

Acne Vulgaris is Associated with the Human β -Defensin I-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

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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.⁷ There is increasing evidence that pro-inflammatory cytokines (such as interleukin-1 β and tumor necrosis factor- α) and bacterial lipopolysaccharides can increase regulation in the human β -defensins.⁸ The DEFB1 gene is one of the main antimicrobial peptides that play a central role in the pathogenesis of acne.⁹ 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 standard⁵ 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 hypothyroidism; (2) patients with androgen-related diseases; (3) patients with infectious diseases; (6) 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.¹⁰ The DNA was stored at -20 °C.

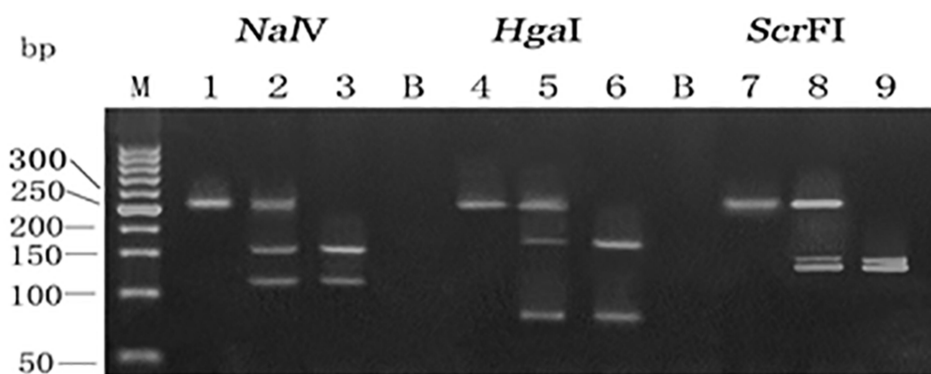


Figure 1 Detection of human β -defensin-I gene variations in the 5'-UTR (G-52A, C-44G, and G-20A) by restriction enzyme digestion with NaIV, HgaI, ScrFI, respectively, followed by 3% agarose gel electrophoresis. Lanes 1 AA genotype, lanes 2 GA genotype, lanes 3 GG genotype of SNP G-52A; lanes 4 CC genotype, 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	G	A
Acne vulgaris group	189 (90.87)	19 (9.13)	138 (66.35)	70 (33.65)
Mild-acne subgroup	54 (84.37)	10 (15.63)	39 (59.37)	25 (40.63)
Moderate-acne subgroup	78 (92.86)	6 (7.14)	55 (65.48)	29 (34.52)
Severe-acne subgroup	57 (95)	3 (5)	44 (73.33)	16 (26.67)
Control group	211 (83.73)	41 (16.27)	144 (57.14)	108 (42.86)

PCR Amplification

The primer sequence was as follows: forward primer 5'-CTT GAC TGT GGC ACC TCC CTT CAG-3' and reverse primer 5'-CAG CCC TGG GGA TGG GAA ACT C-3'.¹¹ 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 U of AmpliTag Gold DNA polymerase adjusted to 30 μL with water. This PCR program consisted of denatured 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 χ^2 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 *NalV*,

HgaI, *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 ($\chi^2 = 1.154$; $P = 0.283 > 0.05$). 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 ($\chi^2 = 0.789$; $P = 0.374 > 0.05$; 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 ($\chi^2 = 5.115$; $P = 0.024 < 0.05$; 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 ($\chi^2 = 4.069$; $P = 0.044 < 0.05$; 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.^{12,13} Antimicrobial gene expression is the result of an undoubtedly complex detection/

Table 2 The Frequencies Distribution of DEFB1 Genotypes for the Acne Vulgaris Group and the Control Group (Numbers with Percentages in Parentheses)

SNP	Genotype	Acne Vulgaris (n=104)	Controls (n=126)	χ^2	P-values	OR	95%CI
		Frequency	Frequency				
G-52A	GG	18(17.31)	25(19.84)	1.154	0.283	1.417	0.749–2.681
	GA	61(58.65)	78(61.91)				
	AA	25(24.04)	23(18.25)				
C-44G	CC	86(82.69)	94(74.61)	0.024	0.126	0.016–1.013	
	CG	17(16.35)	23(18.25)				
	GG	1(0.96)	9(7.14)				
G-20A	GG	36(34.62)	29(23.02)	0.041	0.205	0.044–0.947	
	GA	66(63.46)	86(68.25)				
	AA	2(1.92)	11(8.73)				

Table 3 The Frequencies Distribution of DEFB1 Allele in the Control Group and the Acne Vulgaris Group (Numbers with Percentages in Parentheses)

SNP	Allele	Acne Vulgaris (n=104)	Controls (n=126)	χ^2	P-values	OR	95% CI
		Frequency	Frequency				
G-52A	G	97(46.63)	128(50.79)	0.789	0.374	1.181	0.818–1.706
	A	111(53.37)	124(49.21)				
C-44G	C	189(90.87)	211(83.73)	5.115	0.024	0.517	0.29–0.922
	G	19(9.13)	41(16.27)				
G-20A	G	138(66.35)	144(57.14)	4.069	0.044	0.676	0.462–0.99
	A	70(33.65)	108(42.86)				

signaling pathway.¹⁴ The variety in the functions of antimicrobials appears to be attributable, in part, to differences in primary structures and charges at physiological pHs.¹⁵ Antimicrobial peptides play an important role as part of mucosal and skin innate immunity. Therefore, the genetic variation that results in altered peptide expression may influence susceptibility to inflammation.

The DEFB1 is also a type of inflammatory marker.¹¹ Dörk¹⁶ identified the genetic variants in 5'-UTR of DEFB1 G-52A, C-44G, and G-20A (rs1799946, rs1800972, and rs11362, respectively). The location of the SNP is in the 5'-UTR of hBD-1. The resultant change does not confer a change in the amino acid composition of the peptide but may be associated with a variation in translation or transcription of hBD-1 or another linked gene. The DEFB1 gene is found at the 8p23.2–p23.1 locus and is approximately 7.3 kb in size. SNPs in this gene have been studied, as their location in the gene suggests that they may induce functional alterations.¹⁷ The up-regulation of DEFB1 in acne vulgaris lesions suggested that DEFB1 may be involved in the pathogenesis of acne vulgaris

by protecting the pilosebaceous unit from microbial invasions.¹⁸ Strong DEFB1 was found in all the suprabasal layers of the epidermis, the distal outer root sheath of the hair follicle, and the pilosebaceous duct.

We concluded that DEFB1 genomic polymorphisms could be considered as important genetic markers for patients with acne. There are three kinds of polymorphic genes respective of DEFB1 G-52A, C-44G, and G-20A. The allele variant might influence DEFB1 actions, which may have a close relationship with the occurrence of acne. Differential DEFB1 expression among individuals could be explained by the presence of one or more SNPs that induces overexpression.¹⁹ Studies had reported that SNPs might induce functional alterations. DEFB1 C-44G in exon –1 is a transversion that creates a putative site for nuclear factor- κ B binding, and a nuclear factor involved in regulating the expression of pro-inflammatory cytokines. DEFB1 G-20A in exon –1, adjacent to the sequence, encodes the signal peptide. Transcripts for nuclear factor- κ B-regulated cytokine genes may be elevated in lesions.

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 -44G 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 leukocytes 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.

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Disclosure

The authors report no conflicts of interest in this work.

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