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The RETN gene rs1862513 polymorphism as a novel predisposing marker for familial *Acne vulgaris* in a Pakistani population

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ABSTRACT

Resistin (RETN), recently found to be relevant to inflammation and inflammatory disorders. We, therefore, aimed to investigate the potential role of RETN gene polymorphism in pathogenesis of acne vulgaris with familial history. We investigated the RETN-420C/G polymorphism in 180 patients with acne vulgaris and 180 healthy individuals in a case-control association analysis. In this study, we also investigated the heritability of the RETN susceptible allele from 140 trio families with acne affected offspring. The genotyping was performed by polymerase chain reaction and direct DNA sequencing. The RETN-420C/G polymorphism was significantly associated with acne in patients compared with healthy controls (P=0.014). The minor allele G at -420 was more prevalent in cases vs. controls (P=0.002). The RETN-420C/G polymorphism was significantly associated with severity of acne vulgaris in patients (P=0.0097). The results of a transmission disequilibrium test revealed a significant association between the RETN-420C/G polymorphism and acne vulgaris (P<0.001). For the first time in the literature, to our knowledge, we demonstrate a significant association of the RETN-420C/G functional polymorphism with familial acne vulgaris.

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The pathogenesis of *Acne vulgaris* is multifactorial; including sebum overproduction, acne proliferation, inflammation, and genetic (1). The identification of genetic variants contributing toward acne may provide insight into novel biological pathways affecting disease initiation or progression and ultimately assist in improved classification, diagnosis, and treatment. Therefore, scientists are in the active pursuit of identifying additional risk factors that may influence the disease pathophysiology. Several single nucleotide polymorphisms in pro-inflammatory cytokine genes have been associated with the pathogenesis of acne (2). Among the cytokines, resistin (RETN) gene is relatively novel and little known about the role of resistin in the pathophysiology of *Acne vulgaris*. Therefore, it could be remarkable to study the link of a functional polymorphism of the RETN gene at -420 with *Acne vulgaris*. The aim of this study was to investigate the presence of RETN-420C/G (rs1862513) promoter gene polymorphism in patients with *Acne vulgaris*.

In this study, 180 patients (mean age 23.6±6.1) with documented *Acne vulgaris* and 180 healthy control subjects (mean age 23.1±5.3) were enrolled in the case-control association of RETN gene polymorphism.The diagnosis of acne was based on physical examination, and Global Acne Grading System (3). Patients were divided into three sub-categories, as: acne comedonica (mild acne), acne papulo-pustulosa (moderate acne), and nodulo-cystic acne (severe acne). Healthy controls with no symptoms of *Acne vulgaris* or other skin diseases in the present as well as in the past were enrolled in this study from the same ethnic region as patients.

After discovering a significant association of minor allele G at RETN-420 with acne, we extended the analysis to investigate transmission of the allele from the parents to affected offspring using the transmission disequilibrium test (TDT) method from 140 trio-families. The genotyping of the RETN gene promoter region was carried out by polymerase chain reaction and direct DNA-sequencing method using forward primer F-5'- TGT CAT TCT CAC CCA

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Table 1. Characteristics of patients with *Acne vulgaris* and healthy controls (n= 360)

Variables	Patients= 180	Controls= 180	*P*-value
Age (years)	23.6 ± 6.1	23.1 ± 5.3	0.4070
Men/ Women (n, %)	49 (27.2)/131 (72.8)	51 (28.3)/129 (71.7)	0.8139
Age of onset of disease (years)	18.2 ± 4.1	-----	-----
Duration of disease (years)	4.9 ± 3.9	-----	-----
Treatment (n)	53 (29.4%)	-----	-----
Scar (n)	116 (64.4%)	-----	-----
Family History (n)	45 (25.0%)	-----	-----
Duration of Pimples (days)	9.8 ± 9.4	-----	-----
Number of Pimples (n) / week	6.1 ± 2.8	-----	-----
Swelling (n)	135 (75.0%)	-----	-----
Occupation (n)			
Students	107 (59.4%)	-----	------
Households	53 (29.4%)	-----	-----
Government servant	20 (11.2%)	-----	-----
Acne breakout (n)			
Facial	95 (52.8%)	-----	-----
Shoulder	24 (13.3%)	-----	-----
Neck	17 (9.4%)	-----	-----
Chest + Back	44 (24.5%)	-----	-----

Data represented by means with ± SD and numbers with percentage in parentheses

GAG AC-3' and reverse primer R-5'- TGG GCT CAG CTA ACC AAATC-3'. PCR was carried out with the following thermal cycling conditions: an initial denaturation step at 94 ˚C for 12 min, followed by amplification for 35 cycles at 94 ˚C for 30 sec, 56 ˚C for 1 min, and 72 ˚C for 2 min, followed by a final extension step at 72 ˚C for 2 min. DNA samples were sequenced on a CEQ™ 8800 Genetic Analysis System using CEQ DTCS Quick Start Kit (Beckman Coulter, Fullerton, Calif), according to the manufacturer's instructions. Conditions included a capillary temperature of 50 ˚C, 2 min denaturation at 90 ˚C, 15 s of injection time at 2 kV, and separation for 60 min at 5 kV. Informed consent was obtained in accordance with the Helsinki Declaration of 1975 as revised in 1997. The results were evaluated by chi-square test, Fisher's exact test, and MecNemar test, respectively.

Table 1 shows the distribution of the basic and clinical characteristics of patients with *Acne vulgaris* and healthy controls. Of the 180 patients, 95 patients had mild to moderate acne and 85 patients had severe acne. The genotypes and allele distribution in both groups is shown in Table 2. The distribution of the genotypes in acne and the control group was consistent with the Hardy-Weinberg equilibrium (*P*=0.0584 for acne group, *P*= 0.1084 for the control group). The observed genotype frequencies in patient group were CC=65.0%, CG=28.3%, and GG=6.7%, resulting in a G-allele frequency of 20.9%. In healthy controls, CC, CG, and GG genotypes were 78.3%, 18.9%, and 2.9%. The minor allele G at RETN-420 was 12.2% in the control group. The incidence of the RETN-420C/G polymorphism was significantly higher in patients with acne compared with healthy controls (*P*=0.014). Regarding RETN-420C/G polymorphism, we observed a statistically significant difference between the C and G alleles in the patient and control groups (*P*=0.002; Table 2). The distribution of RETN-420C/G polymorphism according to the severity of acne is shown in Table 3. A significant association between RETN-420C/G genotypes and severity of *Acne vulgaris* was noted (*P*=0.0097; Table 3). Transmission of the C and G allele at the RETN-420 locus from heterozygous parents to offspring with acne is summarized in Table 4. Among 260 parents, 165 transmitted the G allele and 13 transmitted C allele to their offspring who had acne.

Table 3. Genotype distribution in sub-groups of patients with *Acne vulgaris* (n=140)

RETN-420C/G	Mild to moderate acne = 95	Severe acne = 85	*P*-value
CC	67 (70.5%)	42 (49.4%)	Chi-Square
CG	23 (24.2%)	31 (36.5%)	= 9.27
GG	5 (5.3%)	12 (14.1%)	*P*= 0.0097

Values are given in numbers and percentage
P-values were calculated by Chi-square test for independence

Table 2. Genotype and allele frequencies of RETN-420C/G polymorphism in all subjects

RETN-420C/G	Patients= 180	Controls= 180	*P*-value
Genotype frequencies			
CC	117 (65.0%)	141(78.3%)	Chi-Square= 8.5
CG	51 (28.3%)	34 (18.9%)	*P*= 0.014
GG	12 (6.7%)	5 (2.8%)	
Allele frequencies	Patients	Controls	
C allele	285 (79.1%)	316 (87.8%)	*P*= 0.002
G allele	75 (20.9%)	44 (12.2%)	OR = 1.8
			95%CI=1.23-2.89

Values are given as a number and percentage, (OR, odds ratio; CI, 95% Confidence Interval)
P-values were calculated by using the chi-square test for genotype, Fisher's exact test for allele distribution

Table 4. Allele transmitted and non-transmitted at RETN-420: All affected offspring in all families with both parents typed

Transmitted				
Not-transmitted	C	G	Total	Chi-Square = 45.8 OR = 2.6
C	20	165	185	95%CI=1.97-3.62
G	62	13	75	*P*<0.001
	82	178	260	

Values are given as a number, (OR, odds ratio; CI, 95% Confidence Interval)
P-values were calculated by using McNemar test

Our TDT analysis revealed a preferential transmission of the G allele compared with the C allele (*P*<0.001; Table 4).

Resistin, originally described as an adipocyte-specific hormone, has been suggested to be an important link between obesity and insulin resistance. RETN gene is located on chromosome 9p13.3 and it has been shown that the occurrence of -420C/G polymorphism is linked to their higher transcription activity and enhanced expression (4, 5). Evidence showed that resistin has an important regulatory role apart from its role in insulin resistance in a variety of biological processes including inflammation (6). Recently, experimental studies showed that resistin is expressed in basal sebocytes which indicates its pathological link with inflammatory skin conditions (7). Likewise, increased levels of serum resistin have been markedly associated with psoriasis (8). Consequently, this published data showed that resistin has a strong inflammatory effect and actively involved in inflammatory skin disorders. The link between RETN gene variants and *Acne vulgaris* has not been investigated prior to this study and there is no report in literature which we can compare our results. Therefore, these findings should be replicated from larger cohorts of different populations to confirm the novel observation of this study. However, our findings of significant association of RETN-420C/G polymorphism with acne are consistent with the prior observations of significant association of TNF-alpha gene variants with acne from other populations (2).

The TDT is a powerful statistical test to detect linkage between the marker allele and susceptibility to the disease. Evidence shows that lower sample size can achieve a significant power in the TDT to demonstrate a linkage with a disease based on allele-sharing compared to traditional linkage tests (9). Data from this study showed that there was a preferential transmission of the RETN G allele at -420 from heterozygous parents to affected offspring (*P*<0.001). To our knowledge, for the first time, we demonstrate a significant association of the RETN variant allele G at -420 with the family history of *Acne vulgaris*. A recent report showed that family history is strongly associated with diagnosis at a young age, and has a crucial role of genetic factors in the pathophysiology of acne in population subgroups (10). However, the exact mechanism of this association remains to be explored, the current family-based study provides credible evidence that genetic variation at RETN locus may contribute to the etiology of acne in our high risk population.

Conclusion

For the first time, the presented data suggest that a variant in the RETN gene plays a significant role in acne pathogenesis. We also demonstrate an excess transmission of mutant allele G at RETN-420 from 130 trio families using TDT analysis. Therefore, we suggest that inherited variation in RETN at -420 appears to be associated with heritable *Acne vulgaris*.

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