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SHORT COMMUNICATION

STAT4 but not TRAF1/C5 variants influence the risk of developing rheumatoid arthritis and systemic lupus erythematosus in Colombians

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The aim of this study was to determine the influence of STAT4 (rs7574865) and TRAF1/C5 (rs10818488 and rs2900180) gene polymorphisms on the risk of developing rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in a Colombian population. This was a case–control study in which 839 individuals with RA (N = 274) and SLE (N = 144) and matched healthy controls (N = 421) were included. Genotyping was performed by using a polymerase chain reaction system with pre-developed TaqMan allelic discrimination assay. STAT4 rs7574865T allele disclosed a significant influence on the risk of developing SLE (P = 0.0005; OR 1.62, 95% CI 1.22–2.16) and RA (P = 0.008; OR 1.36; 95% CI 1.08–1.71), whereas no effect on these autoimmune diseases was observed for the TRAF1/C5 polymorphisms examined. Our data strengthen STAT4 rs7574865 polymorphism as a susceptibility factor for RA and SLE and provide further evidence for a common origin of autoimmune diseases.
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Keywords: *STAT4; TRAF1/C5; polymorphisms; systemic lupus erythematosus; rheumatoid arthritis; autoimmunity*

Introduction

Last advances in the genomic research have generated a great expectation in Biomedicine, especially in search of new susceptibility genes involved in complex diseases. Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are common autoimmune and complex rheumatic diseases.[1] Although RA and SLE are considered as idiopathic diseases, both the higher concordance rates and familial aggregation indicate a significant genetic influence on their pathogenesis.[2–4] Human leukocyte antigen genes are the strongest genetic locus known to be associated with susceptibility to both diseases.[5,6] Nevertheless, genome-wide linkage studies have shown several non-human leukocyte antigen loci influencing their susceptibility risk.[7,8]

Signal transducers and activators of transcription (STATs) regulate the entire haematopoietic process through cytokines binding to specific cell-surface receptors.[9] Recently, *STAT4* gene polymorphism (rs7574865), at 2q32, has been found to be associated with RA and SLE.[10,11] Other studies have reported chromosome 9q as a susceptibility locus for RA mainly due to the effect of two polymorphisms (rs2900180 and rs10818488) in the *TRAF1/C5* locus.[12,13] Complement component 5 (*C5*) and TNF receptor-associated factor 1 (*TRAF1*) are adjacent to each other on chromosome 9q33–34, and their products are potent chemoattractant complement component and essential effectors of the TNF signalling cascade, respectively. As C5 and TNF blockade represents a therapeutic target for RA and SLE,[14–16] the study of their gene polymorphisms represent a rational to improve our understanding of the pathogenesis of these diseases. Replication of promising initial results is a necessary step to assess the genetic contribution to human disease. With this goal, this study was conducted to assess the influence of *STAT4* and *TRAF1/C5* gene polymorphisms on RA and SLE in a Latin American (Colombian) population.

Results and discussion

This was a case–control study in which 839 individuals belonging to a well-defined and homogeneous Colombian population were included and in whom *STAT4* and *TRAF1/C5* polymorphisms were simultaneously examined. Deviation from Hardy–Weinberg proportion

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Table 1 Genotype and allele frequencies of the rs7574865 *STAT4* polymorphism in healthy controls and RA and SLE patients

STAT4 rs7574865	*Controls* n = *410 (%)*	*RA* n = *257(%)*	P_{value}	*OR (95% CI)*	*SLE* n = *144(%)*	P_{value}	*OR (95% CI)*
GG	198 (48.3)	100 (38.9)	0.02	0.68 (0.58–0.92)	48 (33.3)	0.001	0.54 (0.35–0.81)
GT	166 (40.5)	116 (45.1)	0.2	1.21 (0.88–1.65)	69 (47.9)	0.1	1.35 (0.91–2.02)
TT	46 (11.2)	41 (16)	0.07	1.50 (0.95–2.35)	27 (18.8)	0.02	1.83 (1.05–3.16)
G	562 (68.5)	316 (61.5)	0.008	0.73 (0.53–0.952)	165 (57.3)	0.0005	0.62 (0.46–0.82)
T	258 (31.5)	198 (38.5)	0.008	1.36 (1.08–1.71)	123 (42.7)	0.0005	1.62 (1.22–2.16)

Abbreviations: CI, confidence interval; OR, odds ratio; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.
A total of 257 RA patients, 144 SLE patients and 421 gender-, age-, socioeconomic and ethnic-matched controls were included. All patients met the international classification criteria for their respective disease.[17,18] All the subjects gave written informed consent for the study. The study was approved by the local ethics committee. Clinical and immunological characteristics of RA and SLE patients have been reported previously.[19,20]
Samples were genotyped for *STAT4* rs7574865, *TRAF1/C5* rs2900180 and rs10818488 variants using a PCR system with pre-developed TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labelled with the fluorescent dyes VIC and FAM, respectively. PCR reaction was carried out in a total reaction volume of 5 μl with the following amplification protocol: denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s and finished with annealing and extension at 60 °C for 1 min. Post-PCR, the genotype of each sample was attributed automatically by measuring the allelic-specific fluorescence on the ABI PRIM 7900 Sequence Detection Systems using SDS 2.3 software for allelic discrimination (Applied Biosystems). Duplicate samples and negative controls were included to check the accuracy of genotyping.
We used the χ^2 test for Hardy–Weinberg equilibrium and statistical analysis to compare allelic and genotypic distributions. ORs and 95% CIs were calculated according to Woolf's method using the Statcalc program (Epi Info 2002, Centers for Disease Control and Prevention, Atlanta, GA, USA). Probability of false report was estimated according to Wacholder *et al*.[21] The power was estimated using Quanto v 0.5 software (Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA).

was not observed in the entire cohort for the three polymorphisms studied. Table 1 show the *STAT4* rs7574865 genotype and allele distributions. We observed a statistically significant increase of the minor T allele in SLE patients compared to healthy controls (42.7 vs 31.5%, $P = 0.0005$, OR 1.62, 95% CI 1.22–2.16). A similar effect was found in our RA cohort (Table 1), where the frequency of the T allele was significantly increased in patients versus controls (38.5 vs 31.5%, $P = 0.008$, OR 1.36; 95% CI 1.08–1.71). *STAT4* rs7574865 minor allele frequency was found to be higher in Colombians than in North Americans[10] and similar to Koreans[11] (22 and 33%, respectively). Nevertheless, the differences in allelic frequencies between patients and controls were similar in Colombian population (7% higher in patients) and in the other populations (5% in North Americans and 6% in Koreans).

The examined *TRAF1/C5* rs2900180 and rs10818488 polymorphisms are shown in Table 2. The minor allele frequencies found for both SNPs were similar to other populations studied. We found no statistically significant differences in the distribution of rs2900180 and rs10818488 allele and genotype frequencies between RA and SLE patients compared with controls. These polymorphism are not in linkage disequilibrium ($r^2 = 0.035$), and the haplotypes of the two variants are not associated with RA and SLE (data not shown).

The *STAT4* genes encode a transcription factor that transmits signals induced by IL-12, IL-23 and type 1 interferon.[22] A major action of IL-12 through STAT4 signalling is to promote the differentiation of naive $CD4^+$ T cells into T-helper 1 cells (Th1), which produce IFNγ. These Th1 cells are thought to drive the chronic autoimmune response. STAT4 is also important for the development of the recently identified IL-17 secreting Th cells in response to IL-23.[23] These Th17 cells play critical roles in autoimmune diseases, such as RA and SLE, through IL-17 production.[23–25] As both lineages are master regulators of RA and SLE ethiopathology in humans, *STAT4* may exert its influence in both through a defective signalling in these pathways.

The *TRAF1* gene mediates signal transduction from various receptors of the TNF receptor superfamily, that is a critical cytokine in the pathogenesis of RA and SLE.[26–27] A genome-wide study identified the *TRAF1/C5* locus on chromosome 9q33–34 as a susceptibility region for RA[12] A fine mapping approach published in the same study noted that a polymorphism, rs2900180, could explain the majority of the association signal across the locus. Almost at the same time, an independent candidate gene approach study identified another SNP, rs10818488, associated with RA.[13] The functional roles of these SNPs are unclear. Both polymorphisms are located in an intergenic region and could exert its effect through a neighbouring gene (*PHF19*), but the biologic evidence exposed before supports a role for either *TRAF1* or *C5*, or both.

This is the first study to attempt to determine the potential implication of the *STAT4* and *TRAF1/C5* polymorphisms with RA and SLE in a Colombian population. The association of rs7574865 variant in the *STAT4* gene was initially reported in Korean patients with RA[11] and then in independent cohorts of Caucasian patients with RA and SLE.[10,28] When our results are interpreted in the context of these previous results,[10,11,28] association between rs7574865 *STAT4* polymorphism and SLE and RA was reproduced and confirmed to be true. These data suggest that *STAT4* could be a good genetic marker of autoimmunity and add further evidence for a common genetic origin of diverse autoimmune diseases.[29] Although it seems clear that *STAT4* plays a key role in several pathways involved in RA and SLE pathogenesis, the functional role(s) of the associated polymorphism(s) remains to be elucidated. This variant

Table 2 Genotype and allele frequencies rs2900180 and rs10818488 of the *TRAF1/C5* polymorphism in healthy controls and RA and SLE patients

TRAF1/C5 rs2900180	*Controls* n = *394 (%)*	*RA* n = *249(%)*	P_{value}	*OR (95% CI)*	*SLE* n = *134(%)*	P_{value}	*OR (95% CI)*
CC	185 (47)	123 (49.4)	0.5	1.10 (0.86–1.41)	67 (50)	0.5	1.13 (0.75–1.70)
CT	165 (41.9)	103 (41.4)	0.9	0.98 (0.71–1.35)	54 (40.3)	0.7	0.94 (0.62–1.42)
TT	44 (11.1)	23 (9.2)	0.4	0.82 (0.48–1.40)	13 (9.7)	0.6	0.85 (0.42–1.71)
C	535 (67.9)	349 (70.1)	0.4	1.11 (0.86–1.40)	188 (70.1)	0.5	1.11 (0.81–1.52)
T	253 (32.1)	149 (29.9)	0.4	0.90 (0.71–1.15)	80 (29.9)	0.5	0.90 (0.66–1.23)
TRAF1/C5 rs10818488	*Controls* n = *421 (%)*	*RA* n = *274(%)*	P_{value}	*OR (95% CI)*	*SLE* n = *131(%)*	P_{value}	*OR (95% CI)*
GG	147 (34.9)	114 (41.6)	0.07	1.33 (0.96–1.84)	53 (40.5)	0.2	1.27 (0.83–1.93)
GA	197 (46.8)	117 (42.7)	0.3	0.85 (0.62–1.15)	57 (43.5)	0.5	0.88 (0.58–1.32)
AA	77 (18.3)	43 (15.7)	0.4	0.84 (0.66–1.03)	21 (16)	0.5	0.85 (0.50–1.50)
G	491 (58.3)	345 (63)	0.08	1.21 (0.97–1.52)	163 (62.2)	0.3	1.18 (0.88–1.28)
A	351 (41.7)	203 (37)	0.08	0.82 (0.66–1.03)	99 (37.8)	0.3	0.85 (0.63–1.14)

Abbreviations: CI, confidence interval; OR, odds ratio; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.
Significant differences were not found.

is located at the third intron of the gene and could be involved in the splicing of the protein. In fact, a bioinformatics approach using *FASTSNP* Software[30] indicates that if this position may be functioned as an intron enhancer, it could affect the mRNA splicing and expression. Nevertheless, this is only an *in silico* approach, and additional functional studies are needed to clarify the role of *STAT4* as a marker for novel common pathways involved in autoimmune diseases.

On the other hand, we found no association of *TRAF1/C5* variants with RA or SLE in Colombians. Although there is a clear trend of association of the rs10818488 minor allele with RA, the lack of power to detect OR below 1.5 of our study and the different direction of the association with other reports (decrease in A allele in Colombians populations and increase in A allele in the others population studied) suggest that the absence of association may be more likely. Our negative findings in SLE could be due to lack of power to detect a true association. Therefore, the use of larger SLE cohorts may be necessary before drawing definite conclusions regarding the role of *TRAF1-C5* variants in SLE. The lack of significant findings in our *TRAF1/C5* study may also be due to environmental or genetic heterogeneity between populations. Related to this, it could be that the *TRAF1/C5* variants analysed do not constitute the real aetiological locus and that population-specific LD between the *TRAF1/C5* variants and other proxies may explain our results.

In conclusion, this study shows the influence of *STAT4* rs7574865T of Colombian patients with RA and SLE and indicates a global risk of this polymorphism on these conditions, whereas no clear effect was observed for *TRAF1/C5* rs2900180 and rs10818488.

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