Association between glutathione S-transferase M1, P1, and NFKBI polymorphisms and systemic lupus erythematosus susceptibility: a meta-analysis

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Abstract: This study aimed to determine whether Glutathione S-transferase M1 (GSTM1), P1 (GSTT1), NFKBI polymorphisms confer susceptibility to systemic lupus erythematosus (SLE). We performed a meta-analysis on the associations between GSTM1 and GSTT1 null genotypes, and NFKBI -94 ins/delATTG polymorphisms and SLE. In total, seven studies were considered for this meta-analysis, which comprised 2,119 SLE patients and 3,014 healthy controls. Meta-analysis of the GSTM1 null polymorphism in 869 SLE and 1,544 control subjects revealed an association between SLE and the GSTM1 null genotype (OR = 1.321, 95% CI = 1.103–1.583, p = 0.002). Stratification by ethnicity indicated an association between the GSTM1 null genotype and SLE in Asians (OR = 1.334, 95% CI = 1.096–1.623, p = 0.004). However, meta-analysis of the GSTT1 null polymorphism, comprising 717 SLE and 1,008 control subjects, revealed no association between SLE and the GSTT1 null genotype overall (OR = 0.850, 95% CI = 0.687–1.051, p = 0.113) or in an Asian population (OR = 0.794, 95% CI = 0.594–1.061, p = 0.119). Meta-analysis of the NFKBI -94 ins/delATTG polymorphism, comprising 1,250 SLE and 1,127 control subjects, revealed an association between SLE and the NFKBI D allele (OR = 1.127, 95% CI = 1.011–1.257, p = 0.031). Ethnicity-specific meta-analysis revealed an association between the NFKBI D allele and SLE in Asians (OR = 1.155, 95% CI = 1.026–1.300, p = 0.017). This meta-analysis demonstrates that the functional GSTM1 and NFKBI polymorphisms are associated with the SLE risk in Asians.

Key words: Systemic lupus erythematosus, GSTM1, GSTT1, NFKBI, Polymorphism, Meta-analysis.

Introduction

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease where immune regulation is disrupted, characterized by multisystem involvement that is mediated by autoantibodies and immune complex deposits. Although the etiology of SLE is not fully understood, it is evident that genetic components play a major role in its development (1). Oxidative modifications of proteins and other biological molecules may lead to the expression of neoantigens and increase the risk of autoimmune diseases (2). Furthermore, oxidation of nucleotides by reactive oxygen species (ROS) increases the DNA immunogenicity (3). Moreover, ROS are involved in the pathogenesis of SLE, because they cause immunogenicity of DNA, lipid oxidation, and immunoglobulin G, generating ligands for which autoantibodies show higher avidity that may turn to meta-analysis. Here, we aimed to determine whether the GSTM1 and GSTT1 null genotypes, and SLE, but results reported are contradictory, owing possibly to the low statistical power of individual studies (9-15). Therefore, to overcome the limitations of individual studies, resolve inconsistencies, and reduce the likelihood of random errors that are responsible for false-positive or false-negative associations (16-18), we turned to meta-analysis. Here, we aimed to determine whether the GSTM1 and GSTT1 null genotypes and NFKBI -94 ins/delATTG polymorphisms confer susceptibility to SLE.
Methods

Identification of eligible studies and data extraction

We performed a search for studies that examined associations between the GSTM1, GSTT1, and NFKB1 polymorphisms and SLE using the MEDLINE, EMBASE databases and the grey literature to identify available reports where GSTM1, GSTT1, and NFKB1 polymorphisms were analyzed in SLE patients (until December 2014). Combinations of keywords, such as, “GSTM1,” “GSTT1,” “NFKB1,” “polymorphism,” “systemic lupus erythematosus,” and “SLE” were entered as Medical Subject Heading (MeSH) components and as text words. References in the identified studies were also investigated to identify additional studies that did not indexed by the electronic databases. Genetic association studies that determined the distributions of the GSTM1, GSTT1 null, and NFKB1 -94 ins/delATTG polymorphisms in SLE patients and normal controls were included. The inclusion criteria were as follows: (1) a case-control study design, (2) original data, and (3) sufficient genotype data to calculate odds ratios (ORs). No language restriction was applied in the meta-analysis. The exclusion criteria were as follows: (1) overlapping data, (2) inability to ascertain the number of null and wild genotypes, and (3) studies of family members based on linkage considerations. The following information was extracted from each study: author, year of publication, ethnicity of the study population, number of cases and controls, and the genotype and allele frequencies of the GSTM1, GSTT1, and NFKB1 polymorphisms.

Evaluations of statistical associations

A chi-square test was used to determine whether observed genotype frequencies conformed to the Hardy-Weinberg equilibrium (HWE) (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). We performed meta-analyses using allelic contrast, homozygote contrast, and recessive and dominant models. Meta-analyses were performed on the association between GSTM1 and GSTT1 null genotypes, and NFKB1 -94 ins/delATTG polymorphisms and SLE. Point estimates of risks, ORs, and 95% and 99% confidence intervals (CI) were estimated for each study. Moreover, Cochran’s Q-statistic was used to assess within- and between-study variations and heterogeneities. This heterogeneity test assesses the null hypothesis that all studies evaluated the same effect. The effect of heterogeneity was quantified using I², which ranges from 0 to 100%, and represents the proportion of between-study variability attributable to heterogeneity rather than chance (19). I² values of 25%, 50%, and 75% were nominally considered low, moderate, and high estimates, respectively. The fixed effects model assumes that a genetic factor has a similar effect on SLE susceptibility across all studies investigated, and that observed variations among studies are caused by chance alone (20). However, the random effects model assumes that different studies show substantial diversity and assesses both within-study sampling errors and between-study variances (21). When study groups are homogeneous, the two models are similar, but if this is not the case the random effects model usually provides wider CIs than the fixed effects model. The random effects model is best used in the presence of significant between study heterogeneity (21). The threshold for statistical significance was 0.05. Statistical manipulations were performed using the Comprehensive Meta-Analysis computer program (Biosta, Englewood, NJ, USA). The power of each study was computed as the probability of detecting an association between the polymorphisms and SLE at a level of significance of p < 0.05, assuming a small effect size (effect size convention w = 0.1). The power analysis was performed using the G*Power statistical program (http://www.gpower.hhu.de).

Evaluation of publication bias

Funnel plots are often used to detect publication bias. However, due to the limitations of funnel plotting, which requires a range of studies of varying sizes involving subjective judgments, we evaluated publication bias using Egger’s linear regression test (22), which measures funnel plot asymmetry using a natural logarithm scale of odds ratios (ORs).

Results

Studies included in the meta-analysis

Seven studies in total were considered in this meta-analysis, which in total involved 2,119 SLE patients and 3,014 controls, and five Asian and two European populations (9-15) (Table 1). Given the populations available, an ethnicity-specific meta-analysis was conducted on the European and Asian populations. Four studies examined the GSTM1 polymorphism, three the GSTT1 polymorphism, and three the NFKB1 polymorphism. Details of the GSTM1, GSTT1, and NFKB1 polymorphism studies included are summarized in Table 1. The statistical power of the studies ranged from 55.9% to 98.8%, and one of the studies had a statistical power exceeding 80%.

Table 1. Characteristics of the individual studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Study [Ref]</th>
<th>Country</th>
<th>Population</th>
<th>Numbers</th>
<th>Studied polymorphism</th>
<th>Association findings</th>
<th>Power (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiyohara, 2012(9)</td>
<td>Japan</td>
<td>Asian</td>
<td>151</td>
<td>GSTM1</td>
<td>GSTM1 (p = 0.370)</td>
<td>66.7</td>
</tr>
<tr>
<td>Zhang, 2010(10)</td>
<td>China</td>
<td>Asian</td>
<td>298</td>
<td>GSTM1, GSTT1</td>
<td>GSTM1 (p = 0.003), GSTT1 (p = 0.119)</td>
<td>67.4</td>
</tr>
<tr>
<td>Kang, 2006(11)</td>
<td>Korea</td>
<td>Asian</td>
<td>330</td>
<td>GSTM1, GSTT1</td>
<td>GSTM1 (p = 0.311), GSTT1 (p = 0.370)</td>
<td>68.7</td>
</tr>
<tr>
<td>Ollier, 1996(12)</td>
<td>UK</td>
<td>European</td>
<td>90</td>
<td>GSTM1, GSTT1</td>
<td>GSTM1 (p = 0.322), GSTT1 (p = 0.779)</td>
<td>72.8</td>
</tr>
<tr>
<td>Cen, 2013(13)</td>
<td>China</td>
<td>Asian</td>
<td>845</td>
<td>NFKB1</td>
<td>NFKB1 (p = 0.047)</td>
<td>98.8</td>
</tr>
<tr>
<td>Gao, 2012(14)</td>
<td>China</td>
<td>Asian</td>
<td>224</td>
<td>NFKB1</td>
<td>NFKB1 (p = 0.176)</td>
<td>59.1</td>
</tr>
<tr>
<td>Orozco, 2005(15)</td>
<td>Spain</td>
<td>European</td>
<td>181</td>
<td>NFKB1</td>
<td>NFKB1 (p = 0.918)</td>
<td>55.9</td>
</tr>
</tbody>
</table>

Ref: reference; UK: United Kingdom; NS: not significant; *: GSTM1, GSTT1 null vs. non-null, or NFKB1 -94 delATTG vs. insATTG allele, *Assuming a small effect size (effect size convention w = 0.1) at a level of significance of 0.05.
Meta-analysis of the association between the GSTM1 and GSTT1 null genotype and SLE susceptibility

We performed meta-analyses of the GSTM1 and GSTT1 null polymorphisms using allelic contrast due to limited data. Meta-analysis of GSTM1 null polymorphism in 869 SLE and 1,544 controls revealed an association between SLE and the GSTM1 null genotype (OR = 1.321, 95% CI = 1.103–1.583, p = 0.002) (Table 2). Stratification by ethnicity indicated an association between the GSTM1 null genotype and SLE in Asians (OR = 1.334, 95% CI = 1.096–1.623, p = 0.004) (Fig. 1, Table 2, 3). The single European study showed no association between SLE and the GSTM1 null genotype (OR = 1.258, 95% CI = 0.798–1.983, p = 0.322) (Fig. 1, Table 2, 3).

Meta-analysis of the GSTT1 null polymorphism, involving 717 SLE and 1,008 control subjects, revealed no association between SLE and the GSTT1 null genotype (OR = 0.850, 95% CI = 0.687–1.051, p = 0.113) (Table 2). Stratification by ethnicity showed no association between the GSTT1 null genotype and SLE in Asians (OR = 0.794, 95% CI = 0.594–1.061, p = 0.119) (Fig. 1, Table 2), and the single European study showed no association between SLE and the GSTT1 null genotype (Fig. 2, Table 2).

Meta-analysis of the NFKB1 -94 ins/delATTG polymorphism and SLE susceptibility

We performed meta-analyses of the NFKB1 -94 ins/delATTG polymorphism using allelic contrast, homozygote contrast, and recessive and dominant models. Meta-analysis of the NFKB1 polymorphism, involving 1,250 SLE and 1,127 control subjects, revealed an association between SLE and the NFKB1 D allele (OR = 1.127, 95% CI = 1.011–1.257, p = 0.031) (Table 2). Stratification by ethnicity indicated an association between the NFKB1 D allele and SLE in Asians (OR = 1.155, 95% CI = 1.026–1.300, p = 0.017) (Fig. 1, Table 2, 3). The single European study showed no association between SLE and the NFKB1 D allele (Table 2). Furthermore, analysis using homozygote contrast model showed no association between SLE and the NFKB1 -94 ins/delATTG promoter polymorphisms and SLE.

Table 2. Meta-analysis of associations between the GSTM1, GSTT1 null, and NFKB1 -94 ins/delATTG promoter polymorphisms and SLE.
delATTG polymorphism and SLE in Asians (Table 2).

**Heterogeneity and publication bias**

The distribution of genotypes in normal control group was not consistent with HWE in one study (13), and the distribution of genotypes in SLE group was not consistent with HWE in another study (14). There were no between-study heterogeneities during meta-analyses, except for the meta-analysis of the NFKB1 DD vs. DI + II genotype (Table 2). In subgroup analysis, there was some heterogeneity with no statistical significance (Table 2). This may be explained that ORs of studies in the subgroup showed the same direction, and the difference of ORs of each study were not big different. The same reason may be the case in the meta-analysis in overall group. ORs of studies in the overall group showed the same direction, and the difference among ORs of each study were not different in overall group. Funnel plots, which are usually used to detect publication bias, were difficult to correlate, presumably because of the small number of studies included (Fig. 2). Egger’s regression test showed no evidence of publication bias in this meta-analysis of the GSTM1, GSTT1, and NFKB1 polymorphisms in any of the studies included (Egger’s regression test p-values > 0.1).

**Discussion**

The increases in ROS levels result in oxidation of DNA and lipids and the production of a variety of cytotoxic products (3). The widely expressed, GST supergene family seems to provide critical cellular protection against ROS. GSTs catalyze the conjugation of glutathione to a variety of substrates, including ROS and other toxins, and thus, facilitate their elimination (5). Previous studies on the GSTM1 and GSTT1 polymorphisms in SLE...
have produced disparate results (9-15), which is not surprising because discordant results are common among genetic studies on complex diseases due to low statistical power, and small sample size. The statistical power of all of the studies except for one study had a statistical power lower than 80%. GSTM1 plays an essential role in the xenobiotics detoxification (5). Because the GSTM1 null genotype lead to a complete lack of enzyme activity, the GSTM1 null genotype is associated with higher ROS levels (23). However, each of the individual studies failed to show an association between the GSTM1 polymorphism and SLE. In this meta-analysis of the GSTM1 null polymorphism in 869 SLE and 1,544 healthy controls, we found an association between SLE and the GSTM1 null genotype (OR = 1.334, 95% CI = 1.096–1.623, p = 0.004). In addition, stratification by ethnicity indicated an association between the GSTM1 null genotype and SLE in Asians. In contrast, with respect to the GSTT1 null polymorphism, we found no association between SLE and the GSTT1 null genotype in all study subjects or in Asians. Our results of no association between the GSTT1 polymorphism and SLE risk are not consistent with previous functional studies on the polymorphism (24). However, epidemiologic results often do not coincide with functional studies because SLE is a complex disease with contributions from multiple genes, different genetic backgrounds, and environmental factors. The negative results for the GSTT1 polymorphisms might also be due to a Type II error. We could not perform a meta-analysis for GSTP1, because only one study on GSTP1 was identified. NFkB plays an important role in the innate and adaptive immune responses, and dysregulated NFkB-signaling may be involved in the pathogenesis of autoimmune diseases, including SLE (25). Moreover, the NFKB1 -94 ins/delATTG promoter polymorphism has functional effects on the transcription of the NFKB1 (8). Our analysis of the NFKB1 -94 ins/delATTG polymorphism, involving 1,250 SLE and 1,127 healthy controls, revealed an association between SLE and the NFKB1 D allele (OR = 1.127, 95% CI = 1.011–1.257, p = 0.031), and in Asians, indicating the functional NFKB1 -94 ins/delATTG polymorphism contributes to SLE susceptibility. Genetic association studies on relations between genetic variants and complex outcomes must be considered with caution because many factors can influence the results. Thus, our results should be interpreted with caution because of the limited number of studies included, which restricted further subgroup analyses. The present study has some limitations that require consideration. First, publication bias or confounding factors may have distorted the meta-analysis, because studies that produced negative results may not have been published or identified in this study. Although we performed the Egger’s regression test, we could not eliminate the possibility of bias. Second, this ethnicity-specific meta-analysis included data from Asian patients, and thus, our results are applicable to only one ethnic group. Third, the small number of studies included in this analysis, especially in the subgroup analysis by environmental factors, prevented our results from reaching a definitive conclusion. In summary, this meta-analysis suggests that the functional GSTM1 and NFKB1 polymorphisms are associated with the susceptibility to SLE in Asians, but the GSTP1 polymorphisms is probably not associated with SLE risk. Further studies of a larger scale in populations with different ethnicities are required to explore the roles played by the GSTM1, GSTT1, and NFKB1 polymorphisms in the pathogenesis of SLE.

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References