Glutathione S-Transferase M1, T1, and P1 Genotypes and Rheumatoid Arthritis

BO RA YUN, AHMED EL-SOHEMY, MARILYN C. CORNELIS, and SANG-CHEOL BAE

ABSTRACT. Objective. To determine the effects of genetic polymorphisms of glutathione S-transferase (GST) M1, GSTT1, and GSTP1 on risk and severity of rheumatoid arthritis (RA) in a Korean population.

Methods. A total of 258 patients with RA and 400 disease-free controls were enrolled. GST genotypes were determined by RFLP-PCR. HLA-DRB1 typing and further subtyping of all alleles was performed using sequence-specific oligonucleotide probe hybridization after PCR. Severity of RA among cases was assessed by Steinbrocker anatomical stage. Risk was assessed by calculating the age and sex adjusted odds ratio (OR) and 95% confidence intervals (CI).

Results. The OR for risk of RA with the GSTM1-null genotype was 1.40 (95% CI 1.02–1.92, p = 0.04), and 1.86 (95% CI 1.12–3.09, p = 0.005) among individuals without the shared epitope (SE). Among patients with RA, the OR for risk of severe RA for the GSTM1-null genotype was 2.45 (95% CI 1.04–5.77, p = 0.02). No association was observed between the GSTT1 or GSTP1 genotypes and either risk or severity of RA.

Conclusion. These results suggest that the deletion polymorphism of GSTM1 is associated with increased susceptibility for RA, particularly among individuals who are not carriers of the HLA-DRB1 SE.

Key Indexing Terms:
RHEUMATOID ARTHRITIS
GENOTYPE

Rheumatoid arthritis (RA) is characterized by symmetrical inflammation of the peripheral joints, resulting in progressive destruction of articular and periarticular joints and structures. Both genetic and environmental factors contribute to the development of this chronic disease, with genetic differences accounting for about 50% of the variability in risk. Among the genetic markers studied to date, the HLA system, particularly the HLA-DRB1 molecule, is one of the most important determinants of risk and severity of RA. The HLA-DRB1 associated alleles encode a conserved amino acid sequence referred to as the shared epitope (SE). Certain combinations of the SE-bearing alleles have been associated with a more severe form of RA.

The inflammation that typically occurs in patients with RA results in an increased production of reactive oxygen species (ROS), which cause oxidative damage to cellular molecules such as DNA and lipids. ROS are produced by phagocytes in the synovial fluid and pannus, and by synovial endothelial cells during hypoxia-reperfusion events, and there is growing evidence that ROS and their byproducts may play a role in the development of RA. Thus, genetic differences in the capacity to detoxify ROS and their byproducts may modulate the risk and severity of RA.

Mammalian cells constitutively express a number of detoxifying enzymes such as the glutathione S-transferase (GST) family. GST are a superfamily of polymorphic enzymes that play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. Common polymorphisms have been identified in the GSTP1, GSTM1, and GSTT1 isoenzymes. An amino acid substitution (Ile→Val) at codon 105 in exon 5 of GSTP1 results in altered heat stability and reduced catalytic activity toward certain substrates. Both the GSTM1 and GSTT1 genes are affected by deletion polymorphisms that result in deficient conjugating activity. Homozygosity for a common deletion in the GSTM1 gene (GSTM1*0) results in a complete lack of GSTM1 activity, while the GSTM1*1 allele encodes an active form of the enzyme. Similarly, GSTT1 has 2 alleles, denoted GSTT1*0 for the null allele and GSTT1*1 for the functional allele. Genetic polymorphisms of these enzymes may contribute to the wide variation seen in the extent of joint damage and functional impairment. Further, differences in the frequencies of the GST polymorphisms exist among various ethnic populations, and could account for some of the differences in disease prevalence.
GSTM1-null is associated with increased production of anti-Ro antibodies in patients with systemic lupus erythematosus, and GSTT1-null confers an increased risk of inflammatory diseases such as ulcerative colitis and Crohn’s disease. Few studies have investigated the role of GST in RA, and no study has examined the role of GST in RA among Asians. We investigated whether genetic polymorphisms of GSTM1 (null), GSTT1 (null), and GSTP1 (Ile105Val) modify the risk or severity of RA in a Korean population.

**MATERIALS AND METHODS**

Korean patients (*n* = 258) who met the 1987 American College of Rheumatology (ACR) classification criteria for RA were recruited consecutively from the outpatient clinic of The Hospital for Rheumatic Diseases, Seoul, Korea. Four hundred healthy volunteers who were ethnically identical were enrolled from the same hospital (nurses, paramedical personnel, and laboratory workers) as the control group. Written informed consent was obtained from each subject. Clinical data including age, sex, age of disease onset, disease duration and duration of treatment, and laboratory data were obtained retrospectively from patient medical records.

**Clinical variables.** Severity of RA was classified into one of 4 stages based on joint damage assessed by Steinbrocker radiographic criteria. For this study, Stage I was regarded as mild RA, and Stages II, III, and IV as severe RA. Functional status was determined using the ACR criteria for classification of global functional status and the Korean Health Assessment Questionnaire (KHAQ).

**Genotyping.** GSTM1 (deletion), GSTT1 (deletion), and GSTP1 (Ile105Val) were assayed without knowledge of case-control status using a multiplex restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method that simultaneously detects the polymorphisms in all 3 genes in a single reaction as described. Briefly, 10 ng of DNA was amplified using a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA) and the HotStar™ DNA polymerase kit (Qiagen, Mississauga, ON, Canada) with PCR buffer containing 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 U Taq, and 8 pmol of each primer set. All primers were synthesized by ACGT (Toronto, ON, Canada). After an initial denaturation at 95°C for 15 min, amplification was achieved using a touchdown PCR protocol with 20 cycles of 94°C for 30 s, 68–48°C for 30 s with a reduction of 1°C in each cycle, and 72°C for 30 s, followed by 20 cycles with the annealing temperature set at 51°C and a final extension at 72°C for 10 min. Following restriction enzyme digestion with 2 U of Alw26I, PCR products were resolved by 2% agarose gel electrophoresis and stained with ethidium bromide. Bands were visualized using a FluorChem™ UV imaging system. GSTM1 and GSTT1 genotypes were determined by the presence or absence of a 275 bp (195 + 80 after digestion) and 480 bp band, respectively. Because the GSTM1 fragment contains a nonpolymorphic Alw26I restriction site, the 275 bp band that is amplified is digested into 195 bp and 80 bp fragments in all samples, providing a positive control for complete digestion. The 294 bp band represents GSTP1, and the Ile105Val (A→G substitution) polymorphism in this gene contains an Alw26I restriction site that produces a 234 bp and 60 bp band after digestion. Amplification of GSTP1 also serves as an internal control for the PCR reactions that have both GSTM1-null and GSTT1-null alleles.

**HLA-DRB1 typing.** HLA-DRB1 typing and further subtyping of all alleles were performed by PCR, sequence-specific oligonucleotide probe hybridization, and direct DNA sequencing analysis. We defined the SE as having HLA-DRB1 *+0101, *+0401, *+0404, *+0405, *+0410, *+1001, and *+1406 alleles.

**Statistical analysis.** All data were analyzed using the Statistical Analysis System software (SAS, version 8.2), with tests of significance at the alpha = 0.05 level. Differences in GST genotype and HLA-DRB1 SE type distributions between RA patients and controls and between mild and severe RA was assessed using (univariate) 2 by 2 contingency tables and the chi-square test. Where significant differences were observed, multiple logistic regression analyses were applied adjusting for age, sex, and disease duration. To distinguish the independent effects of the GST genotype from the SE on risk or severity of RA, we examined the effect of GST genotype within each SE type, with the wild-type GST as the reference group. For these analyses, homozygous and heterozygous risk alleles were grouped together. Odds ratios with 95% confidence intervals (95% CI) were calculated from 2 by 2 contingency tables by chi-square test.

**RESULTS**

Clinical characteristics of subjects by case-control status and RA severity are shown in Table 1. Compared to controls, RA patients were older and more likely to be female. According to Steinbrocker’s radiographic criteria, 91% of cases were classified as having severe RA. Patients with severe RA were more likely to be female and younger at age of disease onset compared to those with mild RA.

Table 2 shows each genotype distribution by case-control status. The GSTP1 genotype distribution among controls followed the Hardy-Weinberg equilibrium (*p* = 0.98). Deviations from Hardy-Weinberg equilibrium were not tested for distribution of GSTM1 and GSTT1 genotype because the PCR assay did not enable discrimination of homozygote from homozygote carriers. The GSTM1-null genotype was associated with an increased risk of RA (Table 2). Compared to the GSTM1-positive genotype, the age and sex adjusted OR for risk of RA was 1.40 (95% CI 1.02–1.92, *p* = 0.04). The GSTM1-null genotype was also associated with greater disease severity (Table 3). The adjusted OR for risk of severe RA for the GSTM1-null was 2.45 (95% CI 1.04–5.17, *p* = 0.02) compared to patients with the GSTM1-positive genotype. GSTT1 and GSTP1 genotypes were not associated with either risk (Table 2) or severity (Table 3) of RA. The effect of combined GST genotypes showed no associations with risk or severity of disease (data not shown). The GSTM1, GSTT1, and GSTP1 genotypes were not associated with the RA functional status (data not shown).

We next examined the independent effects of the GST genotype from the SE in terms of the risk and severity of RA. As anticipated, RA patients had a greater frequency of the SE compared to controls, and patients with severe RA had greater frequency of this allele compared to those with mild RA (Table 1). The OR for risk of RA with the GSTM1-null genotype was 1.46 (95% CI 0.91–2.35, *p* = 0.12), and 1.86 (95% CI 1.12–3.09, *p* = 0.005) among individuals with and without the SE (Table 4). Among patients with RA, the OR for risk of severe RA for the GSTM1-null genotype was 2.57 (95% CI 0.82–8.04, *p* = 0.057) and 2.68 (95% CI 0.71–10.17, *p* = 0.096) for subjects with and without the SE, respectively (Table 5). The absence of a statistically significant association with severity may be due to the reduced sample size after stratifying by the presence or absence of the SE.
Table 1. Characteristics of Korean cases with RA and controls.

<table>
<thead>
<tr>
<th></th>
<th>Control (%, n = 400)</th>
<th>RA (%, n = 258)</th>
<th>p</th>
<th>Mild RA* (%, n = 24)</th>
<th>Severe RA** (%, n = 234)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SEM (range), yrs</td>
<td>41.8 ± 14.1 (19–76)</td>
<td>49.3 ± 11.2 (24–72)</td>
<td>0.001</td>
<td>48.7 ± 8.4 (38–71)</td>
<td>49.3 ± 11.4 (24–72)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, female:male</td>
<td>351/49 (7:1)</td>
<td>249/30 (8:1)</td>
<td>0.011*</td>
<td>213/76 (38:1)</td>
<td>228/6 (38:1)</td>
<td>0.00049*</td>
</tr>
<tr>
<td>Mean age at onset ± SEM (range), yrs</td>
<td>—</td>
<td>41.7 ± 11.1 (13–68)</td>
<td>0.001</td>
<td>42.0 ± 9.1 (29–67)</td>
<td>41.5 ± 11.0 (13–68)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean disease duration ± SEM (range), yrs</td>
<td>—</td>
<td>12.9 ± 7.0 (1–40)</td>
<td>0.001</td>
<td>9.1 ± 5.0 (2–20)</td>
<td>13.2 ± 7.0 (2–40)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean treatment duration ± SEM (range), yrs</td>
<td>—</td>
<td>7.6 ± 3.6 (1–18)</td>
<td>0.011*</td>
<td>6.8 ± 4.2 (2–14)</td>
<td>7.8 ± 3.6 (2–18)</td>
<td>NS</td>
</tr>
<tr>
<td>RF-positive, %</td>
<td>—</td>
<td>80.1</td>
<td>75.9</td>
<td>80.7</td>
<td>NS</td>
<td></td>
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<tr>
<td>Functional class (n = 247)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>—</td>
<td>55 (22)</td>
<td>7 (29)</td>
<td>48 (22)</td>
<td>—</td>
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<tr>
<td>II</td>
<td>—</td>
<td>64 (26)</td>
<td>7 (29)</td>
<td>57 (25)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>—</td>
<td>81 (33)</td>
<td>7 (29)</td>
<td>74 (33)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>—</td>
<td>47 (19)</td>
<td>3 (12)</td>
<td>44 (20)</td>
<td>—</td>
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<tr>
<td>Anatomical class</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>Stage I</td>
<td>—</td>
<td>24 (9)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>—</td>
<td>64 (25)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>—</td>
<td>121 (47)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>—</td>
<td>49 (19)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>SE, n (%)</td>
<td>—</td>
<td>&lt; 0.0001</td>
<td>0.049</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>+/+</td>
<td>7 (2)</td>
<td>28 (11)</td>
<td>4 (0.16)</td>
<td>24 (0.10)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>+/−,−/+</td>
<td>120 (30)</td>
<td>139 (54)</td>
<td>10 (0.42)</td>
<td>129 (0.55)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>−/−</td>
<td>273 (68)</td>
<td>91 (35)</td>
<td>10 (0.42)</td>
<td>81 (0.35)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>


Table 2. Frequencies of GSTM1, GSTT1, and GSTP1 genotypes and risk of RA.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control (n = 400), n (%)</th>
<th>RA (n = 258), n (%)</th>
<th>OR (95% CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-null</td>
<td>186 (46.5)</td>
<td>99 (38)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Null</td>
<td>214 (53.5)</td>
<td>159 (62)</td>
<td>1.40 (1.02–1.92)</td>
<td>0.04</td>
</tr>
<tr>
<td>GSTT1 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-null</td>
<td>186 (46.5)</td>
<td>134 (52)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Null</td>
<td>214 (53.5)</td>
<td>124 (48)</td>
<td>0.80 (0.59–1.10)</td>
<td>0.25</td>
</tr>
<tr>
<td>GSTP1 (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ile/Ile</td>
<td>269 (67)</td>
<td>155 (60)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Ile/Val + Val/Val</td>
<td>131 (33)</td>
<td>103 (40)</td>
<td>1.36 (0.99–1.89)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Non-null: heterozygous or homozygous for the presence of the gene; null: homozygous deletion. * p value was adjusted for age and sex.

Table 3. GSTM1, GSTT1, and GSTP1 genotypes and severity of RA.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mild RA (n = 24), n (%)</th>
<th>Severe RA (n = 234), n (%)</th>
<th>OR (95% CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-null</td>
<td>14 (58)</td>
<td>85 (36)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Null</td>
<td>10 (42)</td>
<td>149 (64)</td>
<td>2.45 (1.04–5.77)</td>
<td>0.02</td>
</tr>
<tr>
<td>GSTT1 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-null</td>
<td>9 (37.5)</td>
<td>115 (49)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Null</td>
<td>15 (62.5)</td>
<td>119 (51)</td>
<td>0.62 (0.26–1.47)</td>
<td>0.096</td>
</tr>
<tr>
<td>GSTP1 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>17 (71)</td>
<td>138 (59)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Ile/Val + Val/Val</td>
<td>7 (29)</td>
<td>96 (41)</td>
<td>1.69 (0.37–4.23)</td>
<td>0.095</td>
</tr>
</tbody>
</table>

* P value was adjusted for age, sex, and disease duration.
DISCUSSION

GST represent a family of detoxifying enzymes that play a major role in protecting cells against the damaging effects of ROS, which have been implicated in the pathogenesis of RA\textsuperscript{9,10}. Common polymorphisms in the genes encoding these enzymes may therefore, alter risk or severity of the disease. Individuals who are homozygous for deletion polymorphisms in GSTM1 and GSTT1 do not express a functional protein\textsuperscript{20,21}. An amino acid substitution (Ile → Val) at codon 105 in exon 5 of GSTP1 results in altered heat stability and reduced catalytic activity toward certain substrates\textsuperscript{19}. We investigated whether genetic polymorphisms of GSTM1, GSTT1, and GSTP1 are associated with the risk or severity of RA in a Korean population. Our data suggest that the GSTM1-null genotype significantly increases the risk of RA in this population. However, no significant associations were observed for either GSTT1 or GSTP1. The lack of an association with either GSTT1 and GSTP1 may be due to differences in tissue-specific gene expression or to a different role of certain substrates of GST in the pathogenesis of RA.

The frequencies of the GSTM1, GSTT1, and GSTP1 genotypes differ among ethnic populations\textsuperscript{33,34}, and few studies have examined the association between GST and RA\textsuperscript{23-25}. The frequency of roughly 50% for GSTM1-null that we observed is similar to frequencies reported in other populations. However, the frequency of the GSTT1-null (~50%) is higher than the 20%–30% reported in most populations examined. We have reported a Val allele frequency of 18% among the present Korean population, which is somewhat lower than frequencies reported in other populations\textsuperscript{23}.

To our knowledge, only one study has examined the role of genetic polymorphisms of GST in RA\textsuperscript{23-25}, which involved a population of Northern European Caucasians. It has been suggested that the presence of a functional allele for GSTM1 may be associated with a reduced risk of RA\textsuperscript{23}, which is consistent with the results of our study. These findings relating GST genotype with risk or severity of RA were independent of rheumatoid factor positivity and the presence of the SE, both of which have been associated with greater disease severity\textsuperscript{3,35-37}. Having observed a significant effect of GSTM1 on both risk and severity of RA, we investigated the independent role between the SE and GSTM1 genotype. We found that these 2 susceptibility genes had independent roles in the development of RA, a result consistent with a previous study\textsuperscript{23}. Also, either candidate gene may have a more pathogenic role, especially among individuals without the other susceptible gene, suggesting HLA-DRB1 and GSTM1-null genotype may be involved in different pathogenic pathways. However, these findings were not statistically significant after adjustment for multiple hypothesis testing.

Using a case-only study design, a recent study reported that while disease outcome in patients with a history of smoking is significantly worse than in those who have never smoked, smoking was associated with the most severe disease in patients who carried the GSTM1-null polymorphism\textsuperscript{25}. This observation provides further evidence that the inability to detoxify certain reactive compounds may promote the development of the disease. In our study, however, most patients were nonsmokers.

One limitation of our study is that we did not use a quantitative method by scoring standard radiographs, such as the Larsen scores in the assessment of radiologic outcome of RA. The cases in our study had a long duration of disease and treatment, and it is likely that many were taking various

\begin{table}
\centering
\caption{Effect of GSTM1 genotypes on RA susceptibility according to shared epitope (SE).}
\begin{tabular}{lcccc}
\hline
HLA-DRB1 SE/SE or SE/X & GSTM1 Controls, N = 400 (%) & RA, N = 258 (%) & OR (95% CI) & p* \\
\hline
+ & Non-null & 62 (53) & 72 (43) & 1.0 \\
+ & Null & 56 (47) & 95 (57) & 1.46 (0.91–2.35) & 0.12 \\
− & Non-null & 124 (44) & 27 (30) & 1.0 \\
− & Null & 158 (56) & 64 (70) & 1.86 (1.12–3.09) & 0.005 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{The effect of GSTM1 genotypes on RA severity according to shared epitope (SE).}
\begin{tabular}{lcccc}
\hline
HLA-DRB1 SE/SE or SE/X & GSTM1 Mild RA, n = 24 & Severe RA, n = 234 & OR (95% CI) & p* \\
\hline
+ & Non-null & 9 (64) & 63 (41) & 1.0 \\
+ & Null & 5 (36) & 90 (59) & 2.57 (0.82–8.04) & 0.057 \\
− & Non-null & 5 (50) & 22 (27) & 1.0 \\
− & Null & 5 (50) & 59 (73) & 2.68 (0.71–10.17) & 0.096 \\
\hline
\end{tabular}
\end{table}
medications. Because certain drugs produce ROS when oxidized, the use of certain drugs may have diminished the effect of GST on disease severity.

Our findings suggest that the deletion polymorphism of GSTM1 is associated with increased susceptibility to RA, particularly among individuals who are not carriers of the HLA-DRB1 shared epitope.

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REFERENCES

33. Lin HI, Han CY, Bernstein DA, Hsiao W, Lin BK, Hardy S. Ethnic distribution of the glutathione transferase Mu 1-1 (GSTM1) genotype in 1473 individuals and application to bladder cancer