Associations between TLR polymorphisms and systemic lupus erythematosus: a systematic review and meta-analysis

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ABSTRACT
Objective. The aim of this study was to determine whether the polymorphisms of Toll-like receptor (TLR) confer susceptibility to systemic lupus erythematosus (SLE).

Methods. The authors conducted a systematic review and meta-analysis of reports on associations between TLR polymorphisms and SLE susceptibility.

Results. A total of 8 studies (11 separate comparisons) were included in this meta-analysis, which included European and Asian populations. Meta-analysis showed an association between the 2 allele of rs3853839 (TLR7) and SLE in Asian subjects (OR 1.246; 95% CI 1.160, 1.388; p=2×10^-7). No studies on rs3853839 (TLR7) and rs352139 (TLR9) have been performed in Europeans. No association was found between SLE and the 2 alleles of the rs5743836 (TLR9) polymorphism in all overall subjects or in Europeans, but one study showed a significant association in Asians (OR 4.243; 95% CI 1.487, 12.10; p=0.007). Furthermore, no association was found between the rs5744168 (TLR5) polymorphism and SLE susceptibility in Europeans or between the rs187084 (TLR8) or rs352140 (TLR9) polymorphisms and SLE susceptibility in Asians.

Conclusions. This meta-analysis suggests that the TLR7 and TLR9 polymorphisms are associated with the development of SLE in Asians. Further studies are required to determine whether the TLR polymorphisms contribute to SLE susceptibility in other ethnic groups.

Introduction
Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease, characterised by intense inflammation and multiple organ damage (1). In addition, significant familial aggregation and convincing demonstrations of multiple genetic linkages demonstrate that SLE has a genetic basis (1). Toll-like receptors (TLRs) constitute a family of transmembrane proteins that are expressed by various cell types including immune cells and function to discriminate pathogens and initiate inflammatory signalling pathways (2). Stimulation of these pathways culmi-
amined the contrast of the allelic effect of 2 (common allele) versus 1 (minor allele) of the TLR polymorphisms. Point estimates of risk, ORs, and 95% confidence intervals (CIs) were estimated for each study. Cochran’s Q-statistic was used to assess within- and between-study variations and heterogeneities (16). This heterogeneity test assessed the null hypothesis that all the studies evaluated the same effect. In addition, we quantified the effect of heterogeneity using $I^2 = 100\% \times (Q - df)/Q$ (17); where $I^2$ ranges between 0% and 100% and represents the proportion of between-study variability attributable to heterogeneity rather than chance. Statistical manipulations were performed using a Comprehensive Meta-Analysis computer programme (Biosta, Englewood, NJ, USA).

**Results**

**Studies included in the meta-analysis**

Eight studies met the inclusion criteria (Table I) (5-12), but one of the eligible studies contained data on three groups (6), and another study data on 2 different groups (9). These groups were treated independently, and thus, a total of 11 separate comparisons were available. Meta-analysis was performed if there were at least 2 comparisons. Due to the limited number of studies on polymorphisms, six types of meta-analyses were performed (Table II). Selected characteristics of the 8 studies are summarised in Table I.

**Table I.** Characteristics of the studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Study (Ref.)</th>
<th>Country (ethnicity)</th>
<th>Allele numbers, SLE/control</th>
<th>TLR polymorphisms</th>
<th>Major findings for association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhou et al. 2010 (5)</td>
<td>China (A)</td>
<td>630/676</td>
<td>TLR9 Rs352139, rs532140</td>
<td>NS</td>
</tr>
<tr>
<td>Shen et al. 2010 (6)</td>
<td>China (A)</td>
<td>8,668/9,880</td>
<td>TLR7 Rs3853839</td>
<td>Rs3853839 (association $p$-value=6.50E-10)</td>
</tr>
<tr>
<td>Song et al. 2009 (7)</td>
<td>China (A)</td>
<td>358/285</td>
<td>TLR9 Rs187084, rs5321397</td>
<td>NS</td>
</tr>
<tr>
<td>Sanchez et al. 2009 (8)</td>
<td>Spain (E)</td>
<td>1,504/1,008</td>
<td>TLR5, 7 Rs5744168, rs179008</td>
<td>NS</td>
</tr>
<tr>
<td>Demirci et al. 2007 (9)</td>
<td>USA (E)</td>
<td>796/446</td>
<td>TLR5, 9 Rs5744168, rs5743836</td>
<td>NS</td>
</tr>
<tr>
<td>Tao et al. 2007 (10)</td>
<td>Japan (A)</td>
<td>730/1,331</td>
<td>TLR9 Rs187084, rs532139, rs5743836, rs5743842, Rs5743843, rs5743844, rs5743845, rs5743846</td>
<td>Rs352139 ($p=0.029$)</td>
</tr>
<tr>
<td>Ng et al. 2005 (11)</td>
<td>China (A)</td>
<td>934/1,598</td>
<td>TLR9 Rs187084, rs5743836, rs532139, rs352140</td>
<td>NS</td>
</tr>
<tr>
<td>Hut et al. 2005 (12)</td>
<td>Korea (A)</td>
<td>700/660</td>
<td>TLR9 Rs187084, rs532139, rs352140</td>
<td>NS</td>
</tr>
</tbody>
</table>

Ref: reference; E: European; A: Asian; NS: not significant; SNP: single nucleotide polymorphism; TLR: Toll-like receptor.

**Table II.** Results of meta-analyses of associations between TLR polymorphisms and lupus.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Population</th>
<th>No. of studies</th>
<th>Test of association</th>
<th>Test of heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR5</td>
<td>Rs5744168</td>
<td>European</td>
<td>3</td>
<td>0.910</td>
<td>0.714, 1.161</td>
</tr>
<tr>
<td>TLR7</td>
<td>Rs3853839</td>
<td>Asian</td>
<td>3</td>
<td>1.246</td>
<td>1.160, 1.388</td>
</tr>
<tr>
<td>TLR9</td>
<td>Rs5743836</td>
<td>Overall European</td>
<td>3</td>
<td>1.787</td>
<td>0.803, 3.975</td>
</tr>
<tr>
<td>TLR9</td>
<td>Rs352139</td>
<td>Asian</td>
<td>5</td>
<td>1.170</td>
<td>0.939, 1.457</td>
</tr>
<tr>
<td>TLR9</td>
<td>Rs187084</td>
<td>Asian</td>
<td>4</td>
<td>1.044</td>
<td>0.923, 1.181</td>
</tr>
<tr>
<td>TLR9</td>
<td>Rs352140</td>
<td>Asian</td>
<td>3</td>
<td>1.046</td>
<td>0.929, 1.177</td>
</tr>
</tbody>
</table>

F: fixed effects model; R: random effects model; NA: not available; TLR: Toll-like receptor.

Meta-analysis of relations between the rs5744168, rs352140, rs5743836, rs187084, rs352139, and rs3853839 polymorphisms and SLE susceptibility

Meta-analysis showed an association between the 2 allele of the rs3853839 (TLR7) polymorphism and SLE in Asian subjects (OR 1.246; 95% CI 1.160, 1.388; $p=2 \times 10^{-9}$) (Table II), and revealed no association between the 2 alleles of the rs3853839 (TLR7) and rs352139 (TLR9) has been performed in Europeans. Furthermore, no association was found between SLE and the 2 alleles of the rs5743836...
(TLR9) polymorphism in the overall population (OR 1.787; 95% CI 0.803, 3.975; p=0.155) (Table II). Stratification by ethnicity showed no association between rs5743836 and SLE in Europeans (OR 1.275; 95% CI 0.680, 2.389; p=0.448), but a significant between it and SLE in Asians (OR 4.243; 95% CI 1.487, 12.10; p=0.007) (Table II). However, only one study examined the relation between the rs5743836 (TLR9) polymorphism and SLE in Asians. No association was found between the rs5744168 (TLR5) polymorphism and SLE susceptibility in Europeans and between rs187084 (TLR8) or rs352140 (TLR9) and SLE in Asians by meta-analyses (Table II).

Family studies of the above-mentioned TLR polymorphisms and other TLR polymorphisms
Two family studies were identified that examined TLR polymorphisms in SLE (19, 20). Xu et al. (18) performed a family-based association analysis on the TLR9 polymorphism (rs352140) in 77 Chinese patients and in 211 other family members from 74 nuclear families with SLE, and found that the T allele of rs352140 was associated with susceptibility to SLE in a dominant model (p=0.018). Hawn et al. (19) performed transmission disequilibrium testing in a cohort containing 199 affected patients and their 75 unaffected siblings and 326 parents, and found that the allele 1174C of TLR5 (rs5744168) was preferentially transmitted to SLE-aFFECTED offspring (p=0.8009). Polymorphisms were not found in rs5743836, rs5743842, rs5743843, rs5743844, rs5743845, or rs5743846 (TLR9) in a Japanese population (10) or in rs4986790 and rs4986791 (TLR9) in a Korean population (12). Furthermore, no association was found between the rs4986790, rs4986791, rs5743708 (TLR4), or TLR2 Arg677Trp polymorphisms and SLE in a Spanish population (Table I).

Discussion
Our results provide evidence of an association between the TLR7 and 9 polymorphisms and SLE in Asians, but do not support associations between the polymorphisms of TLR and SLE susceptibility in Europeans. These findings suggest that the TLR7 and 9 polymorphisms are associated with the development of SLE in Asians. However, further studies are needed to determine whether polymorphisms of TLR confer susceptibility to SLE in other ethnic groups. SLE is considered to result from a breakdown of tolerance to self antigens, including DNA and RNA (3). The defective clearance of cell debris or hyperactive B cell receptor (BCR) may underlie the development of SLE. It is known that the administration of endogenous RNA or DNA can activate TLR7 and RLR9 and induce an autoimmune response similar to that characteristic of SLE (20). The TLR9 rs5743836 polymorphism is located upstream of the gene, where it could influence transcription regulation (21). Evidence also suggests that TLR7 contributes to the development of autoimmunity (6). In addition, the TLR7 rs3853839 polymorphism has been reported to be functional, for example, G-allele carriers had increased TLR7 transcripts and more pronounced interferon signatures than C-allele carriers (6). Polymorphisms of candidate genes may play a role in the pathogenesis of autoimmune diseases (22, 23). Based on function of TLR7 and TLR9 in SLE, it appears that genetic variations in TLR7 and TLR9 could affect susceptibility to SLE.

In conclusion, this systematic meta-analysis based review reveals the possibility that the TLR7 and TLR9 polymorphisms are associated with SLE susceptibility in Asians. Since the allelic frequencies of genes often differ substantially between populations of different ethnicities, this analysis demonstrates that further studies are required to determine whether the polymorphisms of TLR contribute to SLE susceptibility in different ethnic groups.

References
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