SUMO4 and MAP3K7IP2 Single Nucleotide Polymorphisms and Susceptibility to Rheumatoid Arthritis

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ABSTRACT. *Objective.* To explore the role of single nuclear polymorphisms (SNP) in 2 candidate genes, *SUMO4* and *MAP3K7IP2*, in susceptibility to rheumatoid arthritis (RA).

Methods. Two cohorts from different Spanish towns totalling 635 patients with RA and 826 controls were studied. Six SNP were genotyped by matrix assisted laser desorption-ionization time-of-flight (MALDI-TOF) with the MassARRAY SNP genotyping system.

Results. We found no association with susceptibility to RA for any of the SNP including a previously described functional variant in the *SUMO4* gene (163A→G). RA susceptibility was independent of the haplotypes defined by the 6 SNP and there was also no association with clinical features of RA.

Conclusion. *SUMO4* and *MAP3K7IP2* SNP did not significantly influence predisposition to and features of RA, in contrast to previous genetic and functional evidence that suggested their involvement. (J Rheumatol 2006;33:1048–51)

Key Indexing Terms:

RHEUMATOID ARTHRITIS		TYPE 1 DIABETES
NUCLEAR FACTOR-κB	GENETIC SUSCEPTIBILITY	IDDM5

Recently, a series of single nucleotide polymorphisms (SNP) in the IDDM5 locus on chromosome 6p25 were found to be strongly associated with susceptibility to type 1 diabetes[1,2]. Among them, a functional SNP in the small ubiquitin-like modifier 4 (*SUMO4*) gene (163A→G or rs237025) was singled out as a susceptibility factor for type 1 diabetes. The IDDM5 locus has also been linked to rheumatoid arthritis (RA) in multicase families[3], indicating shared genetic factors between the 2 autoimmune diseases. Other commonalities between these 2 diseases include their excessive coincidence within families[4], the role of the HLA locus and of the protein tyrosine phosphatase nonreceptor-type 22[5,6], and the characterization of both as Th1 autoimmune diseases. The central role of the transcription factor nuclear factor-κB (NF-κB) in RA inflammation[7] adds to interest in the IDDM5 locus, as the 2 known candidate genes in the locus, *SUMO4* and the mitogen-activated protein kinase kinase kinase 7-interacting protein 2 (*MAP3K7IP2*), regulate this transcription factor. *SUMO4* boosts IκBα-negative regulation of NF-κB transcriptional activity[1] and *MAP3K7IP2* is part of a protein complex that activates NF-κB in response to interleukin 1[8]. These observations all suggest that the IDDM5 locus may have a role in susceptibility to RA.

MATERIALS AND METHODS

Samples. DNA samples were obtained from peripheral blood of Spanish patients with RA and healthy controls. They were from teaching hospitals in 2 widely separated Spanish towns, University Clinical Hospital in Santiago de Compostela and "12 of October" Hospital in Madrid. Patients with RA were classified according to the 1987 American College of Rheumatology criteria[9]. In Santiago, we sought to enroll all patients with RA followed in the Rheumatology Unit, and the first 455 recruited patients (from June 2001 to July 2004) were included. In Madrid, patients were recruited among outpatients of the Rheumatology Unit over an extended period. Control samples, in Santiago, were collected from 643 subjects older than age 55 years undergoing preoperative preparation for elective surgery excluding orthopedics. In Madrid, controls were blood bank donors. Clinical data for patients and controls are provided in Table 1. The Ethical Committee for Clinical Research of Galicia approved this study and all participants gave their written informed consent according to the Declaration of Helsinki.

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Table 1. General characteristics of patients with RA and controls from 2 Spanish towns.

| | Santiago | | Madrid | |
	RA, n = 455	Controls, n = 643	RA, n = 180	Controls, n = 183
Female, %	77.2	53.2	72.2	68.3
Age at recruitment*	60 (43–79)	69 (62–76)	—	—
Age at onset*	49 (37–58)		49 (39–57)	
RF, %	58.5		87.1	
Bone erosions, %	67.0		73.1	
DMARD use, %	96.7		—	
Tobacco use, %	20.3		—	

* Median (interquartilic range). The last 4 clinical characteristics from the table were available for more than 75% of the RA patients from Santiago and about 50% of the RA patients from Madrid.

SNP genotyping. Six SNP from Guo, *et al*[1] were analyzed with the MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA, USA) following the manufacturer's instructions[10] as described[11]. Briefly, the 6 SNP were amplified in a multiplex polymerase chain reaction, followed by minisequencing assays, designed with SpectroDesigner software (Sequenom Inc.) (primer sequences are available at http://bioinformatics.cesga.es/supmat/; accessed March 10, 2006). Products were separated by mass spectrometry analysis with the Bruker Bi-flex matrix assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Spectral output was analyzed using SpectroTyper–RT v3.1 software (Sequenom Inc.) and by manual review. Samples from the 2 recruiting centers were analyzed simultaneously. As quality control, re-genotyping of 25 samples was undertaken throughout the study. Repeat genotypes (150) were in 100% agreement with original results. The 6 SNP were successfully genotyped in more than 99% of the 1460 individuals studied. Genotype frequencies of each were in accordance with Hardy-Weinberg equilibrium when considered globally or stratified by status, sex, or town of recruitment.

Statistical analysis. Allelic frequencies were compared using chi-squared tests from contingency 2 × 2 tables. Correlation analysis and logistic regression were used to evaluate the relationship between genotypes and clinical features of RA following an additive genetic model with Statistica software (Statsoft, Tulsa, OK, USA). Adjustment by age of onset and sex was done with logistic regression. Accordance with Hardy-Weinberg equilibrium was tested using the Markov chain algorithm[12]. Cocaphase software[13] was used to infer haplotypes by the expectation-maximization algorithm and to compare haplotype frequency distribution by the homogeneity likelihood ratio test. Haplotypes with frequencies below 5% in any of the strata were grouped.

RESULTS

We studied the 163A→G SNP and 5 other flanking SNP that span 150 kb in the IDDM5 locus and include the *MAP3K7IP2* and *SUMO4* genes. They were genotyped in 2 independent case-control collections of DNA samples from Spanish subjects. The first collection included 455 patients with RA and 643 healthy controls from Santiago de Compostela and the second comprised 180 patients with RA and 183 controls from Madrid. Women constituted 77.2% of the RA patients in the Santiago cohort and 72.2% in the Madrid cohort. We observed significant differences between controls from the 2 collections for 2 of the SNP (163A→G and 012Taq; $p < 0.03$ for both). Upon analysis, we realized these differences were due exclusively to differences in the women. Consequently, we analyzed data from the 2 cohorts separately and stratified by sex. There were no significant differences between patients and controls in the allelic frequencies of any of the 6 SNP (Tables 2 and 3) or in the genotypes (available at http://bioinformatics.cesga.es/supmat/). In addition, clinical features of RA were independent of the IDDM5 SNP genotypes, as evaluated by age at disease onset, presence of rheumatoid factor, or radiographic signs of erosion (data not shown).

All 6 SNP were in linkage disequilibrium, but this was much more marked between the 4 central SNP (from 001Msp to 012Taq) that consisted of a haplotype block (mean pairwise D′ = 0.94) covering the *SUMO4* and *MAP3K7IP2* genes. There were no significant differences in haplotype frequencies between patients and controls from either Santiago or Madrid, either for all 6 SNP (8 haplotypes with estimated frequencies > 5% in at least one of the groups) or for only the 4 SNP of the haplotype block (5 haplotypes with estimated frequencies > 5%). When the haplotypes were also stratified by sex (Table 4) there was a difference between male RA patients and controls from Santiago that did not reach significance ($p = 0.06$); this was not corroborated in male patients and controls from Madrid.

We also analyzed differences in allele frequencies between the 2 collections of samples. There were significant differences between women from the 2 towns; both controls and RA patients from Madrid showed increased allele frequencies. This dissimilarity included the 4 SNP that formed the haplotype block. In contrast, there were no differences between men in any of the subgroups. The differences found in women showed the importance of carefully avoiding population stratification in genetic epidemiology studies to prevent spurious results.

DISCUSSION

The hypothesis implicating these SNP in susceptibility to RA has been supported by the RA linkage to the IDDM5 locus[3,4], in the association of the latter with these SNP in other studies[1,2,14,15], and in the functional consequences of the 163A→G *SUMO4* SNP. This SNP has been shown to affect

Table 2. Lack of difference in IDDM5 SNP allele frequencies between patients with RA and controls from Santiago de Compostela. Values are expressed as percentages.

| | | Women | | | Men | | |
| | | Controls, n = 684 | RA, n = 702 | p | Controls, n = 602 | RA, n = 208 | p |
SNP	Allele						
373 Taq	G	34.5	34.0	NS	33.4	30.6	NS
001Msp	T	42.1	43.7	NS	38.7	37.4	NS
268Hha	T	43.2	45.8	NS	41.9	38.5	NS
163A→G	G	45.2	42.4	NS	48.7	50.5	NS
012Taq	A	45.2	42.1	NS	48.3	49.5	NS
454Msp	G	39.2	41.5	NS	37.0	37.5	NS

n: number of chromosomes; NS: not significant (values of p < 0.1 are shown).

Table 3. Lack of difference in IDDM5 SNP allele frequencies between patients with RA and controls from Madrid. Values are expressed as percentages.

| | | Women | | | Men | | |
| | | Controls, n = 250 | RA, n = 260 | p | Controls, n = 116 | RA, n = 100 | p |
SNP	Allele						
373 Taq	G	30.4	32.6	NS	31.9	36.0	NS
001Msp	T	34.4	35.4	NS	41.4	41.0	NS
268Hha	T	36.2	35.8	NS	43.1	42.0	NS
163A→G	G	55.2	56.1	NS	49.1	50.0	NS
012Taq	A	55.2	56.1	NS	49.1	49.0	NS
454Msp	G	32.8	38.7	NS	37.9	45.0	NS

n: number of chromosomes; NS: not significant (p < 0.1).

Table 4. Haplotype frequencies of IDDM5 SNP in patients with RA and controls from Santiago and Madrid stratified by sex. Only haplotypes with frequency > 5% in at least one of the groups were considered.

| | Santiago | | | | Madrid | | | |
| | Women | | Men | | Women | | Men | |
Haplotypes	Control	RA	Control	RA	Control	RA	Control	RA
ACCAGA	37.1	32.6	41.7	38.0	47.9	45.1	40.9	36.0
GTTGAG	16.2	16.7	15.7	11.6	14.1	18.9	14.8	18.3
GTTGAA	11.2	9.9	12.6	10.2	8.2	6.0	11.9	12.8
ACCGAG	9.3	10.0	8.3	8.4	6.2	6.7	8.7	9.3
ATTGAG	7.7	7.9	6.2	3.4	6.2	5.0	10.4	5.9
ATTGAA	6.8	9.3	4.4	11.7	5.5	4.7	3.3	2.6
GCCAGA	4.8	5.3	3.3	4.1	3.5	5.2	3.2	2.6
ACCAGG	3.0	3.6	3.2	6.7	2.7	5.1	2.9	9.5

the activity of NF-κB[1], which is a central transcription factor in RA[16]. In addition, a second candidate gene in the type 1 diabetes-associated region, *MAP3K7IP2*[1,2], has also been reported to affect NF-κB function, although no functional polymorphism has been described in this large gene (about 95 kb). Despite this hypothesis, we did not find evidence of an association between RA susceptibility and SNP in these 2 genes. Our sample sizes, the concordant results in the 2 sample collections, and the absence of association with haplotypes or clinical features of RA all support our conclusion that these SNP are not associated with susceptibility to RA. Even the female-specific subpopulation heterogeneity we detected was unlikely to have affected our results because we observed similar results in men and women and because we stratified the study by town and by sex. In addition, our results corroborate an independent report of lack of association with RA susceptibility of the 163A→G *SUMO4* SNP in a UK study[17].

The genetic heterogeneity we found between the 2 cohorts was surprising because it was restricted to women and because all samples were Spanish and this population has low genetic heterogeneity[18]. The differences were found in 2 independent samples consisting of controls and patients with RA, respectively. This duplication excludes population stratification, as cases and controls were selected independently. Also,

systematic errors in genotyping are very unlikely as the samples were studied without previous grouping by sex. Consequently, we believe complex genetic mechanisms including but not restricted to transmission ratio distortion (TRD) could be a possible explanation. There was already evidence of TRD in the 6q25 region[19-21] and TRD among offspring seems widespread in the human genome[22]. However, TRD is not sufficient to explain our results because the differences between sexes were town-dependent and unknown site-specific factors would be required. If indeed complex genetics affect the IDDM5 locus in many populations, they could explain the controversial results of *SUMO4* 163A→G SNP association with type 1 diabetes in studies showing a susceptibility effect for the G allele[1,14,15], or the A allele[2], or lack of association[23,24].

We did not find evidence of an association between *SUMO4* or *MAP3K7IP2* SNP and RA susceptibility or severity. In addition, we did find marked heterogeneity of some SNP in the IDDM5 locus among Spanish women that could interfere with association and linkage studies.

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