group included 104 patients with acne vulgaris (41 males and 63 females). The control group included 126 healthy subjects (63 males and 63 females). The control group was matched in age with the patient group.

**The Electrophoretogram of DEFBI After Restriction Enzyme Digestion**

The human β-defensin-1 gene variations were detected in the 5’-UTR by restriction enzyme digestion with NaeI, HhaI, ScrFI, respectively, followed by 3% agarose gel electrophoresis. The sizes of expected restriction fragments were as follows: for DEFBI G-52A [268 bp (AA), 153 + 115 bp (GG)], for DEFBI C-44G [268 bp (CC), 30 + 79 + 159 bp (GG)], and for DEFBI G-20A [268 bp (AA), 143 + 125 bp (GG)] (Figure 1).

**Genotype and Allele Frequencies of DEFBI SNPs**

The distribution of genotypes in each group was in Hardy–Weinberg equilibrium.

As shown in Table 1, for the genotype frequency comparison of DEFBI G-52A, AA, there is no significant difference between the acne vulgaris group and the control group (χ² = 1.154; P = 0.283 > 0.05). For the genotype frequency comparison of DEFBI C-44G, GG, there is a significant difference between the acne vulgaris group and the control group (P = 0.024 < 0.05). For the genotype frequency comparison of DEFBI G-20A, AA, there is a significant difference between the acne vulgaris group and the control group (P = 0.041 < 0.05) (Table 2).

For the DEFBI G-52A allele (A) frequency comparison of the acne vulgaris group and control group, there is no significant difference (χ² = 0.789; P = 0.374 > 0.05; OR = 1.181; 95% CI 0.818–1.706). For the DEFBI C-44G allele (G) frequency comparison of the acne vulgaris group and the control group, there is a significant difference (χ² = 5.115; P = 0.024 < 0.05; OR = 0.517; 95% CI 0.29–0.992). For the DEFBI G-20A allele (A) frequency comparison of the acne vulgaris group and control group, there is a significant difference (χ² = 4.069; P = 0.044 < 0.05; OR = 0.676; 95% CI 0.462–0.99) (Table 3).

**Discussion**

Antimicrobial peptides are a universal feature of the defense systems of almost all life forms. Antimicrobial gene expression is the result of an undoubtedly complex detection/
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Our study found an allele frequency comparison of −44G and −20A, respectively (there is a significant difference). The −44G or −20A allele was a low expression in acne vulgaris, which has already been shown to correlate with the low risk of acne vulgaris. However, it is also possible that the −44G or −20A allele is active against *Cutibacterium acnes* and plays a protective role in this disease. Here, we can hypothesize that the DEFB1 −44G or G-20A allele is associated with an increased constitutive expression of DEFB1 mRNA, increased antimicrobial activity in acne vulgaris, and increased reporter protein expression in transfected cells. This suggests that the GG genotype of DEFB1 C-44G or AA genotype of DEFB1 G-20A results in enhanced transcription of the DEFB1 gene or enhanced post-transcriptional events. We found that the GG or AA genotype appears to protect against acne vulgaris (OR = 0.126 or 0.205), and the DEFB1 −44 G or −20A allele does up-regulate hBD-1 expression. This result could account for the increased potential protection factor in patients with acne vulgaris (OR = 0.517 or 0.676). The outcomes did not support a direct association of DEFB1 G-52A with acne vulgaris. It is also possible that SNP G-52A is not active in the expression of DEFB1 mRNA.

Our observation suggests that the −44G allele of DEFB1 C-44G and the −20A allele of hBD1 G-20A is a protective factor for acne vulgaris in Han Chinese patients. Our results suggest that the DEFB1 C-44G and the DEFB1 G-20A genotype might influence acne vulgaris, further supporting the contribution of inflammatory cytokines to the pathogenesis of acne vulgaris.

The results of this study are mainly applicable to the epidemiology genetics of acne. In our clinical analysis, genomic DNA was extracted from peripheral blood leucocytes collected from patients with acne and then analyzed by using gene chip technology to examine the allele frequency of DEFB1. According to the test results, effective treatments can be employed in the early stage of acne to avoid damaging the patients’ physical appearance. In brief, our research can provide strong measures to establish an early warning mechanism to prevent or reduce the possibility of acne scarring. To conclude, we explored the SNPs of the DEFB1 gene and the genetic susceptibility of acne vulgaris in the Han Chinese ethnic group, which is important in the clinical formulation of personalized treatment programs and the development of defensin-related drugs.

**Limitations**

First, this trial was not a randomized controlled trial. Second, this study was only a single-center trial, and the sample size was limited. Third, the relationship between the SNPs of the DEFB1 gene and the genetic susceptibility of acne vulgaris in other countries should be studied further.

**Conclusion**

The −44G or −20A allele showed low expression in acne vulgaris, which has already been shown to correlate with the low risk of acne vulgaris in Han Chinese patients.

**Funding**

National Natural Science Foundation of China (No.81674039, No.81873347); China Postdoctoral Science Foundation (No.2016M602271, No.2018T110747); Natural Science Foundation of Hubei Province (No.2015CFB577); Natural Science Foundation of Shennongjia Hubei (No.20181510-2); Wuhan Health Bureau Research Project (WX12B20); Training Project Funding Plan of Young and Middle-aged Talent of Health System in Wuhan City (2015); and Training Project Funding Plan of Young-aged Talent of Health System in Hubei Province (2019).

**Disclosure**

The authors report no conflicts of interest in this work.

**References**

1. Togo S, Sugawara K, Tsuruta D. Acne keloidalis in an Asian female patient. *Clin Case Rep*. 2019;7:1412–1414. doi:10.1002/ccr3.2170
2. Fox L, Csongradi C, Aucamp M, Du Plessis J, Gerber M. Treatment modalities for acne. *Molecules*. 2016;21:pii: E1063. doi:10.3390/ molecules21081063
3. Plewig G. How acne vulgaris develops. *Hautarzt*. 2010;61:99–100, 102–4, 106. doi:10.1007/s00105-009-1829-7
4. Dagnelie MA, Montassier E, Khammari A, Mounier C, Corvec S, Dréno B. Inflammatory skin is associated with changes in the skin microbiota composition on the back of severe acne patients. *Exp Dermatol*. 2019;28:961–967. doi:10.1111/exd.13988
5. James WD. Clinical practice. *Acne N Engl J Med*. 2005;352(14):1463–1472.
6. Kim J, Kim BE, Ahn K, Leung DYM. Interactions between atopic dermatitis and *Staphylococcus aureus* infection: clinical implications. *Allergy Asthma Immunol Res*. 2019;11:593–603. doi:10.4168/aair.2019.11.5.593
7. Krishnakumari V, Guru A, Adicherla H, Nagaraj R. Effects of increasing hydrophobicity by N-terminal myristoylation on the antibacterial and hemolytic activities of the C-terminal cationic segments of human-β-defensins 1-3. *Chem Biol Drug Des*. 2018;92:1504–1513. doi:10.1111/cbdd.13317
8. Kalus AA, Fredericks LP, Hacker BM, et al. Association of a genetic polymorphism (−44 C/G SNP) in the human DEFB1 gene with expression and inducibility of multiple β-defensins in gingival keratinocytes. *BMC Oral Health*. 2009;9:21. doi:10.1186/1472-6831-9-21
9. Philpott MP. Defensins and acne. *Mol Immunol.* 2003;40:457–462. doi:10.1016/s0161-5890(03)00154-8
10. Rauchhaus M, Gross M, Schulz S, et al. The E-selectin SER128ARG gene polymorphism and restenosis after successful coronary angioplasty. *Int J Cardiol.* 2002;83:249–257. doi:10.1016/s0167-5273(02)00073-6
11. Leung TF, Li CY, Liu EK, et al. Asthma and atopy are associated with DEFB1 polymorphisms in Chinese children. *Genes Immun.* 2006;7(1):59–64. doi:10.1038/sj.gi.6364279
12. Dessinioti C, Katsambas A. Propionibacterium acnes and antimicrobial resistance in acne. *Clin Dermatol.* 2017;35:163–167. doi:10.1016/j.clindermatol.2016.10.008
13. Jenssen H, Hamill P, Hancock RE. Peptide antimicrobial agents. *Clin Microbiol Rev.* 2006;19:491–511. doi:10.1128/CMR.00056-05
14. Ceral SM, Dreher-Lesnick SM, Gillespie JJ, Rahman MS, Azad AF. New tick defensin isoform and antimicrobial gene expression in response to Rickettsia montanensis challenge. *Infect Immun.* 2007;75:1973–1983. doi:10.1128/IAI.01815-06
15. Kim C, Kaufmann SH. Defensin: a multifunctional molecule lives up to its versatile name. *Trends Microbiol.* 2006;14:428–431. doi:10.1016/j.tim.2006.08.001
16. Dörk T, Stuhrmann M. Polymorphisms of the human β-defensin-1 gene. *Mol Cell Probes.* 1998;12:171–173. doi:10.1006/mcpr.1998.0165
17. Jurevic RJ, Bai M, Chadwick RB, White TC, Dale BA. Single-nucleotide polymorphisms (SNPs) in human β-defensin 1: high-throughput SNP assays and association with Candida carriage in type 1 diabetics and non-diabetic controls. *J Clin Microbiol.* 2003;41:90–96. doi:10.1128/JCM.41.1.90-96.2003
18. Trivedi NR, Gilliland KL, Zhao W, Liu W, Thiboutot DM. Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling. *J Invest Dermatol.* 2006;126:1071–1079. doi:10.1038/sj.jid.5700213
19. Prado-montes de Oca E, García-Vargas A, Lozano-Inocencio R, et al. Association of beta-defensin 1 single nucleotide polymorphisms with atopic dermatitis. *Int Arch Allergy Immunol.* 2007;142:211–218. doi:10.1159/000097023