Evaluation of a Shared Autoimmune Disease-associated Polymorphism of TRAF6 in Systemic Sclerosis and Giant Cell Arteritis

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ABSTRACT. ***Objective.*** We evaluated whether a single-nucleotide polymorphism (SNP) of the *TRAF6* gene previously associated with systemic lupus erythematosus and rheumatoid arthritis may be a common risk factor for systemic sclerosis (SSc) and giant cell arteritis (GCA).

Methods. A total of 1185 patients with SSc, 479 patients with biopsy-proven GCA, and 1442 unrelated healthy controls of white Spanish origin were genotyped for the rs540386 variant using a specifically designed TaqMan© allele discrimination assay.

Results. No significant associations of this SNP with global SSc or GCA were found. This was also the case when the potential associations of the *TRAF6* polymorphism with the main clinical phenotypes of the 2 diseases (e.g., limited cutaneous and diffuse cutaneous SSc, or presence of polymyalgia rheumatica and visual ischemic manifestations in GCA) were assessed.

Conclusion. Our data do not support a role of the rs540386 *TRAF6* variant as a key component of the genetic network underlying SSc and GCA. (J Rheumatol First Release May 15 2012; doi:10.3899/jrheum.120038)

Key Indexing Terms:
GIANT CELL ARTERITIS SYSTEMIC SCLEROSIS *TRAF6* rs540386 AUTOIMMUNITY

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Supported by GENFER from the Spanish Society of Rheumatology, SAF2009-11110 from the Spanish Ministry of Science, CTS-4977 and CTS-180 from Junta de Andalucía, Fondo de Investigaciones Sanitarias (Spain) through grants PI06-0024 and PS09/00748; and by RETICS Program RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII); and by the Orphan Disease Program grant from EULAR. Dr. Callejas and Dr. Martín are funded by Consejería de Salud, Junta de Andalucía, through PI-0590-2010. F.D. Carmona was supported by Consejo Superior de Investigaciones Científicas (CSIC) through the JAE-DOC program.

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Accepted for publication March 7, 2012.

Autoimmune diseases are complex multifactorial disorders caused by a combination of environmental and genetic factors, each with generally modest effects independently, that lead to an imbalance of the immune system[1]. Accumulating knowledge suggests a shared genetic basis underlying autoimmunity, and the hypothesis that a common network of genetic risk variants may influence the development of different autoimmune diseases is gaining interest[2].

Recent studies have reported an association between the tumor necrosis factor (TNF) receptor-associated factor 6 (*TRAF6*) gene on 11p12 and rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)[3,4]. TRAF proteins are cytoplasmic adapter molecules with a pivotal role in the immune response that have been shown to interact with numerous members of the TNF receptor family, although TRAF6 is also involved in the regulation of other receptors, including interleukin 1R, interleukin 18R, and Toll-like receptors (TLR)[5].

To evaluate the possible involvement of *TRAF6* in the pathogenesis of general autoimmunity, we analyzed whether an intronic single-nucleotide polymorphism (SNP) of this gene that has been associated with SLE and RA is also involved in other autoimmune diseases; these included systemic sclerosis (SSc), a chronic fibrotic autoimmune disorder with a genetic background similar to that in SLE[6], and giant cell arteritis (GCA), a vasculitis characterized by inflammatory lesions of medium- and large-size arteries that shares some genetic associations with RA[7].

MATERIALS AND METHODS

Study population. A white Spanish cohort of 1185 patients with SSc, 483 patients with GCA, and 1442 unrelated healthy controls was analyzed. All patients fulfilled the respective American College of Rheumatology criteria for each disease[8,9]. Additionally, the GCA condition was confirmed by a positive temporal artery biopsy. The Spanish National DNA Bank provided the control samples, which had the same criteria for sex and geographic origin. Our study was approved by the local ethical committees and informed written consent was obtained from all participants. Clinical features of the patients have been described previously[10,11].

SSc subgroups were established based on the extent of skin involvement and autoantibody status as limited cutaneous SSc (lcSSc), diffuse cutaneous SSc (dcSSc), positive for anticentromere antibodies (ACA), and positive for antitopoisomerase antibodies (ATA), as well as for the presence of pulmonary fibrosis[12]. GCA subsets were established according to the presence/absence of polymyalgia rheumatica, visual ischemic manifestations, severe ischemic manifestations (comprising visual manifestations, cerebrovascular accidents, jaw claudication, or limb claudication of recent onset), and irreversible occlusive disease (if patients experienced at least 1 of the following complications: permanent visual loss, stroke, or limb claudication of recent onset), as described[10].

Genotyping methods. DNA was extracted from peripheral blood cells using standard procedures. All participants were genotyped for the *TRAF6* variant rs540386 using a predesigned TaqMan© allele discrimination assay (ID: C___2408956_10) in a 7900HT Fast Real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA, USA).

Statistical analyses. PLINK (v1.07) software (Harvard University, Cambridge, MA, USA; http://pngu.mgh.harvard.edu/purcell/plink/) was used to construct 2 × 2 contingency tables and chi-square test and/or Fisher's exact test, when necessary. OR and 95% CI were obtained according to Woolf's method. P values < 0.05 were considered statistically significant.

RESULTS

The overall statistical power of the study is shown in Table 1. No deviation from Hardy-Weinberg equilibrium was observed (p = 0.05).

We first investigated the possible implication of the rs540386 *TRAF6* variant in the genetic susceptibility to SSc and GCA and their major clinical subphenotypes by comparing the allele frequencies of the different case sets with that of the control population (Table 2). No statistical significance was observed for the global disease analyses (SSc vs controls: p = 0.39, OR = 0.93; GCA vs controls: p = 0.94, OR = 0.99) or when the different clinical subgroups were tested.

To further examine the potential role of the rs540386 genetic variant in SSc and GCA, a new comparison between patients positive for each specific clinical characteristic and patients without the corresponding manifestation was performed (Table 3). These analyses yielded similar negative results.

Table 1. Overall statistical power of the study for rs540386 in each analyzed disease at the 5% significance level.

Condition	OR 1.1	OR 1.2	OR 1.3	OR 1.4	OR 1.5
SSc	0.23	0.66	0.94	0.99	1.00
GCA	0.14	0.41	0.73	0.92	0.98

SSc: systemic sclerosis; GCA: giant cell arteritis.

Finally, no significant heterogeneity between cases and controls was detected when the genotype, recessive, and dominant models were applied (Table 4).

DISCUSSION

TRAF6 is a ubiquitin ligase that mediates signal transduction pathways from the TNF and interleukin 1/TLR superfamilies, which implies that this protein is an important regulator of a wide spectrum of physiological processes including innate and adaptive immunity. TRAF6 is a key component of B cell activation and it has been reported that development of regulatory T cells (Treg), which are crucial in the maintenance of immune tolerance, requires *TRAF6* expression in thymocytes[13,14]. *TRAF6* has been associated with SLE and RA; members of the TLR signaling pathway upstream and downstream of *TRAF6*, such as *TNFAIP3*, *IRF5*, *IRF7*, and *IRAK1*, are known risk factors for SSc and other autoimmune diseases[1,3,4,6].

Taking this into account, we considered *TRAF6* an interesting candidate gene that could be involved in the predisposition to general autoimmunity. However, our data show no significant association of the analyzed polymorphism with the susceptibility and main clinical manifestations of SSc and GCA, although this same SNP has been described as strongly associated with SLE and RA[3,4]. Since the cohorts in our study were large and well defined, it is unlikely that the observed lack of association might have been due to a type II error as a consequence of low statistical power. Supporting this assumption, data from a genome-wide association study (GWAS) of SSc from our group[15] found no positive association signals within the *TRAF6* genomic region, which included 15 SNP (most of them closely linked with rs540386 in the CEU population of the HapMap project, Figure 1). However, no GWAS data are available for GCA, and replication in other populations, desirably of white origin, would be needed to draw definitive conclusions. Moreover, the possibility exists that a different *TRAF6* SNP from rs540386 could be associated with SSc and GCA, as reported recently in SLE by Namjou, *et al*[4].

Cumulative evidence indicates that the different clinical autoimmune outcomes would be a consequence of the presence in the genome of a set of common and disease-specific susceptibility loci interacting with epigenetic and environmental triggers[2]. In this regard, an increasing number of genetic loci outside the HLA region have been convincingly associated with a diverse range of autoimmune diseases, such as *PTPN22*, *TNFAIP3*, *STAT4*, *CTLA4*, *IRF5*, *IL23R*, *IL2/IL21*, and *IL2RA*, among others[1]. Our results suggest that *TRAF6*, or at least the SLE- and RA-associated rs540386 genetic variant, may not be a shared autoimmunity locus but a susceptibility factor specifically associated with the pathogenesis of certain autoimmune diseases. Further studies are needed to elucidate the extent of the common genetic contribution to autoimmunity.

Table 2. Genotype and minor allele frequency (MAF) of *TRAF6* rs540386 in patients with systemic sclerosis (SSc) and giant cell arteritis (GCA) and healthy controls from Spain.

	Genotype, N (%)				Allele test	
	CC	TC	TT	MAF, %	p*	OR (95% CI)**
Controls (n = 1442)	1102 (76.42)	319 (22.12)	21 (1.46)	12.52		
SSc						
SSc, n = 1185	924 (77.97)	244 (20.59)	17 (1.43)	11.73	0.385	0.93 (0.79–1.10)
lcSSc, n = 818	634 (77.51)	170 (20.78)	14 (1.71)	12.10	0.684	0.96 (0.80–1.16)
dcSSc, n = 367	290 (79.02)	74 (20.16)	3 (0.82)	10.90	0.232	0.85 (0.66–1.11)
ACA+, n = 545	425 (77.98)	111 (20.37)	9 (1.65)	11.83	0.559	0.94 (0.76–1.16)
ATA+, n = 259	206 (79.54)	52 (20.08)	1 (0.39)	10.42	0.180	0.81 (0.60–1.10)
PF+, n = 286	223 (77.97)	59 (20.63)	4 (1.40)	11.71	0.594	0.93 (0.70–1.22)
GCA						
GCA, n = 479	364 (75.99)	111 (23.17)	4 (0.84)	12.42	0.938	0.99 (0.79–1.24)
PMR+, n = 213	166 (77.93)	46 (21.60)	1 (0.47)	11.27	0.464	0.89 (0.64–1.22)
VIM+, n = 130	100 (76.92)	30 (23.08)	0 (0.00)	11.54	0.647	0.91 (0.61–1.36)
SIM+, n = 242	183 (75.62)	58 (23.97)	1 (0.41)	12.40	0.941	0.99 (0.74–1.32)
IOD+, n = 85	69 (81.18)	16 (18.82)	0 (0.00)	9.41	0.232	0.73 (0.43–1.23)

* All p values have been calculated for the allelic model. ** OR for the minor allele. lcSSc: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; ACA: anti-centromere antibodies; ATA: antitopoisomerase antibodies; PF: pulmonary fibrosis; PMR: polymyalgia rheumatica; VIM: visual ischemic manifestations; SIM: severe ischemic manifestations; IOD: irreversible occlusive disease.

Table 3. Genotype distribution and minor allele frequency (MAF) of *TRAF6* rs540386 in systemic sclerosis (SSc) and giant cell arteritis (GCA) patients according to the presence or absence of specific clinical manifestations.

Manifestation	With Manifestations		Without Manifestations		Allele Test	
	Genotypic Frequencies	MAF, %	Genotypic Frequencies	MAF, %	p**	OR (95% CI)***
SSc						
SSc subtype*	3/74/290	10.90	14/170/634	12.10	0.400	0.89 (0.67–1.17)
Anticentromere antibodies	9/111/425	11.83	7/125/465	11.64	0.886	1.02 (0.79–1.32)
Antitopoisomerase antibodies	1/52/206	10.42	14/178/662	12.06	0.310	0.85 (0.62–1.17)
Pulmonary fibrosis	4/59/223	11.71	11/170/623	11.94	0.885	0.98 (0.73–1.32)
GCA						
Polymyalgia rheumatica	1/46/166	11.27	3/62/192	13.23	0.363	0.83 (0.56–1.24)
Visual ischemic manifestations	0/30/100	11.54	4/78/255	12.76	0.612	0.89 (0.57–1.39)
Severe ischemic manifestations	1/58/183	12.40	3/50/170	12.56	0.941	0.99 (0.67–1.46)
Irreversible occlusive disease	0/16/69	9.41	4/85/269	12.99	0.202	0.70 (0.40–1.22)

* With: diffuse cutaneous SSc; without: limited cutaneous SSc. ** P value for the allelic model. *** OR for the minor allele.

Table 4. Genotype and recessive and dominant models for the minor allele of *TRAF6* rs540386 in global systemic sclerosis (SSc) and giant cell arteritis (GCA) compared with controls.

	Controls	SSc			GCA		
	N (%)	N (%)	p	OR (95% CI)	N (%)	p	OR (95% CI)
CC	1102 (76.42)	924 (77.97)			364 (75.99)		
CT	319 (22.12)	244 (20.59)	0.632*	NA	111 (23.17)	0.572*	NA
TT	21 (1.46)	17 (1.43)			4 (0.84)		
CC + CT	1421 (98.54)	1168 (98.57)	0.963	0.98 (0.51–1.88)	475 (99.16)	0.305	0.57 (0.19–1.67)
TT	21 (1.46)	17 (1.43)			4 (0.84)		
CC	1102 (76.42)	924 (77.97)	0.346	0.92 (0.76–1.10)	364 (75.99)	0.848	1.02 (0.80–1.31)
TT + CT	340 (23.58)	261 (22.03)			115 (24.01)		

* P value for the genotype distribution. NA: not applicable.

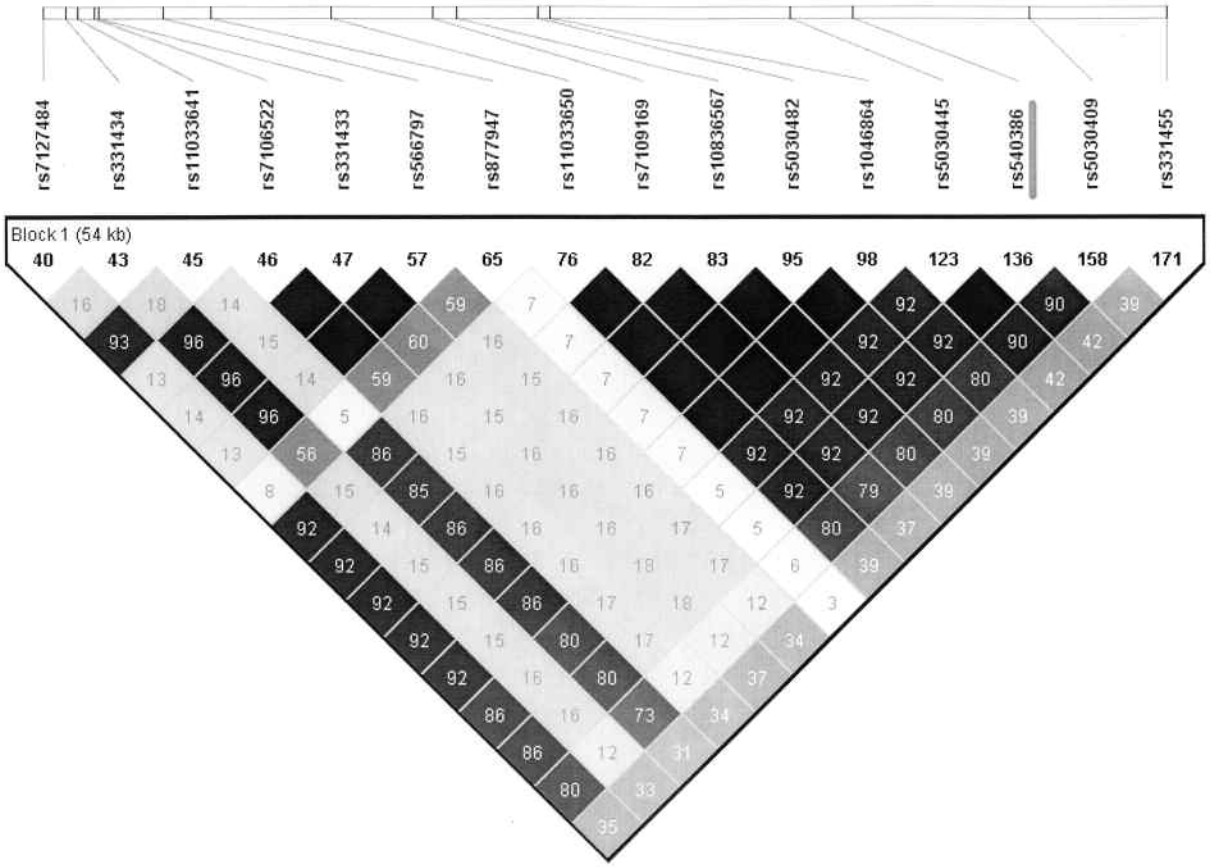

Figure 1. Linkage disequilibrium plot of *TRAF6* variants in the CEU population of the HapMap project, including rs540386 and 15 additional polymorphisms analyzed in a systemic sclerosis genome-wide association study within this locus[15]. R^2 values are shown. The underlined polymorphism corresponds to the *TRAF6* variant analyzed in this study.

ACKNOWLEDGMENT

The authors thank Sofía Vargas, Sonia García, and Gema Robledo for excellent technical assistance, and all the patients and controls for their essential collaboration. Banco Nacional de ADN (University of Salamanca, Spain) is thanked for supplying the control material.

APPENDIX

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