Int.J.Curr.Microbiol.App.Sci (2016) *5*(11): 183-195

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 11 (2016) pp. 183-195**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.511.021

Association of the RETN–420C>G Polymorphism with Rheumatoid Arthritis in an Egyptian Population

Marwa M.E. Abd-Elmonsef[1]*, Naglaa F. Ghoname[1], Abeer Shahba[2], Maha M. Hagras[3], Lamees M. Dawood[4] and Mohamed M.E. Abd-Elmonsef[5]

[1]Department of Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt
[2]Department of Internal Medicine, Faculty of Medicine, Tanta University, Egypt
[3]Department of Clinical Pathology, Faculty of Medicine, Tanta University, Egypt
[4]Department of Biochemistry, Faculty of Medicine, Tanta University, Egypt
[5]Department of Mathematics, Faculty of Science, Tanta University, Egypt
**Corresponding author*

Keywords

Rheumatoid arthritis, RETN gene, polymorphism, resistin.

Article Info

Accepted:
12 October 2016
Available Online:
10 November 2016

A B S T R A C T

To investigate the relation of the resistin gene promoter single nucleotide polymorphism (*RETN*–420C>G) with the serum resistin concentration and rheumatoid arthritis (RA) risk, and if there is any association with the disease activity and autoimmunity. This study included 128 patients with RA (64 active and 64 in remission) and 64 controls. Serum resistin was measured for all subjects by ELISA. Other markers of inflammation and disease activity were also assessed. All subjects were genotyped for *RETN*–420C>G polymorphism using PCR-RFLP. **S**erum resistin was significantly higher in active RA patients compared to inactive patients and controls. Patients with CG and GG genotypes had increased risk of RA compared to CC carriers (OR = 1·88, *P*= 0.049, OR = 4.84, *P* = 0.046, respectively). Highly significant level of resistin was detected with variant genotypes. Highly significant association was found between the investigated polymorphism and the parameters of the disease activity, but not autoimmunity. This is the first study suggesting the relationship between *RETN* – 420C>G polymorphism and both the risk of RA and its activity, but not autoimmunity. More studies of different populations and larger samples are required to confirm these observations.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease primarily affecting the joints (Yoshino *et al.*, 2011). It is characterized by symmetrical synovitis, pannus formation, joint pain, stiffness, swelling and damage due to a chronic inflammatory process (Krysiak *et al.*, 2012; Klein-Wieringa *et al.*, 2011). Actually, the detailed mechanisms involved in the pathogenesis of RA are still obscured. However, inflammatory cytokine production is clearly involved in the pathogenesis of RA (Feldmann *et al.*, 1996).

Among the cytokines, there are indications that resistin may be involved in the pathophysiology of RA (Šenolt *et al.,* 2007a). It is a 12.5kDa cysteine-rich polypeptide (Koerner *et al.,* 2005). Human resistin has 108 amino acids (Steppan *et al.,* 2001). It is secreted by adipocytes and in high levels by peripheral-blood mononuclear cells (Tilg and Moschen, 2006). In rodents, resistin has shown links between obesity and insulin resistance (hence the name resistin) (Steppan *et al.,* 2001), while in humans, its pro-inflammatory properties are superior to its metabolic effects.

Human resistin gene (*RETN*) is located on chromosome 19 at 19p13·3 – 19p13·2 (Steppan *et al.,* 2001; Cepica *et al.,* 2002). Its locus has nine single-nucleotide polymorphisms (SNPs) (Engert *et al.,* 2002). Among others, the –420 C to G (rs1862513) polymorphism in the *RETN* promoter has recently been suggested to play a potential role in pro-inflammatory conditions (Tang *et al.,* 2007). However, the link between this polymorphism and the RA is still unrevealed.

In this study, we aimed to find the association of resistin gene variation with the risk of RA and its activity, serum resistin level and different rheumatoid inflammatory markers.

Materials and Methods

Study population

A total of 128 patients with RA were enrolled in this study, 64 of them were in the active form of the disease (active group) and the other 64 were in remission (inactive group). Also, fifty healthy persons were enrolled as the (control group). The RA was diagnosed according to 1987 revised criteria of the American Rheumatism Association (Arnett *et al.,* 1988),and the clinical activity was assessed according to the 28 count Disease Activity Score (DAS28). It was calculated with the following equation: $DAS28 = 0.56 \times \sqrt{28TJC} + 0.28 \times \sqrt{28SJC} + 0.7 \times \ln ESR + 0.014 \times GH$, where 28TJC and 28SJC are the tender joint count and swollen joint count from 28 joints and general health (GH) is the patient's global assessment on a 100-mm visual analog scale (VAS) (Fransen and van Riel, 2005). Body mass index (BMI) was calculated as the ratio of body weight to body height and expressed in kg/m^2.

The included subjects were randomly chosen from Internal Medicine Department of Tanta University Hospital, Egypt during the period from May 2015 to April 2016. This study fulfilled the requirement of the local Ethical Committee. Informed consent was obtained from each participant. Exclusion criteria were infection, other inflammatory or metabolic diseases, pregnancy, and anti-inflammatory or immunosuppressive medications within previous 24 hours.

Sampling

Peripheral blood was drawn from each participant, and was divided into 2 portions: 1ml of whole blood was collected into evacuated tubes containing EDTA for DNA extraction, and the remaining portion of the blood was used to separate serum immediately. Separated serum was stored at −20 °C until further use.

Measurement of serum resistin

Serum resistin concentration was assayed by enzyme-linked immunosorbent assay (ELISA) using (Sunred biological technology, Shanghai) kits according to the instructions of the manufacturers.

Measurement of other rheumatoid inflammatory parameters

Rheumatoid factor (RF) was measured by a quantitative immunonephelometry (Behring, Marburg, Germany). Anti-citrullinated peptide antibody (ACPA) was assessed by microparticle enzyme immuno essay with the Abbott AxSym(Chicago, IL, USA). C-reactive protein (CRP) was measured by semiquantitative latex test (Omega, Avitex, UK). The erythrocyte sedimentation rate (ESR) was measured by the Westergren method; the reading of the 1st hour was included in the study (Westrgren, 1975).

DNA extraction and genotyping

DNA was isolated and from whole blood (EDTA) using QIAamp-spin-columns according to the manufacturer instructions (QIAamp DNA Mini Kit (250); Qiagen GmbH, Hilden, Germany). Isolated DNA was stored at −20 °C until use.

The *RETN*–420 C>G polymorphism was detected using the polymerase chain reaction - restriction fragment length polymorphism method (PCR-RFLP) as described before by Kunnari *et al.,* 2005. The following primers were used: forward primer 5′-TGT CAT TCT CAC CCA GAG ACA-3′ and reverse primer 5′-TGG GCT CAG CTA ACC AAA TC-3′. The PCR reaction was carried out in a total volume of 50 μl containing 200 ng of genomic DNA, 0.25 μM of each primer (Promega, Madison, WI) and 1× PCR mix (Taq PCR Master Mix Kit, QIAGEN, GmbH, Hilden, Germany). The amplification cycle was performed as follows: denaturation at 95 °C for 7 min, preannealing at 64 °C for 1 min, and then elongation at 72 °C for 2 min followed by 35 cycles of 30 s at 95 °C, 30 s at 64 °C and 1 min 15 s at 72 °C, and finally, elongation at 72 °C for 10 min. The PCR product was digested with 5 U Bbs I (MBI-Fermentas, United Kingdom) at 37 °C for 16 h. The 534-bp PCR products were cleaved into two fragments (327-bp and 207-bp fragments) for the C homozygote, three fragments (534-bp, 327-bp and 207-bp) for CG heterozygote, and the G homozygous remained uncleaved (534-bp). The digestion products were separated by 1.5% agarose gel electrophoresis stained with 0.5 mg/mL of ethidium bromide to visualize the bands (Tang *et al.,* 2007).

Statistical analysis

The results for quantitative variables were expressed as the mean±SD and were analyzed using Student's t and one-way ANOVA tests. Qualitative variables were expressed as percentages and were compared using the Chi-square test. This test was also used to identify significant departures from the Hardy-Weinberg equilibrium. The correlation coefficients were calculated using Spearman correlation. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the associations of the *RETN*–420C>G SNP with the risk of RA, and with the different autoantibodies. A value of $P<0.05$ was considered statistically significant. All data were evaluated using the statistical package for social sciences (SPSS) 23.

Results and Discussion

Clinical and biochemical characteristics of the study population (Table 1)

In this cross-sectional study, the 3 included groups (active RA, inactive RA and healthy controls) were homogenous as regards age, sex and BMI. The levels of serum resistin, ESR and CRP were significantly higher in the active RA group compared to both inactive RA and control groups. Also, active

RA patients had significantly higher levels of RF, ACPA, and DAS28 score rather than inactive patients. There was no significant difference between both patient groups as regards the duration of the disease.

Correlations between serum resistin and the different clinical and biochemical parameters of the RA patients (Table 2)

In all included RA patients, serum resistin level was positively correlated with levels of ESR, CRP, RF, ACPA and DAS28. There were no correlations between resistin and either age, BMI, or the disease duration in the RA patients.

***RETN* –420C>G polymorphism frequencies in the study population (Table 3)**

Genotype frequencies of *RETN* –420C>G polymorphism was in Hardy–Weinberg equilibrium in all included groups. In RA patients, the frequencies of *RETN* –420 GG and CG genotypes were significantly higher than in control group (10.15% vs. 3.12%, *P* = 0.046; 53.13% vs. 42.19%, *P* =0.049, respectively). G-allele frequency was 36.72% among RA patients and 24.22% among controls. Subjects carrying G allele were significantly more likely to develop RA (OR=1.82, 95% CI=1.13-2.93, *P*=0.014).

Relation between *RETN*–420C>G polymorphism genotypes and the different clinical and biochemical characteristics of the RA patients (Table. 4)

There were no significant differences between the genotypes as regards age, BMI, RF, ACPA, and duration of the disease in all RA patients. Levels of resistin, ESR, CRP and DAS28 were significantly different between the different genotypes. RA patients with GG and CG genotypes showed a significant increase in CRP level compared to CC genotype carriers. In addition, there were significant associations of resistin, ESR and DAS28 with GG genotype.

Relation between *RETN* –420C>G polymorphism genotypes and the different parameters of activity in active RA patients (Table 5)

In this study, we detected significant associations between GG genotype and the different parameters of disease activity (ESR, CRPand DAS28). Also, serum resistin was significantly associated with GG genotype in active RA patients. There were no significant differences between the genotypes as regards RF and ACPA.

Relation between *RETN* –420C>G polymorphism genotypes and the autoimmune antibodies in all RA patients (Table 6)

G allele had no association with RF+ve (OR = 0.98, 95% CI = 0.53-1.83, *P* = 0.956), nor with ACPA+ve(OR = 1.53, 95% CI = 0.92-2.55, *P* = 0.105). Although it was higher in ACPA +ve cases compared to negative cases (41.54% vs 31.75%, respectively), it did not reach asignificant level.

RA is a systemic disease that is associated with a high risk of the development of cardiovascular, neurological, and metabolic disorders (Kaplan, 2006). Involvement of inflammatory mediators in the pathogenesis of RA is still a matter of research that remains poorly understood. Knowledge on the link between resistin and RA might contribute to better understand this disease and the possible use of this mediator as a potential target for the treatment of RA.

This study showed a significant increase in resistin levels in the serum of active RA patients compared to inactive RA patients and healthy subjects ($P< 0.001$, for each) (Table 1). This was in accordance with other studies who found significantly higher serum resistin level in RA patients compared to control subjects ($P < 0.001$) (Migita *et al.,* 2006; Kassem *et al.,* 2010). In addition, another study detected marked increase in the production of resistin at synovial tissue and synovial fluid in RA patients which was associated with the increase in the serum resistin of these patients; hence, It supposed that resistin was produced not only by macrophages but also by synovial fibroblasts and other inflammatory cells (Šenolt *et al*., 2007b).

On the contrary, no significant difference was found in the serum resistin between RA patients and controls by Alkady *et al.,* 2011.We cannot explain this discrepancy, however, their study differed in the characteristics of the study population regarding the sex (only female patients were included in their study), also it differed in the ELISA kit type. In addition, our data differ from those presented by Yoshino *et al.* 2011, although they reported a significant correlation between serum resistin and CRP in RA patients, referring to the conclusion that resistin could be a good inflammatory marker.

When we assessed the correlations between serum resistin and the other inflammatory markers in RA patients, we found highly significant positive correlations with ESR ($P = 0.002$), CRP ($P = 0.002$), RF ($P = 0.001$), and ACPA ($P < 0.001$) (Table 2). Also, a positive correlation was found between the serum resistin and the disease activity (DAS28) ($P < 0.001$). However, the correlations of resistin with the age, BMI and the duration of the disease were not significant ($P = 0.563$, 0.819 and 0.687, respectively). Some (Migita *et al.,* 2006; Kassem *et al.,* 2010; Yoshino *et al.,* 2011; Dong *et al.,* 2012), but not other (Bokarewa *et al.,* 2005; Alkady *et al.,* 2011; Fadda *et al.,* 2013) authors found that the increased serum levels of resistin correlated with CRP, ESR, RF and DAS28 in RA patients. However among these studies with opposing results, Bokarewa *et al.,* 2005 found a significant correlation between the synovial resistin levels in RA and both the synovial total leukocyte count(TLC) (P=0.003) and interleukin-6 (IL-6) levels (P= 0.014). In addition, Fadda *et al.,* 2013 found that serum resistin level correlated with TLC (P= 0.018) and that also synovial fluid resistin level correlated with RF, ACPA and TLC (P= 0.038, 0.04, 0.004, respectively).

To the best of our knowledge, this is the first study to examine the association between the *RETN* –420C>G polymorphism and the risk of RA. Our results showed a significant increase in the frequency of *RETN* –420 CG and GG genotypes in RA patients compared to control group (P=0.049 and 0.046, respectively) (Table 3), suggesting an association between the *RETN* –420C>G mutation and the increased risk of RA development. The association between *RETN* –420C>G polymorphism and RA has not been investigated before and there is not any report in literature to compare with our results. However, this SNP was reported to be associated with other inflammatory diseases like polycystic ovary syndrome (Baba *et al.,* 2009), periodontitis (Patel and Raju, 2014), colorectal cancer risk (Mahmoudi *et al.,* 2014), familial acne vulgaris (Hussain *et al.,* 2015), and idiopathic dilated cardiomyopathy (Hussain *et al.,* 2016).

On the contrary, other studies reported lack of association between this SNP and other inflammatory diseases such as multiple sclerosis (Hossein-Nezhad *et al.,* 2013) and

intracerebral hemorrhage (Dong *et al.*, 2012). This discrepancy in results could be attributed to selection bias or due to differences in the size of the study sample, ethnicity, or disease status (El-Shal *et al.*, 2013).However in both studies, serum resistin was significantly higher in *RETN* gene variants, that was consistent with our results. We found that RA patients with genotype GG had significantly higher levels of serum resistin than CC carriers (*P*= 0.035) (Table 4), which was supported by several studies in other diseases (Cho *et al.*, 2004, Osawa *et al.*, 2007; Ukkola *et al.*, 2008; Hussain *et al.*, 2010; Lau and Muniandy, 2011; El-Shal *et al.*, 2013; Patel and Raju, 2014) .

Also in our study, ESR and CRP levels were significantly higher in variant genotypes than CC genotype (Table 4); similar results concerning CRP were obtained by Osawa *et al.*2007 and Tang *et al.* 2007.This positive associations between *RETN* –420C>G polymorphism and both resistin and CRP levels have been recently explained by the findings reported by Osawa *et al.* 2004, that the *RETN*–420C>G variant seemed to gain the ability to bind the Sp1/3 transcription factor which may markedly enhance the *RETN* gene promoter activity and increase the transcription of the RETN gene.

Also, the *RETN* –420G allele was associated with higher mRNA expression of resistin and serum resistin levels (Azuma *et al.*, 2004; Cho *et al.*, 2004). In addition, resistin can strongly upregulate the expression of IL-6 and tumor necrosis factor-alpha (TNF-α) in humans (Pilz *et al.*, 2007). These two pro-inflammatory cytokines were found to be important inducers of CRP (Maachi *et al.*, 2004). Moreover, resistin was documented to significantly upregulate the expression of pentraxin 3, a close homolog of CRP, most probably via the nuclear factor (NF)-kB pathway (Kawanami *et al.*, 2004). All these data support the association between *RETN* –420C>G polymorphism and the increased levels of resistin and CRP.

Conversely, other studies noted non-significant association between this SNP and both resistin and CRP levels (Ukkola *et al.*, 2008; Qasim *et al.*, 2009; Hlavna *et al.*, 2011). The reason for this discrepancy is difficult to explain but may be attributed to several factors. First factor is the differences in the metabolic phenotypes of the study population across these different studies; since several studies documented the strong association of resistin level with BMI (Engert *et al.*, 2002; Mattevi *et al.*, 2004; Xita *et al.*, 2004).

Second factor is the extent of the inflammatory condition; some pro-inflammatory agents, such as TNF-α (Fasshauer *et al.*, 2001), IL-6 (Kaser *et al.*, 2003) and lipopolysaccharide (Lu *et al.*, 2002) can regulate resistin gene expression. Thirdly, heritable factors were reported to account for ~70% of the observed variation in resistin levels; it affects resistin expression and/or circulating levels (Menzaghi *et al.*, 2006).

The assessment of RA activity is based on several parameters. Among these parameters, the most widely used are the inflammatory markers mainly ESR and CRP and the disease activity scores (Verma *et al.*, 2002).Various questions remain regarding the role of autoantibodies as possible markers of RA activity (da Mota *et al.*, 2009).In our active RA patients, There were significant associations of GG genotype with ESR, CRP and DAS28 (*P*= 0.016, 0.02 and 0.022, respectively), but not with RF and ACPA (*P* = 0.91, for each) (Table 5).

Table.1 Clinical and biochemical characteristics of RA patients and healthy controls

Parameters	Active RA cases (group I) n = 64	Inactive RA cases (group II) n = 64	Healthy Controls (group III) n = 64	*P* value
Age (ys)	41.34±8.31	39.66±8.91	39.03±8.77	0.549 I vs II: 0.435 I vs III: 0.283 II vs III: 0.778
Sex (female/male)	56/8	58/6	59/5	0.664 I vs II: 0.571 I vs III: 0.38 II vs III: 0.752
BMI (kg/m2)	20.01±2.52	19.9±2.19	21.08±2.54	0.106 I vs II: 0.853 I vs III: 0.07 II vs III: 0.052
Resistin (ng/ml)	56.68±16.58	23.98±6.19	12.33±3.88	<0.001* I vs II:<0.001* I vs III:<0.001* II vs III:<0.001*
ESR (mm/h)	37.34±20.95	27.69±16.95	12.03±2.49	<0.001* I vs II: 0.047* I vs III <0.001* II vs III:<0.001*
CRP (mg/dl)	16.39±16.67	9.15±11.72	3.38±1.2	<0.001* I vs II: 0.049* I vs III <0.001* II vs III: 0.007*
RF (IU/ml)	242.46±243.9	52.88±32.53	-	<0.001*
ACPA (IU/ml)	73.47±15.81	51.71±19.53	-	0.001*
DAS28	4.45±1.46	3.54±1.92	-	0.001*
Disease duration (ys)	7.05±4.36	6.84±6.15	-	0.879

BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibodies; DAS28, Disease Activity Score including 28 joints.

** Statistically significant at P < 0.05*

Table.2 Correlations of serum resistin level with clinical and biochemical parameters of RA patients

Parameters	*R*	*P* value
Age	0.074	0.563
BMI	0.029	0.819
ESR	0.374	0.002*
CRP	0.385	0.002*
RF	0.462	0.001*
ACPA	0.68	<0.001*
DAS28	0.458	<0.001*
Disease duration	-0.051	0.687

*BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibodies; DAS28, Disease Activity Score including 28 joints. * Statistically significant at P < 0.05*

Table.3 Frequencies of RETN –420C>G in RA patients and healthy controls

Genotypes	RA Cases n = 128	Controls n = 64	*P* value	OR	95 % CI
CC	47 (36.72)	35 (54.69)			
CG	68 (53.13)	27 (42.19)	0.049*	1.88	1-3.5
GG	13 (10.15)	2 (3.12)	0.046*	4.84	1.03-22.84
C-allele	162 (63.28)	97 (75.78)			
G-allele	94 (36.72)	31 (24.22)	0.014*	1.82	1.13-2.93

*Data represented as n (%). OR, odd ratio; 95% CI, 95% confidence interval. * Statistically significant at P < 0.05*

Table.4 Relation between RETN –420C>G polymorphism and the clinical and biochemical characteristics of all RA patients (n = 128)

Parameters	CC (group IV) n = 47	CG (group V) n = 68	GG (group VI) n = 13	*P* value
Age (ys)	38.81±9.82	42.63±7.53	37.86±6.26	IV vs V: 0.103 IV vs VI: 0.064
Sex (female/male)	43/4	62/6	9/4	IV vs V: 0.953 IV vs VI: 0.037*
BMI (kg/m^2)	19.89±2.43	19.75±2.46	21.1±1.03	IV vs V: 0.824 IV vs VI: 0.213
Resistin (ng/ml)	39.28±23.18	41.45±18.34	60.98±23.71	IV vs V: 0.695 IV vs VI: 0.035*
ESR (mm/h)	30.04±17.7	27.73±15.78	62.57±15.64	IV vs V: 0.605 IV vs VI: <0.001*
CRP (mg/dl)	7.55±5.77	16.52±18.02	16.84±18.94	IV vs V: 0.017* IV vs VI: 0.031*
RF (IU/ml)	154.58±207.62	143.7±192.79	204.86±200.426	IV vs V: 0.651 IV vs VI: 0.586
ACPA (IU/ml)	56.5±24.31	65.21±14.67	80.57±18.1	IV vs V: 0.271 IV vs VI: 0.673
DAS28	3.8±1.3	4.51±1	5.17±0.73	IV vs V: 0.115 IV vs VI: 0.012*
Disease duration (ys)	7.54±6.13	7.03±4.76	4.29±3.45	IV vs V: 0.729 IV vs VI: 0.19

*BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibodies; DAS28, Disease Activity Score including 28 joints. * Statistically significant at P < 0.05*

Table.5 Relation between RETN –420C>G polymorphism and the parameters of the disease activity in active RA patients (n = 64)

Parameters	CC (group VII) n = 26	CG (group VIII) n = 30	GG (group IX) n = 8	*P* value
Resistin (ng/ml)	60.66±14.02	57.08±12.65	79.73±1.12	VII vs VIII: 0.485 VII vs IX: 0.018*
ESR (mm/h)	39.23±17.2	28±17.7	66.25±17.97	VII vs VIII: 0.102 VII vs IX: 0.016*
CRP (mg/dl)	9.58±7.36	19.6±19.86	26.5±20.62	VII vs VIII: 0.098 VII vs IX: 0.02*
RF (IU/ml)	268.22±260.03	228.82±248.54	286±244.07	VII vs VIII: 0.61 VII vs IX: 0.91
ACPA (IU/ml)	77.5±13.92	64.78±15.41	87±6.48	VII vs VIII: 0.128 VII vs IX: 0.91
DAS28	3.75±1.4	5.27±0.37	5.58±0.17	VII vs VIII: 0.139 VII vs IX: 0.022*

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibodies; DAS28, Disease Activity Score including 28 joints.
** Statistically significant at P < 0.05*

Table.6 Relation between RETN –420C>G polymorphism genotypes and the autoimmune antibodies in all RA patients (n = 128)

Genotypes	RF + ve Cases n = 101	RF - ve Cases n = 27	*P* value	OR	95 % CI
CC	38 (37.62)	9 (33.33)			
CG	52 (51.49)	16 (59.26)	0.576	0.77	0.31-1.93
GG	11 (10.89)	2 (7.41)	0.757	1.3	0.24-6.94
C-allele	128 (63.37)	34 (62.96)			
G-allele	74 (36.63)	20 (37.04)	0.956	0.98	0.53-1.83
Genotypes	**ACPA +ve Cases n = 65**	**ACPA -ve Cases n = 63**	***P* value**	**OR**	**95 % CI**
CC	24 (36.92)	23 (36.51)			
CG	28 (43.08)	40 (63.49)	0.296	1.05	0.32-1.42
GG	13 (20)	0 (0)	-	-	-
C-allele	76 (58.46)	86 (68.25)			
G-allele	54 (41.54)	40 (31.75)	0.105	1.53	0.92-2.55

Data represented as n (%). OR, odd ratio; 95% CI, 95% confidence interval.
** Statistically significant at P < 0.05*

This demonstrate that the *RETN* –420C>G variant might be associated not only with the increased occurrence, but also with the progression of RA. On the contrary, this SNP was not associated withthe studied autoimmune antibodies of RA (RF and

ACPA) (Table 6). This refers to the possible link between resistin and the rheumatoid inflammation rather than the autoimmunity. This may be supported by Fadda *et al.*, who reported that resistin levels were significantly higher in the serum and synovial fluid of RA (an inflammatory rheumatologic disease) patients than in those with osteoarthritis (a degenerative rheumatologic disease) (fadda *et al.*, 2013).

In conclusion, this study highlights for the first time, the possible role of *RETN* – 420C>G polymorphism as a risk factor for the development of RA and disease activity. Further large studies are needed to confirm our results.

References

Alkady, E.A., Ahmed, H.M., Tag, L., and Abdou, M.A. 2011. Serum and synovial adiponectin, resistin, and visfatin levels in rheumatoid arthritis patients. Relation to disease activity. *Z. Rheumatol.*, 70(7): 602-608.

Arnett, F.C., Edworthy, S.M., Bloch, D.A., Mcshane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H. Luthra, H.S., Medsger, T.A., Mitchell, D.M., Neustadt, D.H., Pinals, R.S., Schaller, J.G., Sharp, J.T., Wilder, R.L. and Hunder, G.G. 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.*, 31: 315-324.

Azuma, K., Oguchi, S., Matsubara, Y., Mamizuka, T., Murata, M., Kikuchi, H., Watanabe, K., Katsukawa, F., Yamazaki, H., Shimada, A. and Saruta, T. 2004. Novel resistin promoter polymorphisms: association with serum resistin level in Japanese obese individuals. *Hormone and Metabolic Res.*, 36: 564–570.

Baba, T., Endo, T., Sata, F., Nagasawa, K., Honnma, H., Kitajima, Y., Hayashi, T., Manase, K., Kanaya, M., Moriwaka, O., Kamiya, H., Yamada, H., Minakami, H., Kishi, R., and Saito, T. 2009. The contributions of resistin and adiponectin gene single nucleotide polymorphisms to the genetic risk for polycystic ovary syndrome in a Japanese population. *Gynecol. Endocrinol.*, 25(8): 498-503.

Bokarewa, M., Nagaev, I., Dahlberg, L., Smith, U., and Tarkowski, A. 2005. Resistin, an adipokine with potent proinflammatory properties. *J. Immunol.*, 174(9): 5789-5795.

Cepica, S., Rohrer, G. A., Masopust, M., Kubickova, S., Musilova, P., and Rubes, J. 2002. Partial cloning, cytogenetic and linkage mapping of the porcine resistin (RSTN) gene. *Anim. Genet.*, 33: 381–383.

Cho, Y.M., Youn, B.S., Chung, S. S., Kim, K.W., Lee, H.K., Yu, K.Y., Park, H. J., Shin, H. D., and Park, K.S. 2004. Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia*, 47(3): 559-565.

DaMota, L.M.H., dos Santos Neto, L.L., and de Carvalho, J.F. 2009. Autoantibodies and other serological markers in rheumatoid arthritis: predictors of disease activity? *Clin. Rheumatol.*, 28: 1127.

Dong, X.Q., Du, Q., Yu, W. H., Zhang, Z. Y., Zhu, Q., Che, Z. H., Wang, H., Chen, J, Yang, S. B. and Wen, J. F. 2012. Plasma resistin, associated with single nucleotide polymorphism -420, is correlated with C-reactive protein in Chinese Han patients with spontaneous basal ganglia hemorrhage. *Genet. Mol. Res.*, 11 (3): 1841-1850.

El-Shal, A.S., Pasha, H.F., and Rashad, N. M. 2013. Association of resistin gene polymorphisms with insulin resistance in Egyptian obese patients. *Gene*, 515: 233–238.

Engert, J.C., Vohl, M.C., Williams, S.M., Lepage, P., Loredo-Osti, J.C., Faith, J.,

Dore, C., Renaud, Y., Burtt, N.P., Villeneuve, A., Hirschhorn, J.N., Altshuler, D., Groop, L. C., Despres, J. P., Gaudet, D., and Hudson, T. J. 2002. 5' flanking variants of resistin are associated with obesity. *Diabetes,* 51: 1629–634.

Fadda, S.M.H., Gamal, S.M., Elsaid, N.Y., and Mohy, A.M. 2013. Resistin in inflammatory and degenerative rheumatologic diseases. Relationship between resistin and rheumatoid arthritis disease progression. *Z. Rheumatol.,* 72(6): 594-600.

Hlavna, M., Kohut, L., Lipkova, J., Bienertova-Vasku, J., Dostalova, Z., Chovanec, J., and Vasku, A. 2011. Relationship of resistin levels with endometrial cancer risk. *Neoplasma,* 58(2): 124-128.

Hossein-Nezhad, A., Varzaneh, F. N., Mirzaei, K., Emamgholipour, S., Varzaneh, F. N., and Sahraian, M. A. 2013. A polymorphism in the resistin gene promoter and the risk of multiple sclerosis. *Minerva Med.,* 104(4): 431-438.

Hussain, S., Asghar, M., and Javed, Q. 2010. Resistin gene promoter region polymorphism and the risk of hypertrophic cardiomyopathy in patients. *Transl. Res.,* 155(3): 142-147.

Hussain, S., Faraz, A., and Iqbal, T. 2015. The RETN gene rs1862513 polymorphism as a novel predisposing marker for familial Acne vulgaris in a Pakistani population. *Iran. J. Basic Med. Sci.,* 18(5): 526–528.

Hussain, S., Haroon, J., Ejaz. S., and Javed, Q. 2016. Variants of resistin gene and the risk of idiopathic dilated cardiomyopathy in Pakistan. *Meta Gene.,* 9: 37–41.

Fasshauer, M., Klein, J., Neumann, S., Eszlinger, M., and Paschke, R. 2001. Tumor necrosis factor alpha is a negative regulator of resistin gene expression and secretion in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.,* 288: 1027-1031.

Feldmann, M., Brennan, F. M., and Maini, R.N. 1996. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.,* 14: 397-440.

Fransen, J., and van Riel, P. L. 2005. The Disease Activity Score and the EULAR response criteria. *Clin. Exp. Rheumatol.,* 23: S93-S99.

Kaplan, M.J. 2006. Cardiovascular disease in rheumatoid arthritis. *Curr. Opin. Rheumatol.,* 18: 289–297.

Kawanami, D., Maemura, K., Takeda, N., Harada, T., Nojiri, T., Imai, Y., Manabe, I., Utsunomiya, K., and Nagai, R. 2004. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. *Biochem. Biophys. Res. Commun.,* 314: 415–419.

Kaser, S., Kaser, A., Sandhofer, A., Ebenbichler, C. F., Tilg, H., and Patsch, J. R. 2003. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. *Biochem. Biophys Res Commun.,* 309: 286-290.

Kassem, E., Mahmoud, L., and Salah, W. 2010. Study of resistin and YKL-40 in rheumatoid arthritis. *J. American Sci.,* 6(10).

Klein-Wieringa, I.R., van der Linden, M.P.M., Knevel, R., Kwekkeboom, J.C., van Beelen, E., Huizinga, T.W.J., van der Helm-van Mil, A., Kloppenburg, M., Toes, R. E. M., and Ioan-Facsinay, A.2011. Baseline Serum adipokine levels predict radiographic progression in early rheumatoid arthritis. *Arthritis Rheum.* 63(9): 2567-2574.

Koerner, A., Kratzsch, J., and Kiess, W. 2005. Adipocytokines: leptin--the classical, resistin--the controversical, adiponectin--the promising, and more to come. *Best Pract. Res. Clin. Endocrinol. Metab.,* 19: 525–546.

Krysiak, R., Handzlik-Orlik, G., and Okopien, B. 2012. The role of adipokines in

connective tissue diseases. *Eur. J. Nutr.*, 51: 513-528.

Kunnari, A., Ukkola, O., and Kesaniemi, Y. A. 2005. Resistin polymorphisms are associated with cerebrovascular disease in Finnish type 2 diabetic patients. *Diabet. Med.*, 22: 583–589.

Lau, C.H., and Muniandy, S. 2011. Adiponectin and resistin gene polymorphisms in association with their respective adipokine levels. *Ann. Hum. Genet.*, 75(3): 370-382.

Lu, S.C., Shieh, W.Y., Chen, C.Y., Hsu, S. C., and Chen, H.L. 2002. Lipopolysaccharide increases resistin gene expression in vivo and invitro. *FEBS Lett.*, 530: 158-162.

Maachi, M., Pieroni, L., Bruckert, E., Jardel, C., Fellahi, S., Hainque, B., Capeau, J., and Bastard, J.P. 2004. Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. *Int. J. Obes. Relat. Metab. Disord.*, 28: 993–997.

Mahmoudi, T., Karimi, K., Arkani, M., Farahani, H., Vahedi, M., Dabiri, R., Nobakht, H., Asadi, A., Mirakhorli, M., Arshi, B. Derakhshan, A., and Zali, M. R. 2014. Resistin -420C>G promoter variant and colorectal cancer risk. *Int. J. Biol. Markers*, 29(3): 233–238.

Mattevi, V.S., Zembrzuski, V.M., and Hutz, M.H. 2004. A resistin gene polymorphism is associated with body mass index in women. *Human Genetics*, 115: 208–212.

Menzaghi, C., Coco, A., Salvemini, L., Thompson, R., De Cosmo, S., Doria, A., and Trischitta, V. 2006. Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. *JCEM*, 91(7): 2792–2795.

Migita, K., Maeda, Y., Miyashita, T., Kimura, H., Nakamura, M., Ishibashi, H., and Eguchi, K. 2006.The serum levels of resistin in rheumatoid arthritis patients. *Clin. Experimental Rheumatol.*, 24: 698-701.

Osawa, H., Yamada, K., Onuma, H., Murakami, A., Ochi, M., Kawata, H., Nishimiya,T., Niiya, T., Shimizu, I., Nishida, W., Hashiramoto, M., Kanatsuka, A., Fujii, Y., Ohashi, J., and Makino, H. 2004. The G/G genotype of a resistin singlenucleotide polymorphism at –420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. *Am. J. Hum. Genet.*, 75: 678–686.

Osawa, H., Tabara, Y., Kawamoto, R., Ohashi, J., Ochi, M., Onuma, H., Nishida, W., Yamada, K., Nakura, J., Kohara, K., Miki, T., and Makino, H. 2007. Plasma resistin, associated with single nucleotide polymorphism -420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. *Diabetes Care*, 30(6): 1501-1506.

Patel, S. P., and Raju, P. A. 2014. Gingival crevicular fluid and serum levels of resistin in obese and non-obese subjects with and without periodontitis and association with single nucleotide polymorphism at −420. *J. Indian SocPeriodontol.*, 18(5): 555–559.

Pilz, S., Weihrauch, G., Seelhorst, U., Wellnitz, B., Winkelmann, B. R., Boehm, B. O., and März, W. 2007. Implications of resistin plasma levels in subjects undergoing coronary angiography. *Clin. Endocrinol.*, (Oxf). 66: 380–386.

Qasim, A. N., Metkus, T. S., Tadesse, M., Lehrke, M., Restine, S., Wolfe, M. L., Hannenhalli, S. Cappola, T., Rader, D. J. and Reilly, M. P. 2009. Resistin gene variation is associated with systemic inflammation but not plasma adipokine levels, metabolic syndrome or coronary atherosclerosis in nondiabetic Caucasians. *Clin. Endocrinol., (Oxf).* 70(5): 698–705.

Šenolt, L., Housa, D., Vernerová, Z., Jirásek, T., Svobodová, R., Veigl, D., Anderlová, K., Müller-Ladner, U., Pavelka, K., and Haluzík, M. 2007a. Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum. *Ann. Rheum. Dis.*, 66(4):458-463.

Šenolt, L., Housa, D., Vernerová, Z., Jirásek, T., Svobodová, R., Veigl, D., Ladner-Müller, U., Pavelka, K. and Haluzík, M. 2007b. Resistin is abundantly present in rheumatoid arthritis synovial tissue, synovial fluid, and elevated serum resistin reflects disease activity. *Ann. Rheum. Dis.*, 66: 458–463.

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel, H. R, Ahima, R. S., and Lazar, M. A. 2001. The hormone resistin links obesity to diabetes. *Nature*, 409: 307-312.

Tang, N.P., Wang, L.S., Yang, L., Zhou, B., Gu, H. J., Sun Q. M., Cong R. H., Zhu, H. J. and Wang, B. 2007. A polymorphism in the resistin gene promoter is related to increased C-reactive protein levels in patients with coronary artery disease. *Clin. Chem. Lab. Med.*, 45(11): 1471–1475.

Tilg, H., and Moschen, A. R. 2006.Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.*, 6: 772–783.

Ukkola, O., Kunnari, A., and Kesäniemi, Y. A. 2008. Genetic variants at the resistin locus are associated with the plasma resistin concentration and cardiovascular risk factors. *Regulatory Peptides*, 149(1-3): 56–59.

Verma, U.N., Misra, R., Singh, R. R., Agarwal, S.S., and Naik, S. 2002. Serological correlates of inflammation in rheumatoid arthritis: usefulness of acute phase reactants in monitoring disease activity. *J. Indian Rheumatol. Assoc.*, 10: 1-4.

Westrgren, A. 1975. Diagnostic tests: the erythrocyte sedimentation rate range and limitations of the technique. *Triangle*, 3(1): 20-25.

Xita, N., Georgiou, I., Tsatsoulis, A., Kourtis, A., Kukuvitis, A. and Panidis, D. 2004. A polymorphism in the resistin gene promoter is associated with body mass index in women with polycystic ovary syndrome. *Fertility and Sterility*, 82: 1466–1467.

Yoshino, T., Kusunoki, N., Tanaka, N., Kaneko, K., Kusunoki, Y., Endo, H., Hasunuma, T., and Kawai, S. 2011. Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. *Intern Med.*, 50: 269-275.

How to cite this article:

Marwa M.E. Abd-Elmonsef, Naglaa F. Ghoname, Abeer Shahba, Maha M. Hagras, Lamees M. Dawood and Mohamed M.E. Abd-Elmonsef. 2016. Association of the RETN –420C>G Polymorphism with Rheumatoid Arthritis in an Egyptian Population. *Int.J.Curr.Microbiol.App.Sci.* 5(11): 183-195. doi: http://dx.doi.org/10.20546/ijcmas.2016.511.021