Association of interleukin-4 gene polymorphism and rheumatoid arthritis in Egyptian patients

Seham M.S. El Nakeeb[a], Reem M. Ahmed[a], Alzahraa A. Elsied[a], Mohsen M. Ali Elsied[b]

Introduction Rheumatoid arthritis (RA) is a progressive disease characterized by chronic joint inflammation and subsequent structural damage. Interleukin (IL)-4-590 promoter polymorphism (rs2243250), a C-to-T base substitution, has been suggested to be associated with RA and has become of great interest to be investigated.

Aim The aim of this study was to find the relationship between IL-4-590 promoter polymorphism and RA in Egyptians, and also to study the relationship of this gene with clinical and laboratory features of the disease.

Patients and methods This study was carried on 180 subjects divided into two groups. The first group included 120 patients with RA and the second group were 60 apparently healthy individuals as controls. Genomic DNA was extracted from blood leukocytes of both groups and genotyped by PCR for amplification of IL-4 gene followed by restriction fragment length polymorphism.

Results IL-4-590 (TT) genotype was significantly more frequent in patients with RA than controls (10 vs. 1.70%, P=0.027, odd ratio (OR)=7.543 and Confidence interval (CI)=0.947–60.049). IL-4-590 (CT) genotype showed no significant difference between patients with RA and controls (31.70 vs. 25%, P=0.195 OR=1.592 and CI=0.786–3.228), whereas IL-4-590 (CC) genotype was significantly less frequent in patients with RA than controls (58.30 vs. 73.30%, P=0.048). Regarding the distribution of different alleles, the frequency of T allele was significantly more in patients with RA than controls ($P<0.01$). In patients with RA, there were significant differences in some clinical and laboratory parameters of RA disease between different IL-4-590 genotypes (e.g. number of tender and swollen joints, duration of morning stiffness, disease activity score 28, serum rheumatoid factor, serum C-reactive protein, and serum anticyclic citrullinated peptide levels), all were higher in TT genotype, which means patients with RA with TT genotype may have more aggressive course of the disease.

Conclusion The T allele and the TT genotype at position −590 of IL-4 gene may be related to development of RA in Egyptians and may be associated with the disease activity.

Sci J Al-Azhar Med Fac, Girls 2019 3:308–316
© 2019 The Scientific Journal of Al-Azhar Medical Faculty, Girls

The Scientific Journal of Al-Azhar Medical Faculty, Girls
2019 3:308–316

Keywords: gene polymorphism, interleukin-4, rheumatoid arthritis

Departments of, [a]Biochemistry, Faculty of medicine (for girls), AL-AzharUniversity, [b]Rheumatology, Al-SayedGalal hospital, Faculty of medicine, Azhar university, Cairo, Egypt

Correspondence to Seham M.S. El Nakeeb, MD in Biochemistry, Professor and Head of Medical Biochemistry Department, Faculty of medicine (for girls), AL-AzharUniversity, Cairo, Egypt.
e-mail: dr.seham_2009@hotmail.com

Received 9 March 2019 **Accepted** 16 June 2019

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic joint inflammation and subsequent structural damage. There may be a ‘window of opportunity’ in early RA to alter the course of the disease if tightly controlled, which diminishes the inflammatory processes. Once RA is well controlled, the ability to sustain remission following the withdrawal of immune-modulatory medications would be an indication of disease modification [1,2]. Although many aspects of RA pathogenesis remain unknown, it is widely accepted that RA onset and response to treatments are determined by the interaction between genetic variations and environmental factors [3]. Dysregulation of the immune responses and the imbalance between proinflammatory and anti-inflammatory cytokines can cause RA, and the production of these cytokines can be influenced by single nucleotide polymorphisms (SNPs) within immune-modulating genes [4]. Interleukin (IL)-4 is the first discovered B-cell pleiotropic cytokine that promotes differentiation of T cells and regulates antibodies production from B cells and plays an important role in the immune system [5].

IL-4, a potent anti-inflammatory cytokine, is produced by activated CD4 lymphocytes, mast cells, and basophils. It exerts immune-modulatory functions on different cell types [6]. It has immunosuppressive action, as it can inhibit inflammation, graft rejection, and other immune responses caused by Th1. IL-4 has a long-term inhibitory effect on Th1 cells in the microenvironment [7]. In addition, IL-4 can inhibit the mRNA transcription of interferon-γ and suppress interferon-γ-inducing B cells to generate immunoglobulin (Ig)G antibody, thereby inhibiting interferon-γ from exerting its biological effects. IL-4

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

increases IgE production and serves as an important regulator of IgG isotypes switching [8,9]. Furthermore, it inhibits the production of destructive protease enzymes and proinflammatory cytokines, such as IL-1, by increasing the expression of IL-1 receptor antagonist [10].

IL-4 is an important factor in the polarization of T helper cells toward Th2 differentiation which is deficient in rheumatoid synovial tissues [11]. Several studies suggest that IL-4 and its receptor may control the inflammation induced by Th17, a third CD4T-cell population, which plays a central role in the pathogenesis of human autoimmune diseases, including RA [12–15].

High expression of intra-articular IL-4 by retroviral transduction in an arthritis model induced clinical and radiological improvement of the arthritis. Moreover, administration of monoclonal antibodies against IL-4 in a mouse model of collagen-induced arthritis resulted in greater severity of disease, so there is a protective role of IL-4 in this model [16].

IL-4 gene has been mapped to the q arm (q23–q31) of chromosome 5 [17]. Polymorphisms affecting gene of IL-4 can be linked with RA risk and has become of great interest to researchers [18]. IL-4 promoter polymorphism, a C-to-T base substitution at −590 position (rs2243250) relative to the transcriptional site, has been identified [19] and has been suggested to be associated with RA [20–22]. Many studies examined the association of IL-4 gene polymorphisms with RA but their data are conflicting [23–27]. Moreover, there is limitation of information about IL-4 polymorphism and its association with RA in different ethnicities [28].

This study aimed to identify the association between this SNP (a C-to-T base substitution) of the IL-4-590 promoter region and RA disease in Egyptians and also to study its relationship to some clinical and laboratory features of the disease.

Patients and methods

Patients

This study was carried on 180 subjects divided into two groups: the first group included 120 (55 male and 65 female) Egyptian patients with RA with ages ranged from 36 to 61 years old; attending the clinic of Rheumatology Departments in Al-Sayed Galal Hospital, Al-Azhar University, Cairo, Egypt, in the period from November 2015 till August 2017. They had definite diagnosis of RA, according to American College of Rheumatology and the European League Against Rheumatism criteria (2010) [29], supported by clinical examination and plain radiograph of the affected joints, and all patients have nonerosive RA. The second group included 60 (19 male and 41 female) apparently healthy individuals as a control group with ages ranged from 35 to 63 years old with no personal or family history of autoimmune diseases or arthritis. They were age, sex, and ethnically matched with patients with RA.

The two groups were subjected to the following: full history taking; clinical examination, including general and joints examination, and plain radiograph for both hands and wrists joints; and routine laboratory investigations for those patients, including complete blood picture, erythrocyte sedimentation rate (ESR), detection of rheumatoid factor (RF) using latex agglutination test, measurement of high-sensitive C-reactive protein (CRP) using ELISA kits, and detection of anticyclic citrullinated peptide (anti-CCP) antibodies using ELISA kits. Modified disease activity score including 28 joint count (DAS28 score) [30] was calculated for all patients with RA using three variables (number of tender joints, number of swollen joints, and ESR).

The protocol of this study was approved by the Medical Ethics Committee at the Faculty of Medicine, Al-Azhar University, Cairo, and a written informed consent was obtained from all patients and healthy controls.

Exclusion criteria

Patients with clinical evidence of other autoimmune disease or allergic diseases.

Specific investigations

Genomic DNA extraction from blood leukocytes was done, genotyping was done by PCR for amplification of IL-4 gene, followed by restriction fragment length polymorphism (RFLP).

Sample preparation

Ten milliliters of venous blood was drawn and divided as follows: 5 ml was added in a sterile plain tube to obtain serum for serological investigations, and the other 5 ml of blood was added to a EDTA sterile tube and was used for molecular testing of the IL-4-590 promoter polymorphism.

Molecular analysis using PCR-restriction fragment length polymorphism technique

Genomic DNA was extracted from EDTA whole blood samples in two steps; leukocytes separation step and the DNA extraction step using quik-g

DNA Miniprep extraction kit (catalog no. D3024, lot no. ZRC186005; The Epigenetic Company, USA). DNA was stored at −20°C till the time of use. Amplification of IL-4-590 promoter polymorphism was done in the following steps:

(1) The region surrounding this polymorphism was amplified with the following primers: forward primer: 5′d ACTAGGCCTCAC CTGA TACG-3′ and reverse primer: 5′d GTTGTA ATGCAGTC CTCCTG-3′ (To have a concentration of 100 p moles/μl of each primer, it was diluted with deionized water in a percentage of 1/10. A volume of 90 μl of deionized water was used to reconstitute the forward primer and another 90 μl was used for the reverse primer vials. After reconstitution, primers were kept at −20°C for subsequent runs).

(2) PCR was carried out on genomic DNA, 100 pmol/μl of each PCR primer, and 1× PCR mix (Dream Taq Green PCR Master Mix #k0181; Thermoscientific, USA), containing 200 μmol/l of each dNTP, 2× Dream Taq PCR buffer, 1.25 U Taq DNA polymerase, and 4 mmol of $MgCl_2$. Thermal cycling conditions were carried out as follows: an initial cycle at 94°C for 5 min (for initial denaturation); 32 cycles under the conditions of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s; and a final cycle at 72°C for 5 min (for final extension). The amplified fragment was 252 bp [31]. PCR product was visualized as shown in Fig. 1. After PCR amplification was performed, the amplified product was digested with fast digest BsmF-1 restriction endonuclease (Thermoscientific), through gentle mixing and incubation at 37°C for 15 min, then inactivation at 80°C for 20 min. The digested products were separated by 3% agarose gel electrophoresis and visualized using UV trans-illuminator. The resulting 252-bp amplified fragment might contain a restriction site for BsmF-1 restriction enzyme, which generated two fragments of 192 and 60 bp after digestion. Thus the following possibilities were present:

(a) If there was one band (252 bp) it was designated.

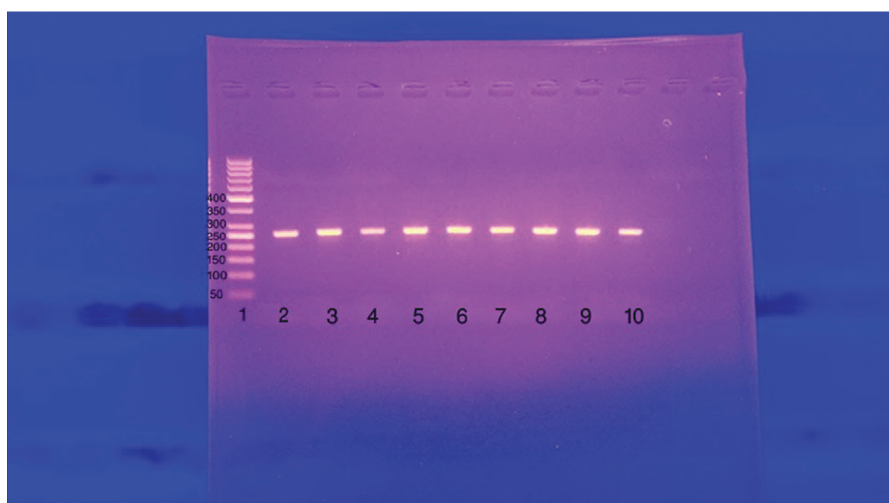
(b) If there were two bands (192 and 60 bp) it was designated CC.

(c) If there were three bands (252, 192 and 60 bp) it was designated CT (i.e. heterozygous genotype), as shown in Figs 2 and 3.

Statistical analysis

Data were analyzed by Microsoft Office 2003 (Excel), SPSS version 16 and statistical package for the social sciences (SPSS) version 16. The analysis of data was done to test statistical significant difference between groups. For qualitative data [frequency and proportion] χ^2-test was used. For quantitative data normally distributed (mean±SD), analysis of variance was used to compare between the groups followed by post-hoc analysis using Least significant differences (LSD) test. P value up to 0.05 is considered significant. For allelic and genotypic frequencies, Hardy–Weinberg equation was used.

Figure 1



Agarose gel electrophoresis showing PCR product-based restriction fragment length polymorphism analysis of IL-4-590 gene amplification guided by marker in the first lane. (1) The first lane is 50 bp ladder (marker). (2) Lane 2–10 represent one band 252 bp PCR product before the action of restriction enzyme BsmF-1, performed on 3% agarose and visualized by UV trans-illumination.

Figure 2

Agarose gel electrophoresis showing PCR product-based restriction fragment length polymorphism analysis of IL-4-590 gene polymorphism digested byBsmF-1guided by marker in the first lane. (1) The first lane is 50 bp ladder (marker). (2) CT genotype: lanes 2, 4, and 8, (there were three bands: 252, 162, and 90 bp). (3) CC genotype: lanes 3, 5, 6, 7, 9, and 10 (there were two bands: 162 and 90 bp).

Figure 3

Agarose gel electrophoresis showing PCR product-based restriction fragment length polymorphism analysis of IL-4-590 gene polymorphism digested by BsmF-1 guided by marker in the first lane. (1) The first lane is 50-bp ladder (marker). (2) TT genotype: lanes 3 and 7 (one band 252 bp). (3) CT genotype: lanes 2, 4, 8, and 10 (there were three bands: 252, 162, and 90 bp). (4) CC genotype: lanes 5, 6, and 9 (there were two bands: 162, 90 bp).

Table 1 Descriptive data for patients with rheumatoid arthritis

	Range	Mean±SD
Age (years)	36–61	48.77±7.17
Sex		
Female (*n*, %)	65 (54.20)	
Males (*n*, %)	55 (45.80)	
Disease duration (years)	3–17	9.43±3.68
Morning stiffness (min)	45–205	88.67±39.43
Number of tender joints	5–18	10.18±3.56
Number of swollen joints	1–8	4.29±1.56
ESR (mm/h)	20–80	43.81±13.71
RF (IU/ml)	69–143	104.28±15.94
CRP (mg/l)	8–43	18.99±4.96
Anti-CCP (μ/ml)	8–90	42.22±17.18
DAS28 score	4.61–6.67	5.05±0.65

Anti-CCP, anti-citrullinated peptide antibodies; CRP, C-reactive protein; DAS28, disease activity score including 28 joint count; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

Results

(1) Agarose gel electrophoresis is shown in Figs 1–3. Figure 1 shows PCR product-based RFLP analysis of IL-4-590 gene before the action of restriction enzyme. Figures 2 and 3 show PCR product-based RFLP analysis of IL-4-590 gene after the action of restriction enzyme.

(2) The descriptive data for patients with RA are shown in Table 1, and a comparison between patients with RA and controls is shown in Table 2. There were no significant differences in sex ratio and age between patients with RA and controls ($P>0.05$).

(3) The genotypes distribution and allele frequencies of IL-4-590 promoter polymorphism in patients with RA and controls are shown in Table 3. The frequency of TT genotype was significantly higher in patients with RA (10.00%) compared with controls (1.70%), whereas there was no significant difference between patients with RA and controls in the distribution of CT genotype (31.70 vs. 25.00%). Frequency of CC genotype was significantly lower among patients with RA than controls (58.30 vs. 73.30%).

(4) Table 4 shows a comparison between IL-4-590 different genotypes regarding some clinical and laboratory parameters of patients with RA. There was no significant difference between IL-4-590 different genotypes in age, sex, and disease duration ($P<0.05$), but there was a highly significant difference in morning stiffness, number of tender joints, and DAS28 score

Table 2 Comparison between control group and rheumatoid arthritis group

	Control group (*n*=60)	Rheumatoid arthritis group (*n*=120)	*P* value
Age			
Mean±SD	46.77±6.89	48.77±7.17	0.076
Range	35–63	36–61	
Sex			
Females (n, %)	41 (68.3)	65 (54.20)	0.069
Males (n, %)	19 (31.7)	55 (45.80)	
Erythrocyte sedimentation rate (mm/h)			
Mean±SD	4.97±1.86	43.81±13.71	0.001
Range	1–10	20–80	
Rheumatoid factor (IU/ml)			
Mean±SD	7.18±2.45	104.28±15.94	0.001
Range	2–15	69–143	
C-reactive protein (mg/l)			
Mean±SD	3.14±1.15	18.99±4.96	0.001
Range	1–6	8–43	
Anticyclic citrullinated peptide			
Positive	0	110	0.001
Negative	60	10	
Anticyclic citrullinated peptide serum level (μ/ml)			
Mean±SD	5.41±1.08	42.22±17.18	0.001
Range	3–7	8–90	

Table 3 Allelic and genotypic frequencies of interleukin-4 promoter gene at position-590 in patients with rheumatoid arthritis and controls

	Controls (*N*=60) [*n* (%)]	Rheumatoid arthritis group (*N*=120) [*n* (%)]	χ^2-Test		Odds ratio	95% confidence interval
			χ^2	*P* value		
Genotypes						
CC	44 (73.30)	70 (58.30)	3.876	0.048	Ref	Ref.
CT	15 (25.00)	38 (31.70)	1.678	0.195	1.592	0.786–3.228
TT	1 (1.70)	12 (10.00)	4.871	0.027	7.543	0.947–60.049
Alleles						
C	103 (85.80)	178 (74.20)	6.357	0.011	2.11	1.171–3.803
T	17 (14.20)	62 (25.80)				

($P<0.01$) and a significant difference in number of swollen joints ($P<0.05$). Moreover, serum CRP, RF, and serum anti-CCP were significantly higher in TT genotype than other genotypes ($P<0.01$).

(5) Post-hoc analysis was done to compare each two genotypes. It showed that TT genotype had significant increase in morning stiffness duration, number of tender and swollen joints, serum CRP, and serum anti-CCP levels than both CC and CT genotypes. DAS28 score was significantly higher in TT genotype than the other two genotypes. Regarding ESR and RF, they were significant higher in TT genotype when compared with CC genotype, but there was no significant difference when compared with CT genotype. There was no significant difference in age, sex ratio distribution, and the disease duration between TT genotype and both CC and CT genotype, as shown in Table 5.

Discussion

RA is a complex autoimmune disease, characterized by synovial inflammation and hyperplasia and cartilage and bone destruction [32]. Cytokine genes or their receptors have received attention as potential markers of susceptibility, severity, and/or protection in RA. Given that RA is a chronic inflammatory process with variable presentation and development, it is possible that these differences are related to individual variations at the level of cytokine production as a result of polymorphic variations of their genes [16]. Various susceptible genes are involved in the early pathogenesis of RA that leads to a series of complex pathophysiological changes. Currently, it is still uncertain which gene plays a decisive role in the pathogenesis and susceptibility of RA [33]. IL-4 and its receptor have been proved to be associated with the development of RA, as diminished production of IL-4 is believed to contribute to the characteristic Th1-mediated autoimmune rheumatoid inflammation [7].

Table 4 Comparison between different genotypes in rheumatoid arthritis group regarding age, sex, disease duration, morning stiffness, number of tender joints and number of swollen joints, disease activity score including 28 joint count score, erythrocyte sedimentation rate, rheumatoid factor, and C-reactive protein

	CC (*N*=70)	CT (*N*=38)	TT (*N*=12)	One-way ANOVA	
				F	*P* value
Age (years)					
Mean±SD	48.87±7.15	48.58±7.12	48.75±8.06	0.020	0.980
Range	37–61	36–60	38–60		
Sex					
Males	33 (47.1)	17 (44.7)	5 (41.7)	0.151	0.927
Females	37 (52.9)	21 (55.3)	7 (58.3)		
Disease duration (years)					
Mean±SD	8.50±3.48	8.89±3.60	9.87±3.73	1.297	0.277
Range	4–13	3–16	4–17		
Morning stiffness (min)					
Mean±SD	56.25±2 1.33	72.63±5.75	102.93±41	14.436	0.001
Range	45–120	45–120	45–205		
Number of tender joints					
Mean±SD	8.58±1.68	9.08±2.73	11.06±3.94	5.535	0.005
Range	7–12	6–17	5–18		
Number of swollen joints					
Mean±SD	3.42±1.16	4.08±1.50	4.56±1.59	3.395	0.037
Range	1–5	1–7	1–8		
Erythrocyte sedimentation rate (mm/h)					
Mean±SD	36.58±7.14	43.03±2.42	45.46±4.86	2.279	0.107
Range	24–47	20–72	20–80		
Rheumatoid factor (IU/ml)					
Mean±SD	88.00±10.10	103.82±4.3	107.31±16.0	8.496	0.001
Range	69–100	83–134	80–143		
C-reactive protein (mg/l)					
Mean±SD	17.71±4.48	20.74±4.90	31.75±8.71	38.42	0.001
Range	8–27	10–30	18–43		
Anticyclic citrullinated peptide					
Positive (*n*, %)	62 (88.6)	37 (97.4)	11 (91.7)	2.495	0.287
Negative (n, %)	8 (11.4)	1 (2.6)	1 (8.3)		
Serum anticyclic citrullinated peptide (μ/ml)					
Mean±SD	31.74±8.58	51.34±8.50	74.42±19.2	115.159*	0.001
Range	8–41	15–60	17–90		
Disease activity score including 28 joint count score					
Mean±SD	5.46±0.45	5.50±0.35	6.06±0.45	10.703	0.005
Range	4.61–6.44	4.96–6.24	5.37–6.67		

*In this table, all comparisons were done by one-way analysis of variance (ANOVA) test except the comparison of serum anticyclic citrullinated peptide level was done by χ^2-test.

Table 5 Post-hoc analysis to compare different genotypes regarding different parameters

	Post-hoc analysis by LSD test		
	P1 TT vs. CT	P2 TT vs. CC	P3 CC vs. CT
Age	0.841	0.957	0.943
Sex	0.970	0.9697	0.833
Disease duration	0.189	0.234	0.746
Morning stiffness	0.001	0.001	0.167
Number of tender joints	0.005	0.023	0.664
Number of swollen joints	0.05	0.018	0. 033
ESR	0.380	0.050	0. 156
RF	0.250	0.001	0.002
CRP	0.001	0.001	0.001
Serum anti-CCP	0.001	0.001	0.001
DAS28 score	0.05	0.001	0.698

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, disease activity score including 28 joint count; ESR, erythrocyte sedimentation rate; LSD, least significant differences; RF, rheumatoid factor.

In this study, IL-4-590 gene polymorphism was studied in Egyptian patients with RA and healthy controls. Regarding the distribution of different genotypes between patients with RA and healthy controls, the TT genotype was statistically significantly more frequent in patients with RA (P=0.027), whereas the CC genotype was statistically significantly more frequent in healthy controls (P=0.048). There was no significant difference found in CT genotype between patients with RA and healthy controls (P=0.195). Regarding the frequency of alleles, the T allele was significantly higher in patients with RA than healthy controls (P<0.01).

This was in accordance with a study conducted by Sun *et al.* [33], who studied the same polymorphism. They revealed that there was a statistically significant difference in CC, CT, and TT genotype distributions between the RA group and the healthy group and the mutation frequency of the T allele in IL-4 of the RA group was significantly higher than that of the healthy group. Moreover, they had found that the mRNA expression of IL-4 in the RA group was significantly lower and was gradually decreased in the order of CC, CT, and TT; the differences among these genotypes were significant.

Canet *et al.* [34] conducted a double-phase case–control study on a population in Spain, and they assessed ~49 genetic variants within or near 17 immune-modulating genes, and among them was IL-4 (–590T/C). They reported that logistic regression analyses showed that carriers of the IL-4 (–590T/T) genotype had a significantly increased risk of developing RA and associated with low serum levels of IL-4 in patients with RA. They suggested that a causative SNP in the IL-4 gene might affect other biological processes such as mRNA processing (splicing or turnover), mRNA stability, and/or mRNA translatability.

Li *et al.* [27] investigated the promoter IL-4-590C/T polymorphism. The genotype distributions and allele frequencies of IL-4-590C/T polymorphism in patients with RA was significantly different from healthy volunteers. They found that the frequency of T allele on IL-4-590 promoter region was significantly increased in patients with RA. They concluded that the IL-4-590 promoter polymorphism may be associated with increased risk of RA and could be used as a genetic marker for assessing the susceptibility and severity of RA in Chinese.

However, in contrary to this results, Trajkov *et al.* [35] revealed that there was positive association of IL-4-590C allele and CC genotype with RA susceptibility in Macedonian patients. Moreover, Qiu *et al.* [36] found a significant association of the C allele of IL-4 (–590C/T) with RA in whites.

In this study, there was a significant differences between the three genotypes regarding some clinical and laboratory parameters of RA disease. Patients with RA with TT genotype have more aggressive course of the disease. They have longer time for morning stiffness and more number of tender and swollen joints than both CC and CT genotypes. Moreover, they have higher serum CRP levels than other genotypes. Moreover, patients with RA having TT genotype with positive anti-CCP have a significant increase in anti-CCP serum level when compared with those having CC or CT genotypes (P<0.01). In addition, patients with RA with TT genotype have significant higher DAS28 score than those with other two genotypes, which means that TT genotype may be associated with RA disease activity.

This was in accordance with a study conducted on Egyptian patients with RA by Hussein *et al.* [31] who studied the association between IL-4 variable number tandem repeat 1/1 (IL-4 VNTR 1/1) and IL-4-590 promoter polymorphism with RA disease susceptibility, severity, and activity. They found in patients with RA, the frequencies of IL-4-590 TT genotype and T allele were significantly increased compared with the control group. Patients with IL-4-590 TT genotype and carriers of T allele were significantly more likely to develop RA. Moreover, they found that patient with TT genotype had a more active RA and lower IL-4 serum level. They concluded that IL-4 VNTR and IL-4-590 promoter polymorphisms may be helpful for assessing RA severity and activity in Egyptians.

Although Pawlik *et al.* [25] had found that the distribution of IL-4-590 genotypes in patients with RA did not differ from control subjects in a study carried on White population from Poland, there was a more severe and active RA form in patients with IL-4-590 TT genotype. The active form of RA was more frequently diagnosed in patients with T allele (genotypes CT and TT) as compared with homozygous CC patients. They found that in carriers of the T allele, parameters of disease activity (DAS28 score, ESR, number of swollen and tender joints) were significantly increased.

Moreno *et al.* [16] found an association of IL-4-590 TT genotype with RA in Colombian population, and

they reported that TT genotype is associated with disease activity and also with early onset of the disease.

Regarding Egyptian population, Hussein *et al.* [31] were the first group who studied this gene polymorphism with RA disease in detail, but still there is shortage of information about this point, and owing to limited sample size in this paper, our results need to be replicated in other Egyptian populations with larger size.

Conclusion

The T allele and the TT genotype at position −590 of IL-4 gene may be related to development of RA in Egyptians and may be associated with disease activity.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Emery P, Burmester GR, Bykerk VP, Combe BG, Furst DE, Barré E, *et al.* Evaluating drug-free remission with abatacept in early rheumatoid arthritis: results from the phase 3b, multicentre, randomized, active-controlled AVERT study of 24 months, with a 12-month, double-blind treatment period. *Ann Rheum Dis* 2015; **74**:19–26.

2 Scrivo R, Difanco M, Spadaro A, Valisini G. The immunology of rheumatoid arthritis. *Ann N Y Acad Sci* 2007; **1108**:312–322.

3 Perricone C, Ceccarelli F, Valesini G. An overview on the genetic of rheumatoid arthritis: a never-ending story. *Autoimmun Rev* 2011; **10**:599–608.

4 Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014; **506**:376–381.

5 Kurk? o J, Besenyei T, Laki J, Glant T, Mikecz T, Szekanecz Z. Genetics of rheumatoid arthritis – a comprehensive review. *Clin Rev Allergy Immunol* 2013; **45**:170–179.

6 Gerli R, Bistoni O, Russano A, Florucci S, Borgato L, Cesarotti M, *et al.* In vivo activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease. *Clin Exp Immunol* 2002; **129**:549–555.

7 Skapenko A, Niedobitek GU, Kalden JR, Lipsky Pechulze-oops H. Generation and regulation of human Th1-biased immune responses in vivo: a critical role for IL-4 and IL-10. *J Immunol* 2004; **172**:6427–6434.

8 Li B, Tournier C, Davis R, Flavell RA. Regulation of Il-4 expression by the transcription factor Jun B during T helper cell differentiation. *EMBO J* 1999; **18**:420–432.

9 Zheng S, Zhu X, Li B, Yang J, *et al.* Correlation of gene polymorphism of interleukin 4 receptor alpha peptide chain and total serum IgE levels in asthmatic children in Guiyang area. *Zhonghua Yi Xue Za Zhi* 2014; **94**:2822–2827.

10 Castro A, Sengupta TK, Ruiz DC, Yang E, Ivashkiv LB. IL-4 selectively inhibits IL-2 triggered Stat5 activation, but not proliferation, in human T cells. *J Immunol* 1999; **162**:1261–1269.

11 Miossec P, van den Berg W. Th1/Th2 cytokine balance in arthritis. *Arthritis Rheum* 1997; **40**:2105–2115.

12 Tesmer LA, Lundy SK, Sarkar S, Fox A. Th17 cells in human disease. *Immunol Rev* 2008; **223**:87–113.

13 Myers LK, Tang B, Stuart JM, Kang AH. The role of IL-4 in regulation of murine collagen-induced arthritis. *Clin Immunol* 2002; **102**:185–191.

14 Wallis S, Cooney L, Endres J, Lee M, Ryu J, Somers E, *et al.* A polymorphism in IL-4 receptor affects the ability of IL-4 to regulate Th17 cells: a possible immunoregulatoty mechanism for genetic control of the severity of rheumatoid arthritis. *Arthritis Res Ther* 2011; **13**:R15.

15 Sarkar S, Tesmer L, Hindnavis V, Endres J, Fox D. Interleukin-17 as a molecular target in immune-mediated arthritis: immunoregulatory properties of genetically modified murine dendritic cells that secret IL-4. *Arthritis Rheum* 2007; **56**:89–100.

16 Moreno O, González CI, Saaibi DL, Otero W, Badillo R, Martín J, Ramírez G. Polymorphisms in the IL-4 and IL-4RA genes in Colombian patients with rheumatoid arthritis. *J Rheumatol* 2007; **34**:36–42.

17 Cantagrel A, Navaux F, Loubet-Lescouli P, NourHashemi F, Enault G, Abbal M, *et al.* Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. *Arthritis Rheum* 1999; **42**:1093–1100.

18 Peng H, Wang W, Zhoul M, Liu CY, Li R, Wen PF, *et al.* Associations of interleukin-4 receptor gene polymorphisms (Q551R, I50V) with rheumatoid arthritis: evidence from a meta-analysis. *Genet Test Mol Biomarkers* 2013; **17**:768–774.

19 Rosenwasser L, Klemm D, Dresback J, Inamura H, Mascli J, Kinnert M, *et al.* Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* 1995; **25**:74–78.

20 Huang J, Kuo M, Hung I, Wu C, Ou L, Cheng J. Lowered IL-4-producing T cells and decreased IL-4 secretion in peripheral blood from subjects with juvenile rheumatoid arthritis. *Chang Gung Med J* 2001; **24**:77–83.

21 Murray KJ, Grom AA, Thompson SD, Lieuwen D, Passo MH, Glass DN. Contrasting cytokine profiles in the synovium of different forms of juvenile rheumatoid arthritis and juvenile spondyloarthropathy: prominence of interleukin-4 in restricted disease. *J Rheumatol* 1998; **25**:1388–1398.

22 Thompson SD, Luyrink LK, Graham TB, Tsoras M, Ryan M, Passo MH, *et al.* Chemokine receptor CCR4 on CD4+ T cells in juvenile rheumatoid arthritis synovial fluid defines a subset of cells with increased IL-4: IFN-γ mRNA ratios. *J Immunol* 2001; **166**:6899–6906.

23 Krabben A, Wilson D, de Rooy D, Zhernakova A, Brouwer E, Lindqvist E, *et al.* Association of genetic variants in the IL4 and IL4R genes with the severity of joint damage in rheumatoid arthritis: a study in seven cohorts. *Arthritis Rheum* 2013; **65**:3051–3057.

24 Inanir A, Yigit S, Tekcan A, Tural S, Kismali G. IL-4 and MTHFR gene polymorphism in rheumatoid arthritis and their effects. *Immunol Lett* 2013; **152**:104–108.

25 Pawlik A, Wrzesniewska J, Florczak M, Gawronska-Szklarz B, Herczynska M. The −590 IL-4 promoter polymorphism in patients with rheumatoid arthritis. *Rheumatol Int* 2005; **26**:48–51.

26 Song G, Bae S, Kim J, Lee Y. Interleukin-4, interleukin-4 receptors, and interleukin-18 polymorphisms and rheumatoid arthritis: a met-analysis. *Immunol Invest* 2013; **42**:455–469.

27 Li X, Chai W, Ni M, Xu M, Lian Z, Shi L, *et al.* The effects of gene polymorphisms in interleukin-4 and interleukin-6 on the susceptibility of rheumatoid arthritis in a Chinese population. *Biomed Res Int* 2014; **2014**: ID265435.

28 Park HK, Kang SK, Kweon HY, Lee KG, Arasu MV, Kim YO. Promoter polymorphism (−590, T/C) of interleukin 4 (IL-4) gene is associated with rheumatoid arthritis: an updated meta-analysis. *Saudi J Biol Sci* 2017; **24**:444–449.

29 Aletaha D, Neogi T, Silman AJ, Funovits J, Felson D, Bingham CO, *et al.* 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010; **62**:2569–2581.

30 Prevoo ML, van't Hof MA, Kuper HH, van Leewen MA, van De Putte LB, van Riel LC. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; **38**:44–48.

31 Hussein Y, El-Shal A, Rezk N, Abdel Galil S, Alzahrani S. Influence of interleukin-4 gene polymorphisms and interleukin-4 serum level on susceptibility and severity of rheumatoid arthritis in Egyptian population. *Cytokine* 2013; **61**:849–855.

32 McInnes IB, Schett G. Mechanisms of disease: the pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011; **365**:2205–2219.

33 Sun YH, Wei ST, Zong SH. Correlation between IL-4 gene polymorphism as well as its mRNA expression and rheumatoid arthritis. *Eur Rev Med Pharmacol Sci.* 2017; **21**:3879–3885.

34 Canet LM, Cáliz R, Lupiañez CB, Canhão H, Martinez M, Escudero A, *et al.* Genetic variants within immune-modulating genes influence the risk of developing rheumatoid arthritis and anti-TNF drug response: a two-stage case-control study. *Pharmacogenet Genomics* 2015; **25**:432–443.

35 Trajkov D, Mishevska-Perchinkova S, Karadzova-Stojanoska A, Petlichkovski A, Strezova A, Spiroski M. Association of 22 cytokine gene polymorphisms with rheumatoid arthritis in population of ethnic Macedonians. *Clin Rheumatol* 2009; **28**:1291–1300.

36 Qiu LJ, Ni J, Cen H, Wen PF, Zhang M, Liang Y, *et al.* Relationship between the IL-4 gene promoter −590C/T (rs2243250) polymorphism and susceptibility to autoimmune diseases: a meta-analysis. *J Eur Acad Dermatol Venereol* 2015; **29**:48–55.