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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; $p=2\times10^{-9}$). 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 culminates in NF-κB activation and in the transcriptions of immune response genes. To date, at least 10 human TLRs have been discovered that can recognise a variety of molecules, such as, viral RNA or DNA, lipoproteins, or flagellin (3). Endogenous RNA or DNA can activate TLRs and induce autoimmune reactions similar to that observed in SLE (4). Based on their functions in the regulation of inflammation and in immune response to pathogens, TLRs are viewed as candidate targets in autoimmune diseases, such as, SLE.

Several authors have examined the contributions made by *TLR* polymorphisms to SLE susceptibility (5-12), but the results produced vary considerably. Meta-analysis can be used to integrate previous findings and increases statistical power and resolution by pooling the results of independent analyses (13-15). In the present study, we explored whether the polymorphisms of *TLR* confer susceptibility to SLE in different populations using a meta-analysis and systematic review approach.

Methods

Identification of eligible studies and data extraction

A search was performed for studies that examined associations between the polymorphisms of *TLR* and SLE using MEDLINE to identify articles (published before October 2010) in which the polymorphisms of *TLR* were investigated in SLE patients and controls. Combinations of keywords, such as, 'Toll-like receptor,' 'TLR,' 'polymorphism,' 'systemic lupus erythematosus' and 'SLE' were entered as Medical Subject Headings (MeSH) and text words. References in identified studies were also investigated to identify additional studies not indexed by MEDLINE. Genetic association studies that determined the distributions of TLR alleles in SLE cases and controls were eligible for inclusion.

Evaluation of statistical associations

The allelic frequencies of the *TLR* polymorphisms as determined by the identified studies were determined using the allele counting method. We ex-

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

Study (Ref.)	Country (ethnicity)	Allele numbers, SLE/control	*TLR* genes	*TLR* polymorphisms	Major findings for association
Zhou *et al*. 2010 (5)	China (A)	630/676	TLR9	*Rs352139, rs352140*	*NS*
Shen *et al*. 2010 (6)	China (A)	8,668/9,880	TLR7	*Rs3853839*	*Rs3853839*(association *p*-value=6.50E-10)
Song *et al*. 2009 (7)	China (A)	358/285	TLR9	*Rs187084, rs3521397*	NS
Sanchez *et al*. 2009 (8)	Spain (E)	1,504/1,008	TLR5, 7	*Rs5744168, rs179008*	NS
Demirci *et al*. 2007 (9)	USA (E)	796/446	TLR5, 9	*Rs*5744168, *rs5743836*	NS
Tao *et al*. 2007 (10)	Japan (A)	730/1,331	TLR9	*Rs187084, rs352139, rs5743836, rs5743842, Rs5743843, rs5743844, rs5743845, rs5743846*	*Rs352139* (*p*=0.029)
Ng *et al*. 2005 (11)	China (A)	934/1,598	TLR9	*Rs187084, rs5743836, rs352139, rs352140*	NS
Hur *et al*. 2005 (12)	Korea (A)	700/660	TLR9	*Rs187084, rs352139, rs352140*	NS

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.

Gene	Polymorphism	Population	No. of studies	Test of association		Test of heterogeneity				
				OR	95% CI	*P*-val	Model	Q	*P*-val	I^2
TLR5	*Rs5744168* 2 vs. 1 allele	European	3	0.910	0.714, 1.161	0.449	F	2.61	0.271	23.3
TLR7	*Rs3853839* 2 vs. 1 allele	Asian	3	1.246	1.160, 1.388	2×10^{-9}	F	0.35	0.838	0
TLR9	*Rs5743836* 2 vs. 1 allele	Overall	3	1.787	0.803, 3.975	0.155	R	8.98	0.011	77.7
		European	2	1.275	0.680, 2.389	0.448	R	2.81	0.093	64.5
		Asian	1	4.243	1.487, 12.10	0.007	NA	NA	NA	NA
TLR9	*Rs352139* 2 vs. 1 allele	Asian	5	1.170	0.939, 1.457	0.161	R	15.9	0.003	74.9
TLR9	*Rs187084* 2 vs. 1 allele	Asian	4	1.044	0.923, 1.181	0.492	F	3.85	0.277	22.2
TLR9	*Rs352140* 2 vs. 1 allele	Asian	3	1.046	0.929, 1.177	0.461	F	1.72	0.422	0

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

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.

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; $p=2\times10^{-9}$) (Table II), and revealed no association between the 2 alleles of the *rs352139 (TLR9)* polymorphism and the risk of developing SLE in Asians (OR 1.170; 95% CI 0.939, 1.457; $p=0.161$) (Table II). However, no similar study on *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=0.0009). 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, rs498679, 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 reaction 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.

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