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ORIGINAL PAPER

The interleukin-6 and interleukin-1A gene promoter polymorphism is associated with the pathogenesis of acne vulgaris

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Abstract Acne vulgaris is a skin disorder with a complex pathogenesis. Better treatment strategies require comprehensive knowledge of molecular factors contributing to the acne pathophysiology. Recent studies are focused on investigating the influence of inflammatory cytokines on the disease. This case–control study investigated the association of IL-6-572 G/C and IL-1A-889 C/T gene polymorphisms with acne in a Pakistani population. Pakistani subjects (380 healthy controls and 430 acne patients) were enrolled in this study. Polymorphism in the promoter region of IL-6-572 and IL-1A-889 was determined by polymerase chain reaction and restriction fragment length polymorphism. The IL-6-572 and IL-1A-889 variant genotypes were significantly associated with the acne pathogenesis. The IL-6-572C and the IL-1A-889T alleles were significantly high in the patient vs. control group ($p < 0.0001$ for both loci). The IL-6-572 G/C and IL-1A-889 C/T variant allele haplotypes showed significantly high prevalence in patients with acne; G-T ($P = 0.0014$), C-C ($P < 0.0001$), and C-T ($P < 0.0001$). This is the first report on the association between the IL-6-572 G/C polymorphism and acne among any population. The IL-1A-889 C/T polymorphism is also significantly linked with acne in the study population; the -889 C/T association with acne has been reported in one ethnic group previously. Our findings suggest that the IL-6-572C and IL-1A-889T alleles may contribute to the pathogenesis of acne in a Pakistani population. Further studies are required to verify these findings in other populations.

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Introduction

Acne vulgaris is a multifactorial skin disease that affects a large number of people around the world. This disease is characterized by the pilosebaceous follicle that results in both inflammatory and non-inflammatory lesions. Environmental and genetic factors can disturb the natural cycling of the sebaceous follicles that could lead to the formation of comedones and inflammatory lesions. Genetic factors and proinflammatory cytokines play an important role in the initiation of acne lesions [17, 29]. The universality of acne has resulted in significant efforts to treat this disease; however, the precise mechanisms that govern the development and progression of acne are not fully understood yet. Of particular interest in understanding the pathogenesis of acne is the onset of an inflammatory process [28, 30]. Recent studies are more focused on investigating the role of inflammatory cytokines in the initiation and development of acne.

Various genes single nucleotide polymorphisms (SNPs) have been implicated in clinical conditions; however, controversy does exist regarding the association of certain SNPs with a disease among ethnic groups. A number of studies have demonstrated that tumor necrosis factor-apha (TNF-alpha) gene polymorphism is linked with different diseases including acne vulgaris [3, 25]. IL-6, a pleiotropic cytokine, has also been widely studied in inflammatory conditions. This cytokine plays a pivotal role in host defense mechanisms, regulates immune responses, hematopoiesis, and inflammation [15]. SNPs in IL-6 gene have been identified and associated with certain diseases [10,

11, 22]. However, there are relatively fewer studies on IL-6 gene polymorphism in acne patients.

IL-1A is another cytokine that has been widely studied in inflammatory conditions. This cytokine is considered to affect the inflammatory process and pathogenesis of acne vulgaris [1, 13]. Studies have indicated that IL-1A polymorphism might be associated with acne; a positive association of the IL-1A-4845 (G/T) polymorphism and acne has been reported [26]. In case of IL-1A-889 (C/T) polymorphism only one study has been reported thus far, that supported the association of the variant genotype at -899 with acne from a Polish population [23].

In the current study, we have investigated the IL-6-572 G/C (rs1800796) and IL-1A-889 C/T (rs 1800587) gene polymorphisms in patients with acne from a Pakistani population. This is the first report demonstrating an association between the IL-6-572 G/C, and IL-1A-889 C/T polymorphisms and acne in the study population.

Materials and methods

This study was reviewed and approved by the Institutional Review Board (IRB), Quaid-i-Azam University, Islamabad, Pakistan. We recruited 430 unrelated Pakistani patients with acne vulgaris, and 380 unrelated healthy control subjects with no past or family history of acne or any other disorder. Over 90 % of the candidates asked agreed to participate fully in this study. The control group comes from same geographical area, and includes blood donors and university students. The blood samples were collected from out-patient department of dermatology from different hospitals in the region. Concerned dermatologists of the enrolled patients characterized acne as non-inflammatory comedones and inflammatory lesions, including papules, pustules, nodules, and cysts, according to the criterion described by Lehmann et al. [18]. Informed written consent was obtained from each participant of the study in accordance with the Helsinki Declaration of 1975 as revised in 1997 and a questionnaire was filled through personal interviews of the participants. Peripheral venous blood samples were drawn from acne patients in 5 mL sterile syringes using aseptic vein puncture technique and transferred immediately to EDTA coated vacutainers to prevent coagulation. Genomic DNA was extracted using standard phenol–chloroform method as described elsewhere [21]. The IL-6 gene -572 G/C polymorphism was genotyped by polymerase chain reaction using the primer pairs FP: AGCCAACCTCCTCTAAGTGG, RP: ACTGAGTTTCCTCTGACTCC, and conditions of PCR cycles were set as follows: an initial denaturation temperature of 94 °C for 4 min, followed by 35 cycles of 95 °C for 40 s, 55 °C for 40 s, and 72 °C for 1 min followed by final extension for 10 min. For IL-1A, -889 C/T was genotyped using primer pairs reported in [33] and annealing temperature used was 60 °C.

Amplified fragment of 300 bp was digested with *NCOI* (5′…C↓CATGG…3′) to detect C/T transition at the -889 position. For IL-6-572 the amplicon was digested with *Mbi*I (BsrBI) restriction enzyme (5′…CCG↓CTC…3′) to determine the G/C transition at -572 position of the IL-6 promoter. The restriction digestion was done according to the procedure mentioned in our previous report [12]. The clinical characteristics (i.e., age and gender parameters) of the patient and control group were compared using Student's *t* test. The Chi square (χ^2) test was used to compare genotype and allele frequencies between the control and patient group. Statistical significance was established at $P < 0.05$. Odds ratios (OR) for the strength of association and risk of acne and their 95 % confidence intervals (CI) were calculated and the statistical tests were carried out using SPSS 16.0 software.

Results

We compared genotype and allele frequencies in two polymorphisms associated with IL-6 and IL-1A genes in 430 acne patients and 380 controls. The mean age of the control group was 23.88 ± 6.69 vs. acne cases 23.27 ± 7.79; male/female ratio was 200/180 in the control group and 200/230 in acne patients, respectively. There was no significant difference between the age and gender parameters in the two study groups.

Genotyping of the IL-6-572 G/C polymorphism in the study population

The genotype and alleles frequency of the IL-6-572 G/C polymorphism from acne cases and control subjects is shown in Table 1. The variant genotypes (GC + CC) at -572 of IL-6 gene were prevalent in 64.0 % acne patients vs. 42.0 % in the control group. The IL-6-572 G/C polymorphism was significantly high in patients vs. controls (OR 3.05, 95 % CI 2.30–4.04, $P < 0.0001$, Table 1). A significant difference was observed between the G and C alleles' frequency at -572 in the patient vs. control group (OR 2.01, 95 % CI 1.61–2.45, $P < 0.0001$; Table 1).

Genotyping of the IL-1A-889 C/T polymorphism in the study population

The genotypes and alleles distribution of the IL-1A-889 C/T polymorphism from patients with acne and healthy control subjects are shown in Table 2. There was an increased prevalence of the variant genotypes (CT + TT)

Table 1 Comparison of genotype and allele frequencies of the IL-6-572 G/C polymorphism in the study population

IL-6-572 G/C	Controls *n* (%) 380 (100)	Patients *n* (%) 430 (100)	OR	95 % CI	*P* value
Genotype frequency (co-dominant model)					
G/G	219 (58)	152 (36)	3.052	2.30–4.04	<0.0001[a]
G/C	145 (38)	230 (53)			
C/C	16 (4)	48 (11)			
Allele frequency					
G	583 (77)	534 (62)	2.011	1.61–2.45	<0.0001[b]
C	177 (23)	326 (38)			
Dominant model					
G/G	219 (58)	152 (36)	3.052	2.30–4.04	<0.0001[b]
G/C + C/C	161 (42)	341 (64)			

n number

[a] *P* value calculated by Chi square test

[b] *P* value calculated by Chi square test (Yates' corrected)

P value (<0.05) indicates statistical significance

of IL-1A-889 in the patient group (59.0 %) compared with controls at 35.0 %. The IL-1A-889 C/T polymorphism was significantly high in patients vs. controls ($P < 0.0001$, Table 2). There was a significant difference between the C and T alleles' frequency at -889 in the patient vs. control group (OR 2.01, 95 % CI 1.60–2.52, $P < 0.0001$; Table 2).

Haplotype analysis

Haplotype analysis of the IL-6-572 G/C and IL-1A-889 C/T polymorphisms from the study population is given in Table 3. The data revealed that the prevalence of variant alleles G-T ($P = 0.0014$), C-C ($P < 0.0001$), and C-T ($P < 0.0001$) were significantly high in acne cases compared with control subjects. The C-T haplotype showed maximal prevalence in patients with acne (19 %) compared with healthy controls at 8 %. These data indicate an association of the disease-susceptible polymorphic alleles of the two genes studied with acne in our population.

Discussion

Acne vulgaris is a complex skin disorder of multifactorial pathogenesis affecting a significant proportion of people. A range of factors, including follicular hyperkeratinization, seborrhea, *P. acnes*, immunologic processes and inflammation [5, 19], could influence the disease pathology. Of particular interest are the implications of inflammatory cytokines, antimicrobial peptides, and matrix metalloproteinases which may trigger acne lesions [28]. Considering the disease's impact on individuals' lives recent studies are aimed at understanding the implications of environmental and genetic factors in triggering the onset of acne lesions. Here, we focus on the role of inflammatory cytokines in the pathogenesis of acne.

The current case–control study is the first report demonstrating a significant association between the IL-6-572 G/C polymorphism in the study subjects with acne vulgaris vs. healthy controls from a Pakistani population ($P < 0.0001$). This SNP in relation to acne has not been previously reported from any population thus far. In the human IL-6 gene, various functional polymorphisms have been observed with controversial conclusions regarding their involvement in different clinical conditions. Four polymorphic sites are commonly identified in the promoter region of IL-6: -597 G/A, -373A(n)T(n), -572 G/C, and-174 G/C. Some SNPs are considered to affect the IL-6 expression and, therefore, may have a role in increasing the vulnerability to the disease. The IL-6-572 SNP has been associated with other inflammatory conditions like osteoporosis in postmenopausal women [8] and multiple sclerosis [32].

The polymorphism sites in the promoter region of IL-6-174 G/C and -572 G/C are associated with the cytokine expression, with the IL-6-572C allele contributing to high levels of IL-6 [4, 9, 20, 27]. The skin keratinocytes release IL-6, and the cytokine appears to affect the local and systemic inflammation [31]. We observed that IL-6-572 variant genotype was significantly associated with acne; therefore, it is reasonable to assume that the IL-6-572 C allele may contribute to an increased risk of the disease. An association between the IL-6-572 G/C polymorphism and periodontitis in the European population [24] suggested that the IL-6-572 G allele played a protective role for the clinical condition investigated. This perspective may support our data that the IL-6-572C allele may be a predisposing factor for acne vulgaris in the study population.

The current investigation also revealed a significant association between the IL-1A-889 C/T polymorphism and acne in the study population ($P < 0.0001$). In vitro and in vivo studies demonstrated that IL-1A is released from developing comedones and can promote comedone formation [2]. Change in the activity of sebaceous glands can trigger the release of IL-1A from keratinocytes lining the infundibular canal in hair follicle [7, 14]. IL-1A may participate in inflammation and rupture of follicular canal [13]. Transcription of IL-1A is influenced by

Table 2 Comparison of genotype and allele frequencies of the IL-1A-889 C/T polymorphism in the study population

IL-1A-889 C/T	Controls *n* (%) 380 (100)	Patients *n* (%) 430 (100)	OR	95 % CI	*P* value
Genotype frequency (co-dominant model)					
C/C	247 (65)	178 (41)	2.62	1.98–3.50	<0.0001[a]
C/T	112(29)	213 (50)			
T/T	21 (6)	39 (9)			
Allele frequency					
C	606 (80)	569 (66)	2.01	1.60–2.52	<0.0001[b]
T	154 (20)	291 (34)			
Dominant model					
C/C	247 (65)	178 (41)	2.62	1.98–3.50	<0.0001[b]
C/T + T/T	133 (35)	252 (59)			

n number

[a] *P* value calculated by Chi square test

[b] *P* value calculated by Chi square test (Yates' corrected)

P value (<0.05) indicates statistical significance

Table 3 Haplotype analysis of the IL-6-572 G/C and IL-1A-889 C/T polymorphism in acne patients and control subjects

IL6-572 G/C/IL-1A-889 C/T	Controls *n* (%) 380 (100)	Patients *n* (%) 430 (100)	χ^2	OR (95 % CI)	*P* value
G-C	492 (65)	402 (47)	Reference		
G-T	94 (12)	126 (15)	10.24	1.64 (1.21–2.21)	0.0014[a]
C-C	112 (15)	168 (19)	18.69	1.84 (1.40–2.41)	<0.0001[a]
C-T	62 (8)	164 (19)	53.87	3.24 (2.35–4.46)	<0.0001[a]

n number

[a] *P* value calculated by Chi square test (Yates' corrected)

P value (<0.05) indicates statistical significance

polymorphism at the -889 locus. The T/T genotype significantly increased the transcriptional activity of IL-1A gene compared with the C/C genotype [6]. One report on the IL-1A-889 C/T polymorphism conducted in 115 acne patients vs. 100 controls demonstrates an association between the -889 SNP and acne vulgaris in a Polish population [23]. We conclude from our study with 380 cases vs. 430 controls that the -889 polymorphism affects pathophysiology of acne in a completely different population of Pakistani patients.

Further support for the IL-1A gene's involvement with acne comes from the cytokine's +4845 G/T polymorphism in the Hungarian and Romanian populations [25]. IL-1A is synthesized as a precursor; pre-IL-1A is present in the nucleus which in response to appropriate stimuli is converted to the mature cytokine exhibiting a cytoplasmic localization [16]. The +4845 SNP results in an alanine to serine substitution of amino acid 114 which facilitates proteolytic cleavage by calpain [26]. This cleavage of IL-1A could influence epidermal homeostasis and hence complicate the acne symptoms. The -889 C/T polymorphism has also been shown to affect the levels of IL-1A protein; the variant T/T genotype produced higher levels of the protein compared with the C/C genotype [6]. Haplotype analysis of the IL-6-572 G/C and IL-1A -889 C/T polymorphism from the study population revealed that the variant alleles G-T ($P = 0.0014$), C-C ($P < 0.0001$), and C-T ($P < 0.0001$) were more prevalent in acne patients compared with the major alleles G-C at the two loci. Maximal prevalence was observed in case of C-T haplotype with 19 vs. 8 % in patients with acne and healthy controls, respectively. These data indicate an association of the disease-susceptible polymorphic alleles of the two genes studied with acne in our population.

There are certain limitations of the current study; (1) the sample size in this investigation is small, which precludes patient stratification by the severity of acnes, and these findings should be replicated in a larger population; (2) more studies should be conducted among different ethnic groups to resolve the controversial role of cytokines in relation to the disease; (3) future studies should include the determination of the cytokines' profiles in the study subjects; (4) additionally, ethically approved immunohistochemical and biochemical studies on biopsies of

consenting acne patients may provide useful information regarding the effect of inflammatory cytokines in the pathogenesis of acne lesions.

Conclusion

This study indicates that the IL-6-572 G/C and IL-1A-889 C/T polymorphisms are significantly associated with acne vulgaris in a Pakistani population. Furthermore, significantly high prevalence of the IL-6-572 and IL-1A-889 variant alleles' haplotypes was observed from acne cases compared with the major alleles. This is the first study reporting on the association of IL-6-572 G/C polymorphism with acne. In case of IL-1A-889 C/T polymorphism, there is one published study from a Polish population. Our findings in association with the data from other studies suggest that inflammatory cytokines have an important role in molecular pathogenesis of acne vulgaris.

What's already known about this topic?

- Inflammatory cytokines affect the pathogenesis of acne vulgaris.
- The IL-6-572 polymorphism has not been investigated, and there is one study on the IL-1A-889 polymorphism in acne patients.

What does this study add?

- This is the first report demonstrating an association between the IL-6-572 G/C, and the IL-1A-889 C/T polymorphism and acne in a Pakistani population.
- The variant allele haplotypes of the cytokines showed high prevalence in acne cases.

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Conflicts of interest None.

References

1. Aldana OL, Holland DB, Cunliffe WJ (1998) Variation in pilosebaceous duct keratinocyte proliferation in acne patients. Dermatology 196:98–99
2. Antilla HS, Reitamo S, Saurat JH (1992) Interleukin 1 immunoreactivity in sebaceous glands. Br J Dermatol 127:585–588
3. Baz K, Emin EM, Yazici AC, Söylemez F, Güvenç U, Taşdelen B, Ikizoğlu G (2008) Association between tumor necrosis factor-alpha gene promoter polymorphism at position -308 and acne in Turkish patients. Arch Dermatol Res 300:371–376
4. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, Lowe GD, Humphries SE (2001) Interleukin-6 gene -174G > C and -572G > C promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 21:1458–1463
5. Degitz K, Placzek M, Borelli C, Plewig G (2007) Pathophysiology of acne. J Dtsch Dermatol Ges 4:316–323
6. Dominici R, Cattaneo M, Malferrari G, Archi D, Mariani C, Grimaldi LM, Biunno I (2002) Cloning and functional analysis of the allelic polymorphismin the transcription regulatory region of interleukin-1. Immunogenetics 54:82–86
7. Downie MM, Sanders DA, Kealey T (2002) Modelling the remission of individual acne lesions in vitro. Br J Dermatol 147:869–878
8. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL (2003) Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab 88:255–259
9. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P (1998) The effect of novel polymorphism in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102:1369–1376
10. Foster CB, Lehrnbecher T, Samuels S, Stein S, Mol F, Metcalf JA, Wyvill K, Steinberg SM, Kovacs J, Blauvelt A, Yarchoan R, Chanock SJ (2000) An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood 96:2562–2567
11. Ghazouani L, Abboud N, Ben Hadj Khalifa S, Added F, Ben Khalfallah A, Nsiri B, Mediouni M, Mahjoub T (2011) 174 G/C Interleukin-6 gene polymorphism in Tunisian patients with coronary artery disease. Ann Saudi Med 31:40–44
12. Hussain S, Bibi S, Javed Q (2011) Heritability of genetic variants of resistin gene in patients with coronary artery disease: a family-based study. Clin Biochem 44:618–622
13. Ingham E, Eady EA, Goodwin CE, Cove JH, Cunliffe WJ (1992) Pro-inflammatory levels of interleukin-1a-like bioactivity are present in the majority of open comedones in acne vulgaris. J Invest Dermatol 98:895–901
14. Jeremy AH, Holland DB, Roberts SG, Thomson KF, Cunliffe WJ (2003) Inflammatory events are involved in acne lesion initiation. J Invest Dermatol 121:20–27
15. Kishimoto T (1989) The biology of interleukin-6. Blood 74:1–10
16. Kobayashi Y, Yamamoto K, Saido T et al (1990) Identification of calcium-activated neutral protease as a processing enzyme of human interleukin 1 alpha. Proc Natl Acad Sci USA 87:5548–5552
17. Koreck A, Pivarcsi A, Dobozy A, Kemeny L (2003) The role of innate immunity in the pathogenesis of acne. Dermatology 206:96–105
18. Lehmann HP, Robinson KA, Andrews JS, Holloway V, Goodman SN (2002) Acne therapy: a methodologic review. J Am Acad Dermatol 47:231–240
19. Leyden JJ (1997) Therapy for acne vulgaris. N Engl J Med 336:1156–1162
20. Mälarstig A, Wallentin L, Siegbahn A (2007) Genetic variation in the interleukin-6 gene in relation to risk and outcomes in acute coronary syndrome. Thromb Res 119:467–473
21. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York
22. Sen A, Paine SK, Chowdhury IH, Mukherjee A, Choudhuri S, Saha A, Mandal LK, Bhattacharya B (2011) Impact of interleukin-6 promoter polymorphism and serum interleukin-6 level on the acute inflammation and neovascularization stages of patients with Eales' disease. Mol Vis 17:2552–2563
23. Sobjanek M, Zablotna M, Glen J (2013) Polymorphism in interleukin 1A but not in interleukin 8 gene predisposes to acne

vulgaris in Polish population. J Eur Acad Dermatol Venereol 27:259–260
24. Song GG, Choi SJ, Ji GD, Lee YH (2013) Association between tumor necrosis factor-a promoter -308 A/G, -238 A/G, interleukin-6 174 G/C and 572 G/C polymorphisms and periodontal disease: a meta-analysis. Mol Biol Rep 40:5191–5203
25. Szabo K, KemÊny L (2011) Studying the genetic predisposing factors in the pathogenesis of acne vulgaris. Hum Immunol 72:766–773
26. Szabó K, Tax G, Kis K, Szegedi K, Teodorescu-Brinzeu DG, Diószegi C, Koreck A, Széll M, Kemény L (2010) Interleukin-1A +4845 (G>T) polymorphism is a factor predisposing to acne vulgaris. Tissue Antigens 76:411–415
27. Terry CF, Loukaci V, Green FR (2000) Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 275:18138–18144
28. Trivedi NR, Cong Z, Nelson AM, Albert AJ, Rosamilia LL, Sivarajah S, Gilliland KL, Liu W, Mauger DT, Gabbay RA, Thiboutot DM (2006) Peroxisome proliferator-activated receptors increase human sebum production. J Invest Dermatol 126:2002–2009
29. Trivedi NR, Gilliland KL, Zhao W, Liu W, Thiboutot DM (2006) Gene array expression in profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodelling. J Invest Dermatol 126:1071–1079
30. Vowels BR, Yang S, Leyden JJ (1995) Induction of proinflammatory cytokines by a soluble factor of *Propionibacterium acnes*: implications for chronic inflammatory acne. Infect Immun 63:3158–3165
31. Yamamoto T, Osaki T, Yoneda K, Ueta E (1994) Cytokine production by keratinocytes and mononuclear infiltrates in oral lichen planus. J Oral Pathol Med 23:309–315
32. Yan J, Liu J, Lin CY, Anzgene AN, Csurhes PA, Pender MP, McCombe PA, Greer JM (2012) Interleukin-6 Gene Promoter-572 C Allele may play a Role in Rate of Disease Progression in Multiple Sclerosis. Int J Mol Sci 13:13667–13679
33. Zhao J, Wang X, Xu J et al (2012) Association of inflammatory response gene polymorphism with atherothrombotic stroke in Northern Han Chinese. Acta Biochim Biophys Sin (Shanghai) 44:1023–1030