Toll-like receptor 4 Asp299Gly polymorphism and rheumatoid arthritis: a replicative study in three different populations

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POLIMORFISMO ASP299GLY DEL GEN DEL RECEPTOR TIPO TOLL 4 Y ARTRITIS REUMATOIDE: UN ESTUDIO REPLICATIVO EN TRES POBLACIONES DIFERENTES

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Toll-like receptors (TLRs) polymorphisms have been extensively studied with regard to genetic predisposition to several human complex diseases. In this context, the role of TLR4 Asp299Gly in the pathogenesis of rheumatoid arthritis (RA) is not clear. The aim of this study was to test the possible implication of this polymorphism in the susceptibility to RA. We genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in three different populations from Granada (Southern Spain), Lugo (Northern Spain), and Colombia. We did not find statistically significant differences in the distribution of alleles and genotypes in any of the cohorts under study. Our data, together with those from other groups, do not support a relevant role of TLR4 Asp299Gly polymorphism in the susceptibility to RA.

KEY WORDS: Rheumatoid arthritis/ Polymorphism/ Toll-like receptor 4 gene.
Rheumatoid arthritis (RA) is among the most common autoimmune inflammatory diseases\(^1\). Although the etiology of RA is still poorly understood, family studies, twin studies and segregation analyses have provided evidence of a strong genetic component\(^2\). However, the genetic background of this disorder is complex and likely involves multiple genes encoding proteins with significant function in the modulation of the immune system.

Toll-like receptors (TLRs) are phylogenetically conserved receptors that are involved in the recognition of pathogen-associated molecular patterns (PAMPs) and endogenous ligands, and play an important role in regulating inflammatory response and signalling the activation of adaptive immunity\(^3\). Therefore, genetic variation of TLR genes may play a role in determining susceptibility to chronic human diseases which have an inflammatory component, such as rheumatoid arthritis (RA)\(^4\).

Radstake and colleagues\(^5\) investigated the influence of a functional TLR4 gene polymorphism, at amino acid 299 (Asp299Gly)\(^6,7\), on the susceptibility and severity/outcome of rheumatoid arthritis (RA). The authors presented data showing a lower frequency of the TLR4 heterozygous condition for the polymorphism in 282 patients with RA (10.6\%) than in 314 control individuals (17.2\%), suggesting that the hypofunctional 299Gly allele may protect against RA. The authors also reported no association between TLR4 genotypes and disease severity and/or outcome. As noted by the authors, validation of these findings in independent cohorts is needed to establish firmly a role of TLR4 polymorphism in susceptibility to RA.

We have reported a similar study with different conclusions\(^8\). Our cohort was composed of 224 RA cases and 199 unrelated healthy control subjects, all Caucasian subjects from the South of Spain. Although we observed a difference in the distribution of TLR4 Asp299Gly heterozygous individuals between RA patients (9\%) and control (13\%), it did not reach statistical significance. In order to replicate Radstake et al. data, we have analysed the TLR4 Asp299Gly polymorphism in an extra group of RA patients and controls from Southern Spain, being the total individuals analysed of 337 RA patients and 275 healthy controls (Table I). We determined TLR4 genotypes by a PCR-based method, as previously described\(^9\). Thirty-three (9.8\%) of the RA patients and thirty-six (13.1\%) of the healthy controls were heterozygous for the Asp299Gly polymorphism. No statistically significant differences in genotype or allele distribution of the TLR4 polymorphism were observed between RA patients and control individuals. To further test the effect of TLR4 Asp299Gly polymorphism on RA susceptibility, we replicated the study in two additional cohorts of patients with RA and healthy subjects from Lugo (Northern Spain) and Colombia (Table 1). Among the population from Lugo, 11.9\% of the patients with RA and 11\% of the controls were heterozygous for the allelic variant. Regarding the Colombian cohort, 6.4\% of the patients with RA and 12.3\% of the healthy controls were heterozygous for the SNP. We did not observe statistically significant differences when we compared allele and genotype frequencies between RA patients and healthy subjects in both cohorts.

In agreement with our data, a study conducted in a British population showed no evidence of association of the TLR4 Asp299Gly polymorphism with RA, in a cohort of 212 RA patients and 879 control subjects\(^10\). Since the number of patients with RA and control subjects included in the present study is higher than that in the Radstake et al study, the lack of statistically significant results is unlikely to have resulted from low power. Based on the previous study, one would expect that a power of 99.0\% would be achieved by including 305 and 270 individuals in the patient and control groups, respectively (\(p=0.05\) and

### Table I. Toll-like receptor 4 allele and genotype frequencies in patients with RA and healthy controls in three independent cohorts

<table>
<thead>
<tr>
<th>TLR4 Asp299Gly Frequencies</th>
<th>Granada</th>
<th>Lugo</th>
<th>Colombia</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA patients</td>
<td>Healthy controls</td>
<td>RA patients</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>n= 337 (%)</td>
<td>n= 275 (%)</td>
<td>n= 211 (%)</td>
<td>n= 100 (%)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>641 (95.1)</td>
<td>510 (92.7)</td>
<td>395 (93.6)</td>
</tr>
<tr>
<td>Gly</td>
<td>33 (4.9)</td>
<td>40 (7.3)</td>
<td>27 (6.3)</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>304 (90.2)</td>
<td>237 (86.2)</td>
<td>185 (87.7)</td>
</tr>
<tr>
<td>Asp/Gly</td>
<td>33 (9.8)</td>
<td>36 (13.1)</td>
<td>25 (11.9)</td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>0</td>
<td>2 (0.7)</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

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206
TT genotype frequency in the control group 43.7%). Lack of replication of a previous association is a common event in the search for genetic determinants of complex human traits. Among the most common causes of irreproducibility are population stratification, publication bias, time-lag bias and methodology bias.

The effects of genetic, population and clinical heterogeneity must be considered when attempting to detect susceptibility genes for RA in different populations. In European Caucasian healthy populations the frequency of TLR4 Asp299Gly heterozygous range from 8% to 13%. A possible bias due to the genotyping rate may explain the contradictory results obtained in relation to TLR4 polymorphism and RA. It is worth noting that the frequency of TLR4 Asp299Gly heterozygous in the group of healthy controls is of 17% in Radstake’s manuscript, which is considerably higher than the reported in most studies. In this regard, it is interesting to mention that a study of TLR4 polymorphisms in familial hypercholesterolaemia has found that only 11% of healthy controls were heterozygous for the Asp299Gly polymorphism. This study was carried out in a Dutch population from the same geographical area as Radstake’s study, suggesting that the association between TLR4 and RA susceptibility observed in Radstake’s study may be due to genotyping uncertainty.

In support of ours and Kilding et al. results, whole-genome scan linkage studies have revealed no evidence of linkage between RA susceptibility and the 9q23 region, where TLR4 gene maps, although the lack of linkage does not exclude the possibility of a disease gene mapping to a region.

In our opinion, the above considerations question the role of the TLR4 Asp299Gly polymorphism in RA disease susceptibility.

CONCLUSIONS

In the light of our findings, replicated in three different cohorts, it seems that the TLR4 Asp299Gly polymorphism does not play a relevant role in the pathogenesis of RA, which is in agreement with other recent reports.

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