SLC22A4, RUNX1, and SUMO4 Polymorphisms Are Not Associated with Rheumatoid Arthritis: A Case-Control Study in a Spanish Population

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ABSTRACT

Objective. To replicate the association reported in Japanese individuals of functional SLC22A4 and RUNX1 polymorphisms with rheumatoid arthritis (RA), and to test the possible role in this trait of a functional variant of the SUMO4 gene that was shown to be associated with another related autoimmune disease, type 1 diabetes (T1D).

Methods. Our study population consisted of 886 patients with RA and 987 healthy controls. All subjects were of Spanish Caucasian origin. We conducted a case-control association study with 6 single-nucleotide polymorphisms (SNP) spanning the SLC22A4 gene. SNP mapping in the RUNX1 gene associated with RA in a Japanese population and a SUMO4 polymorphism associated with T1D were also studied.

Results. No statistically significant differences between patients with RA and healthy controls were observed when comparing the distribution of the genotypes or alleles of any of the SLC22A4 polymorphisms tested. Similarly, no evidence of association between RA and the SLC22A4 haplotype previously reported to be associated in a Japanese population was found. With regard to the RUNX1 and SUMO4 SNP, we did not observe statistically significant differences in the distribution of genotypes or alleles between patients with RA and healthy controls.

Conclusion. These results suggest that the SLC22A4, RUNX1, and SUMO4 polymorphisms analyzed do not confer a relevant role in susceptibility to RA in the Spanish population. (J Rheumatol 2006;33:1235–9)

Key Indexing Terms:
RHEUMATOID ARTHRITIS SUSCEPTIBILITY POLYMORPHISM
SLC22A4 RUNX1 SUMO4

Rheumatoid arthritis (RA) is a chronic complex inflammatory disease thought to have an autoimmune origin. Although the precise etiology of RA is unknown, a strong genetic component is well established. The genetic background of systemic autoimmune diseases such as RA is complex and probably involves multiple genes encoding proteins with significant functions in the regulation of the immune system. A genetic approach to identify genes associated with autoimmune disorders is proposed as one of the promising methodologies to elucidate the cause of these diseases.

The chromosomal region 5q31 is particularly interesting with regard to RA genetic predisposition because it contains many genes involved in immune and inflammatory pathways. This region has been reported to be associated with Crohn’s disease, which, like RA, has an inflammatory and autoimmune pathogenesis. A recent study in a Japanese population reported an association between RA and a functional variant of the SLC22A4 gene (solute carrier family 22, member 4), which maps in the 5q31 region and encodes the organic cation transporter. This polymorphism disrupts a
RUNX1 binding site and affects the expression of SLC22A4. Further, in the same study, an association between RA and a single nucleotide polymorphism (SNP) located in the RUNX1 gene was also found. RUNX1 is an essential hematopoietic transcription factor, whose abnormality is frequently found in leukemia\(^5\). Recently, regulatory polymorphisms mapping in RUNX1 binding sites have been independently reported to be associated with systemic lupus erythematosus and psoriasis\(^6,7\).

These findings support the hypothesis that autoimmune diseases may share a common pathogenesis and susceptibility genes\(^8\).

Besides replication studies, considering the possible role of a gene previously associated with a related trait is a useful tool to clarify the genetic component of RA. We have therefore chosen SUMO4 as a candidate gene for susceptibility to RA. Members of the SUMO (small ubiquitin-related modifiers) gene family encode a family of proteins involved in post-translational modification\(^9\). A new member of this gene family, SUMO4, located on 6q25, has recently been identified\(^10,11\). SUMO4 protein conjugates to IkB and negatively regulates nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) transcriptional activity\(^10\). NF-\(\kappa\)B activates transcription of different genes encoding proteins involved in the immune response. Therefore, impaired control of NF-\(\kappa\)B function may lead to the development of autoimmune inflammatory disorders. Recently, evidence was reported for an association of SUMO4 common nonsynonymous SNP 163 A→G, resulting in the amino-acid substitution M55V, with susceptibility to type 1 diabetes\(^10,11\). Further, the SUMO4 M55V substitution was shown to result in an increased NF-\(\kappa\)B transcriptional activity and a higher expression of IL12B gene\(^10\).

The aim of our study was to: (1) replicate the reported association of functional SNP of SLC22A4 and RUNX1 with RA in a Caucasian population, and (2) test the possible role of the SUMO4 polymorphism in RA.

**MATERIALS AND METHODS**

*Subjects.* A total of 886 patients with RA meeting the American College of Rheumatology (ACR) 1987 revised classification criteria for RA\(^12\) were recruited from 5 Spanish hospitals: Hospital Virgen de las Nieves (Granada), Hospital Universitario Virgen del Rocio (Seville), Hospital Xeral-Calde (Lugo), Hospital 12 de Octubre (Madrid), and Hospital Universitario La Paz (Madrid). RA patients had been genotyped for HLA-DRB1. Among the RA patients 75.3% were women; the mean age at disease onset was 50.3 ± 14 years; 55.7% carried the shared epitope; 75.8% were rheumatoid factor-positive; 27% presented extraarticular manifestations; and 20% presented nodular disease. A total of 987 blood bank and bone marrow donors from corresponding cities were included as healthy controls. Patients and controls were all of Spanish Caucasian origin and were included after giving written informed consent. We obtained approval for the study from all participating hospital ethical committees.

*Genotyping.* DNA from patients and controls was obtained from peripheral blood using standard methods. SNP were selected according to previous studies in autoimmune diseases, including SNP studied in Japanese patients with RA spanning the SLC22A4 region (rs3763112 [slc2-2-E1], rs1007602 [slc2-1], rs3792876 [slc2-2-F2], rs2073838 [slc2-2-F1], and rs2269822 [slc2-3])\(^4\), and the SLC22A4 SNP associated with Crohn’s disease in a Caucasian population (rs1050152 [SLC22A4*L503F])\(^13\) (Figure 1). We also tested the RUNX1 rs2268277 variant, which has been reported to be associated with RA\(^5\), and the SUMO4 163 A→G polymorphism previously shown to be associated with type 1 diabetes (T1D)\(^10\).

Samples were genotyped for SLC22A4, RUNX1, and SUMO4 polymorphisms using a TaqMan 5′ allelic discrimination Custom TaqMan\(^8\) SNP Genotyping Assay method (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with the fluorescent dyes VIC and FAM, respectively. PCR reaction was carried out in a total reaction volume of 8 μl with the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and to finish, annealing and extension at 60°C for 1 min. Following PCR, the genotype of each sample was attributed automatically by measuring the allelic specific fluorescence on the ABI Prism 7000 Sequence Detection System using SDS 1.1 software for allelic discrimination (Applied Biosystems).

*Statistical analysis.* Allelic and genotypic frequencies of all the genetic variants were obtained by direct counting. Statistical analysis to compare allelic and genotypic distributions was performed by the chi-square test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated according to Woolf’s method. The software used was the Statacalc program (EpiInfo 2002; Centers for Disease Control and Prevention, Atlanta, GA, USA). p values < 0.05 were considered statistically significant. In all tables, uncorrected p values are presented. For nonparametric data analysis, the Mann-Whitney U test was used for ordinal variables, and Fisher’s exact test was used for dichotomous variables. For haplotype analysis, pairwise linkage disequilibrium measures were investigated and haplotypes constructed by the expectation-maximization algorithm implemented using Unphased software\(^14\). Sample sizes were estimated a priori by Quanto 0.5 software (Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA) according to previously reported allele frequencies\(^3,10\), so that each association study had at least 80% power to detect an association with the same OR as detected in previous studies (OR 1.5–2.0) at the 5% significance level assuming a dominant inheritance model.

**RESULTS**

SLC22A4 genotypes were in Hardy-Weinberg equilibrium in patients and controls. We observed that the SLC22A4 rs3792876 and rs2073838 SNP were in complete linkage disequilibrium, as described in a Japanese population. No statistically significant differences in allele and genotype frequencies of different SNP tested in the SLC22A4 region were found between RA patients and controls (Table 1). Of note, the frequencies of these SLC22A4 polymorphisms in our population differed significantly from those found in the Japanese population\(^4\).

Additionally, we carried out a haplotype analysis of 5 SNP common to the Japanese study, which define the SLC22A4 haplotype associated with RA in the Japanese population (rs3763112, rs1007602, rs3792876, rs2073838, and rs2269822; Table 2). Four haplotypes with frequency > 5% were found in the Spanish population. We did not observe statistically significant differences in the distribution of these haplotypes when comparing RA patients with the control group. The RA-associated SLC22A4 haplotype in the Japanese study was present at an extremely low frequency in our population.

With regard to the rs2268277 RUNX1 polymorphism, genotypes were in Hardy-Weinberg equilibrium in patients and controls. Similarly, no statistically significant differences
between RA patients and controls were observed when the distribution of the genotypes or alleles of this RUNX1 SNP were compared (Table 1). We did not observe the epistatic effect reported by Tokuhiro, et al.4 concerning the susceptible alleles of both SLC22A4 and RUNX1 genes. The number of individuals bearing the combination of these genotypes was much lower in our population than in the Japanese population, due to the marked difference between allelic and genotypic frequencies.

Regarding SUMO4, genotype and allele frequencies of the 163A→G SNP in patients with RA and controls are shown in Table 1. The genotype frequencies were not found to be significantly different from those predicted by Hardy-Weinberg equilibrium testing in controls. The observed allele frequencies in our control population were in concordance with those found in other Caucasian populations10,11,15. However, they differ significantly from those described in Asian populations (Spanish vs Taiwanese, p < 10^-7; Spanish vs Chinese, p = 6·10^-6; Spanish vs Japanese, p = 7·10^-8).

Table 1. Genotype and allele frequencies of different SLC22A4, RUNX1, and SUMO4 SNP among RA patients and healthy controls.

<table>
<thead>
<tr>
<th>Genotypes and Alleles</th>
<th>RA Patients, n = 886 (%)</th>
<th>Healthy Controls, n = 987 (%)</th>
<th>p</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC22A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3763112 (slc2-E1)</td>
<td>G 992 (57.4)</td>
<td>1055 (53.4)</td>
<td>0.11</td>
<td>1.11 (0.97–1.26)</td>
</tr>
<tr>
<td></td>
<td>A 780 (42.6)</td>
<td>919 (46.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC22A4</td>
<td>C 1127 (63.6)</td>
<td>1235 (62.6)</td>
<td>0.51</td>
<td>1.05 (0.91–1.20)</td>
</tr>
<tr>
<td>rs1007602 (slc2-1)</td>
<td>T 645 (36.4)</td>
<td>739 (37.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC22A4</td>
<td>C 1643 (92.7)</td>
<td>1844 (93.4)</td>
<td>0.40</td>
<td>0.9 (0.69–1.16)</td>
</tr>
<tr>
<td>rs3792876 (slc2-F2)</td>
<td>T 129 (7.3)</td>
<td>130 (6.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC22A4</td>
<td>G 1643 (92.7)</td>
<td>1844 (93.4)</td>
<td>0.40</td>
<td>0.9 (0.69–1.16)</td>
</tr>
<tr>
<td>rs2073838 (slc2-F1)</td>
<td>A 129 (7.3)</td>
<td>130 (6.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC22A4</td>
<td>C 997 (56.3)</td>
<td>1130 (57.2)</td>
<td>0.54</td>
<td>0.96 (0.84–1.10)</td>
</tr>
<tr>
<td>rs1050152 (SLC22A4*L503F)</td>
<td>T 775 (43.7)</td>
<td>844 (42.8)</td>
<td>0.98</td>
<td>1.00 (0.83–1.21)</td>
</tr>
<tr>
<td>SLC22A4</td>
<td>C 1527 (86.2)</td>
<td>1701 (86.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2269822 (slc2-3)</td>
<td>T 245 (13.8)</td>
<td>273 (13.8)</td>
<td>0.89</td>
<td>1.01 (0.88–1.15)</td>
</tr>
<tr>
<td>RUNX1 rs2268277</td>
<td>C 711 (40.1)</td>
<td>788 (39.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G 1061 (59.9)</td>
<td>1186 (60.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUMO4 163A→G</td>
<td>A 856 (48.3)</td>
<td>915 (46.4)</td>
<td>0.23</td>
<td>1.08 (0.95–1.23)</td>
</tr>
<tr>
<td></td>
<td>G 916 (51.7)</td>
<td>1059 (53.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spanish vs Korean, $p = 12\cdot10^{-6}$)$^{10}$. No statistically significant differences in the distribution of the alleles or genotypes of the SUMO4 163A→G polymorphism were found when we compared RA patients with the control group (Table 1).

Next, we analyzed demographic and clinical characteristics of RA patients according to their SLC22A4, RUNX1, and SUMO4 genotypes (gender, age at disease onset, presence of shared epitope, rheumatoid factor, rheumatic nodules, and extraarticular disease); however, no significant differences were observed (data not shown).

**DISCUSSION**

In our study, no evidence of an association with RA of the reported SLC22A4, RUNX1, and SUMO4 susceptibility SNP was observed. With regard to SLC22A4 and RUNX1, failure to replicate reported associations is a common event in the search for genetic determinants of complex diseases, due either to genuine population heterogeneity or a different sort of bias, such as publication bias or time-lag bias$^{16}$. The first published report usually suggests a stronger genetic effect, and subsequent studies often fail to confirm the original findings$^{16}$. The lack of replication in our study may have arisen due to a type 2 error (false negative). According to the a priori calculation, our sample size had at least 80% power to detect the relative risk for the individual SNP reported in the Japanese study at the 5% significance level. Nevertheless, we found a very low minor allele frequency of the RA-associated polymorphism (slc2-F1) in our population (6.7%) compared with that found in the Japanese population (31%). Because of our low minor allele frequency, our sample size was underpowered to detect the homozygous slc2-F1, a risk genotype found in the Japanese population. Indeed, according to the frequency of homozygous AA reported in our population and other Caucasian populations, more than 5000 patients and 5000 controls would have to be tested to find an association with similar OR to that described in the Japanese population. Regarding rs3792876 (slc2-F2), this SNP was in complete linkage disequilibrium with rs2073838 (slc2-F1), and considerations about the posteriori power were the same. Regarding the rest of the comparisons, we had more than 80% power to detect a relative risk similar to the Japanese study at the 5% significance level in every case.

The genetic heterogeneity between populations is clearly present in this case, since SLC22A4 allele and genotype frequencies are significantly different between the Spanish and the Japanese populations, which may also account for the failure to replicate the SLC22A4 association with RA. In this sense, there are several reported RA genetic associations in the Japanese population, such as peptidyl-arginine deiminase 4 (PADI4)$^{17}$ or inhibitor of κB-like$^{18}$ gene variants, which were not replicated in Caucasian populations$^{19,20}$. Although an association of the SLC22A4 gene with Crohn’s disease has been reported in both Japanese$^{21}$ and Caucasian populations$^{13}$, in Japanese the associated disease polymorphism was the rs3792876, while in the Europeans it was rs1050152. It is possible that disease-relevant genes or alleles may be specific for certain populations, and vary among different ethnic groups.

During the course of this work, and in agreement with our results, 2 studies showing lack of association of SLC22A4 with RA in other Caucasian populations have been reported$^{22,23}$. All 3 studies in Caucasian populations have the same power calculation problems. Our study and data reported in the Canadian population show a trend similar to the Japanese study. In these 3 studies slc2-F1 AA homozygotes are overrepresented among patients, whereas in the UK population they are underrepresented. It seems inadequate to draw conclusions using SNP with a very low frequency of the minor allele, taking into account the moderate OR found in Japanese. Nevertheless, for the rest of the SNP studied in the region having a higher minor allele frequency, no association was detected.

With regard to the RUNX1 rs2268277 polymorphism, the lack of replication of the association with RA was due neither to a lack of power nor genetic heterogeneity, because the minor allele frequency found in the Spanish population (35%) was very similar to that found in the Japanese population (37%); thus our RA sample size (886 patients) was large enough to reach a 98% statistical power to detect a relative risk similar to the Japanese study at the 5% significance level. In addition, the association of RUNX1 polymorphism with RA has not been replicated in another Caucasian population$^{24}$.

Another possibility to explain discrepancies among studies is environmental heterogeneity. Some genes may play a role in susceptibility to RA only in the presence of specific envi-

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>RA Patients, 2n = 1772 (%)</th>
<th>Healthy Controls, 2n = 1974 (%)</th>
<th>p*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCCGC</td>
<td>780 (44)</td>
<td>830 (42)</td>
<td>0.22</td>
<td>1.08 (0.95–1.24)</td>
</tr>
<tr>
<td>ATCGC</td>
<td>638 (36)</td>
<td>730 (37)</td>
<td>0.53</td>
<td>0.96 (0.84–1.10)</td>
</tr>
<tr>
<td>ACCGC</td>
<td>106 (6)</td>
<td>138 (7)</td>
<td>0.21</td>
<td>0.85 (0.65–1.11)</td>
</tr>
<tr>
<td>GCCGT</td>
<td>88 (5)</td>
<td>118 (6)</td>
<td>0.18</td>
<td>0.82 (0.61–1.10)</td>
</tr>
</tbody>
</table>

* Overall p = 0.24.
ronmental factors to which Japanese, but not the Spanish population, are exposed. Therefore, investigation of possible gene-environmental interaction would be very useful to determine this effect.

Regarding SUMO4, our study attempted to assess the potential implication of the functional variant 163 A→G of the gene, which has been associated with T1D, in susceptibility to a related systemic autoimmune disorder such as RA. No evidence of an association of SUMO4 163 A→G SNP with RA susceptibility was found, which is in accordance with a recent study in a British population. This lack of association is not attributable to the sample size, because the power of our study to detect a difference with OR = 1.5 at α = 0.05 was > 99%. The allele and genotype frequencies observed in our study were similar to those described in other Caucasian populations.

The reported association of the SUMO4 gene to T1D is now under debate. Of note, Guo, et al did not find an association of the SUMO4 polymorphism and T1D in a case-control study carried out in a Spanish population. Therefore, it seems that SUMO4 does not play a relevant role in the genetic predisposition to susceptibility to autoimmune disorders such as RA and T1D in the Spanish population, although a small effect cannot be excluded; this can be verified only in an extremely large data set.

We have been unable to replicate the association of functional variants of SLC22A4 and RUNX1 with RA as previously described in a Japanese population. In addition we did not find an association between RA and a functional polymorphism of SUMO4, which has been associated with T1D.

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REFERENCES