

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.512.045>

Resistin (RETN) Gene rs1862513 Polymorphisms and Acne vulgaris Patients

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ABSTRACT

Acne vulgaris is a multifactorial inflammatory skin disease and the inflammation play an important role in its pathogenesis, many pro-inflammatory adipokines and cytokines contribute to this pathogenesis, from these IL-8 and resistin in which act as pro-inflammatory mediators and encoded by different genes. The present study was conducted to investigate whether RETN-420 C>G gene polymorphism as a predisposing factor to acne vulgaris. 49 Iraqi patients who were suffered from acne vulgaris have enrolled and compared with 25 healthy controls. RFLP-PCR technique was performed to detect the RETN-420 gene polymorphisms. The frequency of the genotypes in acne patients were (CC=6%, CG=14% and GG=80%), while in control group they were (8%, 24% and 68%) respectively. The frequency of all genotypes was not found any significant differences between acne patients and the healthy controls ($P=0.53$). No significant difference in genotype frequency regarding the gender between patients and the healthy group can be noted also between the same gender of patients and healthy group, as well as there was no association detected between this type of polymorphism and the degree of severity of acne (P value=0.74). We conclude that RETN-420G>C single nucleotide polymorphism do not contribute with the pathogenesis of acne vulgaris and there was no significant association between different alleles and the severity of the disease in two gender.

Keywords

Acne vulgaris,
RETN,
Resistin,
Allele,
Genotype,
RFLP-PCR.

Article Info

Accepted:
18 November 2016
Available Online:
10 December 2016

Introduction

Acne vulgaris is one of the most common chronic skin diseases affecting adolescents, 90% of an adolescent being affected by some degree (Al-Salih, 2008), however, acne can remain throughout adulthood in 8 % of the patients (Grange *et al.*, 2010). Clinically, adolescent acne consists of a combination of non-inflammatory (open and closed comedons) and inflammatory (papules, pustules, and nodules) lesions (Webster, 2005). Although acne is not a

life-threatening disease, but still can lead to serious physical (permanent scarring and hyper pigmentation) and psychosocial difficulties (depression, anxiety, anger, impairment in self-image) (Fried, 2006). The pathogenesis of acne is currently attributed to multiple factors such as increased sebum production and alteration in its quality, increased androgen activity, follicular hyperkeratinization, proliferation of *Propionibacterium acnes* (*P. acnes*) and

exhibition of pro- and anti-inflammatory properties (Kurokawa *et al.*, 2009). The affected persons' genetic background may also play an important role in acne predisposition, some studies showed that 81% of the variance of the disease was attributable to additive genetic effects and the remaining 19% was attributable to environmental factors (emotional stress, drugs, food, menstruation, smoking) (He *et al.*, 2006).

Resistin, also called adipocyte-secreted factor (ADSF), found in inflammatory zone 3 (FIZZ3), is a novel hormone secreted by adipocytes (Holcomb *et al.*, 2000). Resistin belongs to a family of cysteine-rich C-terminal proteins, termed RELMs (Resistin-Like Molecule) (Gerstmayer *et al.*, 2003). It was found that human resistin is mainly synthesized by non-adipocytes inflammatory resident cells such as peripheral blood mononuclear cells (PBMCs), bone marrow cells, monocytes and most predominantly by macrophages (Nagaev *et al.*, 2006; Kunnari *et al.*, 2009).

The resistin acts as an inflammatory cytokine have potent proinflammatory properties as it triggers the release of TNF- α , IL-1, and IL-6 (Bokarewa *et al.*, 2005). In effect, mounting evidence has identified neutrophils as dominant sources of resistin at the site of inflammation and even systemically during severe bacterial infections (Bostrom *et al.*, 2009; Johansson *et al.*, 2009). It promote inflammation (Lehrke *et al.*, 2004; Bokarewa *et al.*, 2005). The human resistin gene (RETN) is sited on chromosome 19p13 (Menzaghi *et al.*, 2006). Several single nucleotide polymorphisms (SNPs) have been associated with resistin levels (Osawa *et al.*, 2007; Azuma *et al.*, 2004; Cho *et al.*, 2004). The levels of resistin and their gene polymorphism may contribute to many inflammatory diseases

such as inflammatory bowel disease (Konrad *et al.*, 2007), chronic pancreatitis (Adrych *et al.*, 2009), and in other disease.

Materials and Methods

Patients

The subjects enrolled in this study was comprised of 49 patients (28 female and 21 male) who were suffering from acne vulgaris and their ages are between 13 and 35 years, and the healthy control individuals of 25 (16 female and 9 male) with ages ranges of 14-40 years old, they were attended at Dermatology Center of Al-Sader medical City and Dr. Mohsen Dalimy Clinic, the acne vulgaris was diagnosed by consultant physicians based on physical examination, and Global Acne Grading System (Jerry KL Tan, 2008). 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 were enrolled in this study from the same ethnic region as patients.

Methods

The DNA was extracted from whole blood, after collected, by special kit and the RETN gene was amplified by PCR technique by the use of specific primers for this gene (forward F primer 5'-TGT CAT TCT CAC CCA GAG ACA-3' and reverse R primer 5'-TGG GCT CAG CTA ACC AAA TC-3'). PCR was carried out with the following thermal cycling conditions: an initial preparation step at 94 °C for 5 min., followed by amplification for 40 cycles at 94 °C for 1 min., 57 °C for 1.5 min., and 72 °C for 1.5 min., followed by the end cycle at 72 °C for 7 min. Then the PCR product was incubated over night with BpI restriction

enzyme to cut the gene to show the polymorphisms under UV light by using gel electrophoresis. This Technique was carried out to detect the resistin gene polymorphism SNP -420 (G>C) in RETN gene in the samples of both patients with acne vulgaris and as well as the control group.

Statistical analysis

Statistical analysis was done by using SPSS (statistical package for social sciences) version 20 in which we use ANOVA (analysis of variance), independent sample T-test, chi square test, and Pearson correlation coefficient as needed. We set P value <0.05 as significant.

Results and Discussion

RFLP-PCR product appearance

The product of RFLP-PCR technique showed the single nucleotide polymorphism (SNP) that occur in RETN-420 (rs1862513) and show three bands in size 533bp, 323bp and 210bp this means there is three genotype polymorphism; CC homozygous when two band 323bp and 210bp will appear, GG homozygous when one band 533bp will appear and CG heterozygous when the three band will appear as shown in figure(1), and this result was agreed with the earlier report of Kumar *et al.*, (2014), that this locus had three genotypes only.

Genotype distributions in subjects under study

In the current study, a direct comparison between resistin genotypes and the clinical features of acne vulgaris (susceptibility & severity) was investigated focusing on the two alleles single nucleotide polymorphism in RETN gene in the context of the promoter

region. The frequency of genotypes in acne patients were as follow: CC(3%), CG(7%) & GG(39%) ; while in the healthy control subjects: CC(2%), CG(6%)& GG(17%), figure(2). The genotype distribution had no deviation from Hardy-Weinberg equilibrium (HWE) in all groups.

The GG genotype frequency was higher in acne patients (80%) when compare with healthy controls (68%), but the difference in the distribution of RETN genotypes between patient and control groups was statistically non-significant, (Chi=1.27 & P-value=0.53), as shown in the table (1). This result was contrast with the results of Hussain *et al.*, (2015 B) on acne vulgaris in Pakistani population (Chi=8.5 & p-value=0.014), this support the null hypothesis that there is no association between the RETN-240 C>G SNP polymorphism and the susceptibility to the disease, these differences may be explained by the difference in the geographically different population studied

The distribution of the Genotypes in acne and healthy control subjects according to gender

The results of this study according to the three genotype distributions in acne patients show that there is no gender-specific differences were detected between male and females in acne patients, the P value=0.15 for all genotypes as shown in table (2), according to our knowledge there is no study demonstrate this association.

When Compare between the study groups regarding the same gender, the data showed that there was no association between the RETN-420 >C polymorphism and the two genders of acne patients (P value=0.15) as shown in figure (3) for female and (4) for male, These results was not completely accordant with the results of Younis *et al.*,

(2016), that show that the variant genotype is associated with acne in female patients ($p = 0.008$) whereas, in male patients the variant genotype frequency, while higher, did not reach statistical significance.

The association between RETN-420 C>G polymorphism and the severity of acne

In this study there is no association was detected between the severity of acne and the distribution of the frequency of the three genotypes in acne patients, in which the P

value=0.74) as shown in the table (3). This result contrast with Younis *et al.*, (2016), show that both G/G genotype and G allele frequencies in -420C>G polymorphism are associated with severity of acne symptoms (G/G: $p = 0.027$; G: $p = 0.020$). In view of acne is a multifactorial disease, it is possible that the severity of acne may be readily influenced by other factors such as environmental and other genetic elements (Cordian *et al.*, 2002; Szabo & Kemeny, 2011).

Table.1 Genotype distribution of resistin RETN-420 gene polymorphism in patients and controls

Genotypes	Controls N(%)	Patients N(%)	OR	P	Chi
CC	2 (8%)	3 (6%)	0.75	0.53	1.27
CG	6 (24%)	7 (14%)	0.59		
GG	17 (68%)	39 (80%)	1.83		
Total	25 (100%)	49(100%)			

Table.2 Genotypes distribution of RETN-420 gene polymorphism among acne patients according to the gender

Genotypes	Female N(%)	Male N(%)	OR	Chi	P value
CC	1 (3.6%)	2 (9.5%)	2.83	3.799	0.15
CG	2 (7.1%)	5 (23.8%)	4.06		
GG	25 (89.2%)	14 (66.7%)	0.24		
Total	28 (100%)	21 (100%)			

Table.3 The association between RETN-420 C>G polymorphism and degree of severity of acne patients

Genotype	Mild N (%)	Moderate N (%)	Severe N (%)	P-value
CC	1 (5.3%)	2 (7.15%)	0 (0%)	0.74
CG	4 (21%)	3 (10.7%)	0 (0%)	
*GG	14 (73.6%)	23 (82.15%)	2 (100%)	
Total	19 (100%)	28 (100%)	2 (100%)	

Fig.2 Case-control comparison in the relative frequency of the three selected genotypes (CC, CG and GG).

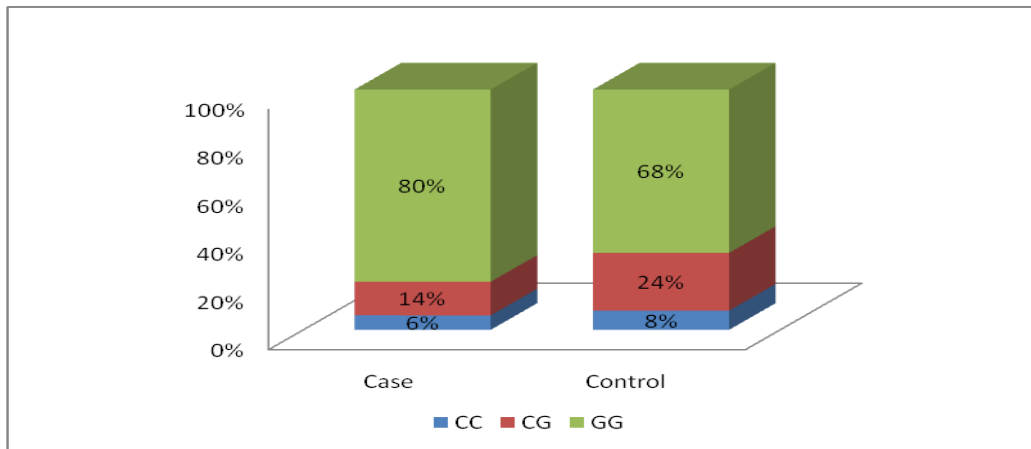


Fig.3 Case –control comparison in the relative frequency of the three selected genotypes in females

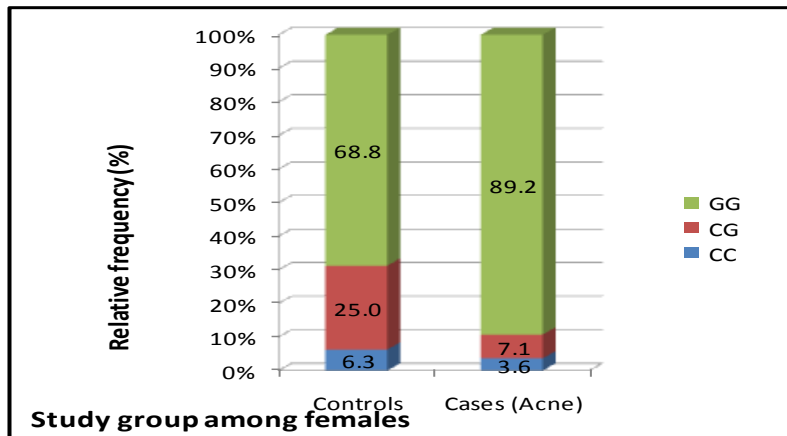
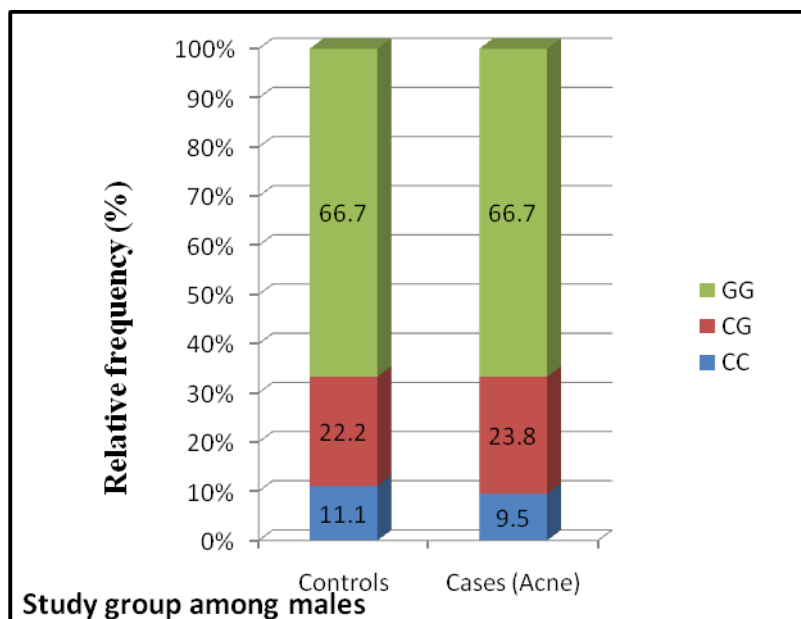


Fig.4 Case –control comparison in the relative frequency of three selected genotypes in males



The association between RETN-420 C>G polymorphism and the severity of acne

In this study there is no association was detected between the severity of acne and the distribution of the frequency of the three genotypes in acne patients, in which the P value=0.74) as shown in the table (3).

This result contrast with Younis *et al.*, (2016), show that both G/G genotype and G allele frequencies in -420C>G polymorphism are associated with severity of acne symptoms (G/G: $p = 0.027$; G: $p = 0.020$). In view of acne is a multifactorial disease, it is possible that the severity of acne may be readily influenced by other factors such as environmental and other genetic elements (Cordian *et al.*, 2002; Szabo & Kemeny, 2011).

In conclusion, there is no prospect association between RETN-420(C>G) single nucleotide polymorphism and the presence or development of acne vulgaris in two genders and there is no association between the presence of different genotypes or alleles

in the population may predict the probability of developing the disease.

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How to cite this article:

Hammadi A. Al-Hilali and Mohammed Jaafar AL-Ansari. 2016. Resistin (RETN) Gene rs1862513 Polymorphisms and Acne vulgaris Patients. *Int.J.Curr.Microbiol.App.Sci*. 5(12): 415-422. doi: <http://dx.doi.org/10.20546/ijcmas.2016.512.045>