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Association of GSTT1, GSTM1 and CYP1A1 polymorphisms with susceptibility to systemic lupus erythematosus in the Chinese population

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ABSTRACT

Background: GSTT1, GSTM1, CYP1A1 are enzymes responsible for the detoxification of the toxicant which may be involved in the development of systemic lupus erythematosus (SLE). We examined the relationship between the risk of SLE and the polymorphisms of these genes in the Chinese population.
Methods: Samples from 298 SLE patients and 284 healthy controls were collected. Polymerase chain reaction-restriction fragments length polymorphism (PCR–RFLP) was used to analyze the genotypes of CYP1A1 m2 and m4, while multiplex PCR was used to analyze the genotypes of GSTT1 and GSTM1.
Results: Statistically significant difference was observed in genotypes for GSTM1 ($p=0.003$, OR 1.66 [95% CI 1.19–2.32]), but not for GSTT1 ($p=0.119$, OR 0.77 [95% CI 0.56–1.07]), in the SLE patients as compared with the controls. Combinational analysis for double-null deletion of both GSTT1 and GSTM1 showed no significant difference ($p=0.863$, OR 1.03 [95% CI 0.70–1.52]). Significant difference was observed in the genotype frequencies ($p=0.013$), but not in the allele frequencies ($p=0.444$, OR 0.90 [95% CI 0.70–1.17]), of CYP1A1 m2. All candidates have a wild-type genotype for CYP1A1 m4.
Conclusions: Polymorphisms of GSTM1 are associated with SLE in the Chinese population.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease featured by inflammation induced by autoantibodies [1–3]. Many organs including the skin, heart, lungs, blood vessels, liver, joints, kidneys and nervous system are affected [3–5]. The etiology of SLE is complex and includes multiple genetic and environmental factors such as medications, hormones, stress, infections, cigarette exposure, UV exposure and toxicants [6–9]. Epidemiological investigations found that the combining outcome of these factors may play a vital role in the development of SLE [10,11].

Toxicants absorbed by the human body can be detoxified by enzymes in human cells. The detoxification process includes 2 phases of metabolism [12]. Phase I of detoxification is mainly catalyzed by the cytochrome P450 (CYP) family of enzymes which generate reactive intermediates that can be subsequently metabolized by phase II enzymes. CYP1A1 is a well understood phase I enzyme that has 4 polymorphisms in the human CYP1A1 gene: m1, C/T in the 3′-noncoding region; m2, A/G at nucleotide 2455 leading to the amino acid change of isoleucine to valine at codon 462, which influences its enzyme activity; m3, also C/T in the 3′-noncoding region; and m4, C/A at nucleotide 2453 resulting in the amino acid change of threonine to asparagines at codon 461 [13–17]. Glutathione S-transferases (GSTs) are phase II enzymes that are responsible for the detoxification of reactive intermediates produced by CYP1A1. In humans, there are at least 13 GSTs that can be divided into 5 subfamilies: α (GSTA), θ (GSTT), π (GSTP), μ (GSTM) and δ (GSTS) [18,19]. It has been shown that the null deletion of GSTT1 or GSTM1 results in the expression of protein without enzyme activity [20,21].

The loss-of-function of GSTT1, GSTM1, and CYP1A1 m2 and m4 may be involved in the development of SLE. Several previous studies investigated the association between SLE and the polymorphisms of these enzymes. Horiuchi et al. found no association between GSTT1 and GSTM1 and the risk of SLE [22], while Kang et al. suggested that deletion of GSTT1 or GSTM1 may influence the manifestation of SLE but not the risk of SLE [23]. Fraser PA and his colleagues reported that

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the GSTM1 homozygous null deletion may alter the effect of occupational sun exposure on the risk of SLE in Caucasians [24]. For the CYP1A1 polymorphisms, it is shown that m2 and m4 polymorphisms both are associated with the risk of SLE [25,26].

2. Materials and methods

2.1. Study populations

The present study included 298 SLE patients and 284 healthy controls. All patients fulfilled the 1997 American College of Rheumatology (ACR) criteria [27] of SLE. Individuals without allergic inflammatory disorders were set as the healthy controls. The study was approved by the ethical committee of the Shenzhen Hospital, Peking University. The individuals gave their written informed consent. The investigations were conducted according to the Declaration of Helsinki principles.

2.2. DNA extraction

Genomic DNA was isolated from the peripheral blood of the candidates using the Genomic DNA Extraction Kit (Sangon, Shanghai) according to the manufacturer's instruction.

2.3. Genotyping of CYP1A1 m2 and m4

The CYP1A1 m2 and m4 polymorphisms were analyzed by PCR–RFLP (polymerase chain reaction–restriction fragments length polymorphism) as previously described [25]. Briefly, PCR was carried out with 40 ng of genomic DNA in 40 μl PCR reaction containing 4 μl 10×Taq PCR buffer, 1.5 mmol/l $MgCl_2$, 200 μmol/l dNTPs, 1.5 U Taq polymerase, and 0.25 μmol/l of primers (forward: 5′-GCC TGT CCT CTA TCC TTT-3′; and reverse: 5′-GTG AGA CTA CCT CTG TGC C-3′). Amplification was performed as follows: 1 cycle of 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s, and 1 cycle of 72 °C for 10 min. The 912 bp PCR products can be confirmed by electrophoresis. Then 10 μl of PCR products were incubated with *Bsr*DI at 37 °C overnight and *Bsa* at 65 °C overnight, respectively, for the detection of CYP1A1 m2 and m4. The digested products were electrophoesed in 2% agarose and visualized by GlodenView staining.

2.4. Genotyping of GSTT1 and GSTM1

Allele-specific PCR, as previously described [28], was used to detect the GSTT1 and GSTM1 polymorphisms. Briefly, the PCR is conducted with 20 ng genomic DNA in a 20 μl PCR reaction mixture containing 1.5 mmol/l $MgCl_2$, 200 μmol/l dNTPs, 1.0 U Taq polymerase, 0.5 μmol/l of GSTT1-specific primers (forward: 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′; and reverse: 5′-TCA CCG GAT CAT GGC CAG CA-3′) or GSTM1-specific primers (forwards: 5′-CTG CCC TAC TTG ATT GAT GGG-3′; and reverse: 5′-CTG GAT TGT AGC AGA TCA TGC-3′). GAPDH gene was amplified as internal control in the same reaction (forward: 5′-CAG CCT CAA GAT CAT CAG CA-3′; and reverse: 5′-TGT GGT CAT GAG TCC TTC CA-3′). Amplification was performed as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 sec, 60 °C for 20 sec, 72 °C for 1 min and a final extension at 72 °C for 10 min.

3. Statistical analysis

From our prototype data ($n^*=50$), the predicted sample size and power (when $df=1$ and alpha = 0.05) are as follows: GSTM1, $n=100$/power = 0.8174, $n=200$/power = 0.953, and $n=300$/power = 0.9981; GSTT1, $n=100$/power = 0.8734, $n=200$/power = 0.992, and $n=300$/power = 0.9993; and CYP1A1, $n=100$/power = 0.8353, $n=200$/power = 0.984, and $n=300$/power = 0.9989. In our study, the sample sizes of patients are 298, and controls are 284. Statistical analysis comparing the allele and genotype distributions was performed using the χ^2 test or Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated according to Woolf's method, using the SPSS 10.0 software for Windows. A $p<0.05$ were considered statistically significant.

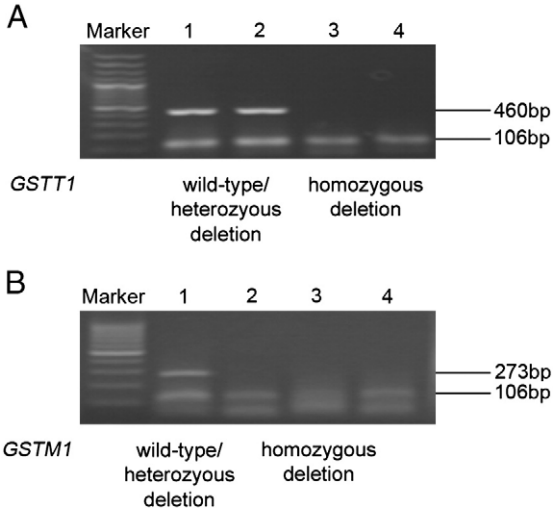

Fig. 1. Representative results for GSTT1 and GSTM1 genotyping. (A)Wild-type and heterozygous deletion of GSTT1 were indicated by the 460 bp PCR product with the co-amplified 106 bp PCR product of GAPDH as internal control. The absence of the 460 bp PCR product indicates homozygous deletion of GSTT1. (B)Wild-type and heterozygous deletion of GSTM1 were indicated by the 273 bp PCR product with the co-amplified 106 bp PCR product of GAPDH as internal control. The absence of the 273 bp PCR product indicates homozygous deletion of GSTM1.

4. Results

Representative genotyping results of GSTT1 and GSTM1 were shown in Fig. 1. Allele-specific PCR was used to distinguish homozygous deletion from wild-type/heterozygote. For these 2 genes, homozygous deletion resulted in the disappearance of the PCR products (Fig. 1). Table 1 shows GSTT1 and GSTM1 genotype frequencies in SLE patients and healthy controls. A statistically significant difference was observed in genotypes for GSTM1 ($p=0.003$, OR 1.66 [95% CI 1.19–2.32]), but not for GSTT1 ($p=0.119$, OR 0.77 [95% CI 0.56–1.07]), in the SLE patients as compared with the controls. Then we did a combinational analysis by comparing the candidates that have double-null deletions of both GSTT1 and GSTM1 with the rest of the population, and no significant difference was observed ($p=0.863$, OR 1.03 [95% CI 0.70–1.52]).

The strategy and representative results of CYP1A1 genotyping were shown in Fig. 2. PCR–RFLP (polymerase chain reaction–restriction fragments length polymorphism) was used to distinguish

Table 1
Genotype frequencies of GSTM1 and GSTT1 in SLE cases and controls.

Genotype frequency, no. (%)					
Genotypes, population	No. of subjects	Wild-type/ heterozygotes	Null deletion	*p*	OR (95% CI)
GSTM1					
Cases	298	108 (36.2)	190 (63.8)	0	1.66 (1.19–2.32)
Controls	284	138 (48.6)	146 (51.4)		
GSTT1					
Cases	298	163 (54.7)	135 (45.3)	NS	0.77 (0.56–1.07)
Controls	284	137 (48.2)	147 (51.8)		
GSTM1 and GSTT1					
Cases	298	228 (76.5)	70 (23.5)[a]	NS	1.03 (0.70–1.52)
Controls	284	219 (77.1)	65 (22.9)[a]		

SLE = systemic lupus erythematosus; OR = odds ratios; 95% CI = 95% confidence interval. *p* value is calculated by Chi-square test between cases and controls.
[a] Double-null deletion of both GSTM1 and GSTT1.