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

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ABSTRACT

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. Serum 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.

Keywords
Rheumatoid arthritis, RETN gene, polymorphism, resistin.

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 × √28TJC + 0.28 × √28SJC + 0.7 × In ESR + 0.014 × 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/m².

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 assay 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 (Westergren, 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, CRP and 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 a significant 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 association 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., 2002). 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* |

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  | 1.3-3.5|
| GG        | 13 (10.15)       | 2 (3.13)        | 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|

C-allele: 36.7% in RA vs 25.8% in controls; G-allele: 63.3% in RA vs 74.2% in controls; * Statistically significant at P < 0.05

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/m²²)     | 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: 0.02* |

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) | | 0.576 | 0.77 | 0.31-1.93 |
| CG | 52 (51.49) | 16 (59.26) | | 0.757 | 1.3 | 0.24-6.94 |
| GG | 11 (10.89) | 2 (7.41) | | 0.956 | 0.98 | 0.53-1.83 |
| C-allele | 128 (63.37) | 34 (62.96) | | | | |
| G-allele | 74 (36.63) | 20 (37.04) | | | | |

| Genotypes | ACPA +ve Cases n = 65 | ACPA -ve Cases n = 63 | P value | OR | 95 % CI |
|------------|---------------------|---------------------|---------|----|---------|
| CC | 24 (36.92) | 23 (36.51) | | 0.296 | 1.05 | 0.32-1.42 |
| CG | 28 (43.08) | 40 (63.49) | | | | |
| 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 with the 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.

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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