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Signal transducer and activator of transcription 4 (*STAT4*) *G>T* gene polymorphism in Egyptian cases with rheumatoid arthritis

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ABSTRACT

Background: The gene encoding signal transducer and activator of transcription 4 (*STAT4*) has been reported to be associated with rheumatoid arthritis (RA) in several populations. This work aimed at assessing the association of *STAT4 G>T* gene polymorphism with the susceptibility, activity and functional disability of RA in Egyptian subjects.
Subjects and methods: This study included 112 unrelated RA Egyptian patients who were compared to 122 healthy unrelated individuals taken from the same locality. For all subjects, DNA was genotyped for *STAT4 G>T* (*rs7574865*) polymorphism using the PCR-RFLP technique. Antibodies to cyclic citrullinated peptides (anti-CCP) were measured by enzyme-linked immunosorbent assay (ELISA).
Results: Cases showed a significantly higher frequency of the *STAT4 T* allele carriage (GT+TT genotypes) compared to controls (51.8% *vs.* 31.1%, OR = 2.37, 95% CI = 1.39–4.05, $p = 0.001$). Also the frequency of the *STAT4 T* allele was significantly higher among cases compared to controls (30.4% *vs.* 16.8%, OR = 2.16, 95% CI = 1.39–3.35, $p = 0.001$). Cases positive to the *STAT4 T* allele (*GT+TT* genotypes) showed no significant difference compared to those with the GG genotype regarding their clinical and immune parameters. Nonetheless, they showed a more functional disability presented in their significantly higher health assessment questionnaire (HAQ) score ($p = 0.02$).
Conclusions: This study gives an extra evidence to the association of the *STAT4 T* allele with the susceptibility and functional disability of RA.

1. Introduction

Rheumatoid arthritis (RA) is the most common chronic autoimmune disease, affecting 1% of the adult population worldwide. It is characterized by chronic inflammation and destruction of the synovial joints, leading to progressive joint damage, and it is associated with significant disability and early mortality [1]. The etiology of RA is complex, and it is not completely understood. However, it is known that RA risk is probably influenced by an interaction between environmental and genetic factors [2,3]. Human leukocyte antigen (HLA) class II molecules were portrayed as being the most powerful genetic factors of RA identified to date. However, family studies suggest that this association accounts for only one-third of genetic susceptibility, and that non-HLA genes are also involved [4].

The polymorphism pertinent to the signal transducer and activator of transcription 4 (STAT4) gene seems to be one of the best examples of a non-MHC common susceptibility allele for autoimmunity [5]. STAT4 is expressed in activated peripheral blood monocytes, dendritic cells and macrophages at the sites of inflammation [6]. STAT4 transmits signals induced by interleukin-12 (IL-12), interleukin-23 (IL-23) and interferon-γ (IFN-γ), which are key cytokines and play important roles in the development of autoimmune diseases [7,8]. The STAT4 gene maps to chromosome 2q33 and encodes a transcription factor, which plays pivotal roles in the differentiation and proliferation of both T helper 1 (Th1) and T helper 17 (Th17) cells [8]. Since Th1 and Th17 lineages are crucial effectors in chronic inflammatory disorders, STAT4 gene may play an important role in the pathogenesis of autoimmune diseases. To date, the SNP rs7574865 in STAT4 gene has been reported to be associated with an increased risk for diverse complex autoimmune diseases in different ethnic populations, such as RA [9–13]. Knowing that the allelic frequencies of genes often differ substantially between populations; we were interested to test the association

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of this seemingly important *STAT4 G>T* polymorphism with RA in Egyptian cases.

2. Subjects and methods

This study included 112 RA patients (93 females and 19 males). All fulfilled the criteria for RA set by the American College of Rheumatology classification [14]. Their age mean ± SD was 47.6 ± 10.1 years. They were recruited from the Outpatient Clinic of Rheumatology and Rehabilitation department; Mansoura University Hospitals, Egypt. Exclusion criteria included cases with abnormal renal or hepatic functions, history of malignancy, alcohol abuse, pregnancy and lactation and cases associated with other autoimmune disorders. For comparison, another control sample was taken in the form of 122 healthy unrelated blood donors (99 females and 23 males) from the same locality. Their age mean ± SD was 42.3 ± 10.3 years.

Disease activity was determined on the basis of multiply defined parameters including the swollen joint count (SJC), tender joint count (TJC), Disease Activity Score 28 (DAS28), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [15,16]. Functional disability was determined on the basis of health assessment questionnaire (HAQ) defined parameters that comprises eight general categories including: dressing and grooming, arising, eating, walking, hygiene, reach, grip and other activities. Each of which consists of one or more specific questions. Each question is scored either (0): "without any difficulty", (1): "with some difficulty", (2): "with much difficulty" and (3): "unable to do" with a summed score ranging 0–24 [17,18].

C-reactive protein (CRP) and rheumatoid factor (RF) were estimated by semi-quantitative latex agglutination with AVITEX-CRP and AVITEX-RF kits (Omega Diagnostics, Alva, Scotland, UK). Serum was considered positive when the titer is 6.0 mg/L for CRP and 8.0 IU/ml for RF. Anti-CCP was detected in serum samples using enzyme-linked immunosorbent assay (ELIZA) kit (Orgentec Diagnostics, Mainz, Germany). A concentration $\geqslant$20 U/ml was considered positive.

For all participants, genotyping of the *STAT4 G>T* gene rs7574865 SNP was performed via the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP), using the following forward (5′-AAA GAA GTG GGA TAA AAA GAA GTT TG-3′) and reverse (5′-CCA CTG AAA TAA GAT AAC CAC TGT-3′) primers [12]. PCR mixture contained 100 ng DNA, 10 pmol of each primer, 50 μm of dNTPs, 1X PCR buffer with $MgCl_2$, 1 U Taq DNA polymerase and H_2O up to 25 μl. PCR amplification protocol consisted of initial denaturation at 95 °C for 5 min, 30 cycles of amplification which included denaturation at 94 °C for 30 s, annealing at 52 °C for 60 s and extension at 72 °C for 60 s, and final extension at 72 °C for 7 min. The amplified product was digested with FastDigest HpaI restriction enzyme (Fermentas, Germany). The digestion product was applied on a 2% agarose gel stained with ethidium bromide permitting the differentiation of the following restriction pattern. The amplification of the STAT4 generate a 147 bp fragment. The G allele was not digested; so the GG genotype appeared as a single band develops at 147 bp while the T allele was cut into 122 and 25 bp fragments; so the TT genotype appeared as single band develops at 122 bp (25 bp fragment was too small to be detected); and the GT genotype appeared as two bands; one developed at 147 bp, and the other at 122 bp.

2.1. Ethical approval and informed consent

The study was commenced on after obtaining an approval from the university ethical and scientific committees. In addition, an informed consent was obtained from all participants before their enrollment into the study.

2.2. Statistical analysis

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 17.0). The frequencies of studied genotypic and allelic variants among cases were compared to that of controls using Fisher's exact test and odds ratio (OR) with the 95% confidence interval (95% CI). Quantitative traits were compared using the Student *t*-test while nominal traits were compared using the Chi square test. Conformity with the Hardy Weinberg law of genetic equilibrium (HWE) was assured by a non-significant Chi square test comparing the observed versus the expected genotype frequencies in cases and controls. A minimum level of statistical significance was considered at a p level of <0.05.

3. Results

The demographic and clinical data of the RA patients showed that 38 cases (33.9%) had a positive family history of rheumatoid arthritis and 50 cases (44.6%) had a positive consanguinity. Analysis of laboratory markers revealed that 104 cases (92.8%) were positive for anti-cyclic citrullinated peptide (anti-CCP) while 83 cases (74.1%) were positive for rheumatoid factor (RF).

The genotypic and allelic frequencies of the *STAT4 G>T* gene SNP in RA patients and controls are shown in Table 1. The frequencies of STAT4 genotypes were in agreement with Hardy–Weinberg equilibrium in both RA and control groups ($p > 0.05$). Testing for the dominant model of inheritance (*GT+TT vs. GG*) showed that cases had a significantly higher frequency of the T allele carriers (GT+TT genotypes) compared to controls (51.8% *vs.* 31.1%, OR = 2.37, 95% CI = 1.39–4.05, $p = 0.001$). Cases had a significantly higher frequency of the GT genotype in RA cases compared to the controls (42.9% *vs.* 28.7%, OR = 2.13, 95% CI = 1.23–3.71, $p = 0.008$). Testing for the recessive model *(TT vs. GG+GT)*, cases showed a significantly higher frequency of the *TT* homozygous genotype compared to the controls (8.9% vs. 2.4%, OR = 3.89, 95% CI = 1.04–14.52, $p = 0.04$). Regarding the allelic frequencies, cases showed a significantly higher frequency of the *STAT4 T* allele compared to controls (30.4% *vs.* 16.8%, OR = 2.16; 95% CI = 1.39–3.35, $p = 0.001$) (Table 1).

Table 1
The genotype and allele frequencies of *STAT4 G>T* in the RA patients and control groups.

	RA patients *n* (%)		Controls *n* (%)	
Genotypes				
GG	54 (48.2)		84 (68.9)	
GT	48 (42.9)		35 (28.7)	
TT	10 (8.9)		3 (2.4)	
Alleles				
G	156 (69.6)		203 (83.2)	
T	68 (30.4)		41 (16.8)	
HWE	$\chi^2 = 0.02$, $p > 0.05$		$\chi^2 = 0.08$, $p > 0.05$	
Statistics		OR (95% CI)		*p*
Recessive	*TT vs. GG+GT*	3.89 (1.04–14.52)		0.04*
Dominant	*GT+TT vs. GG*	2.37 (1.39–4.05)		0.001**
Codominant	*GT vs. GG*	2.13 (1.23–3.71)		0.008*
	TT vs. GG	5.19 (1.36–19.69)		0.02*
Allele	*T vs. G*	2.16 (1.39–3.35)		0.001**

OR: odds ratio; CI: confidence intervals; HWE: Hardy–Weinberg equilibrium.
* $p < 0.05$ = significant.
** $p < 0.001$ = highly significant.