Genetic Polymorphism of C452T (T127I) in Human γ-Glutamyl Hydrolase in a Japanese Population

Hideki Hayashi,a Chihiro Fujimaki,a Kazuyuki Inoue,b Toshio Suzuki,b and Kunihiko Itoh*a

a Department of Clinical Pharmacology and Genetics, School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan; and b Department of Pharmaceutical Science, Akita University Hospital; 1–1–1 Hondo, Akita 010–8543, Japan.

Received November 27, 2006; accepted January 8, 2007; published online January 11, 2007

We investigated the genotype distribution and allele frequency of C452T polymorphism of γ-glutamyl hydrolase (GGH) gene, which causes the decreased enzymatic activity affecting the efficacy of methotrexate (MTX), in a Japanese population. The polymerase chain reaction–restriction fragment length polymorphism assay was applied to determine the genotype of C452T polymorphism in 269 Japanese healthy individuals. The genotype distribution was as follows: C/C, 89.2% (n=240); C/T, 10.4% (n=28); T/T, 0.4% (n=1). The frequency of C and T allele was 0.944 and 0.056, respectively. The obtained genotype distribution was well agreed with those expected by Hardy–Weinberg equilibrium. The genotype distribution and allele frequency in a Japanese population were found to be similar to those of African–Americans but significantly different from Caucasians. Although the frequency of variant T allele in a Japanese population is not so high as compared to Caucasians, determination of C452T polymorphism of GGH may be useful for monitoring of efficacy and side-effects of MTX for treatment of diseases such as rheumatoid arthritis or childhood acute leukemia. To our knowledge, this is the first report about the examination of C452T polymorphism of GGH in a Japanese population.

Key words γ-glutamyl hydrolase; genetic polymorphism; methotrexate; rheumatoid arthritis; childhood acute leukemia

MATERIALS AND METHODS

Methods of Genotyping Whole venous blood was obtained from 269 unrelated healthy Japanese volunteers using EDTA-2Na Venoject II tubes (Terumo, Tokyo, Japan). Leukocyte genomic DNA was extracted directly from the blood specimen using a QIAamp DNA Blood Mini Kit (Qia-gen, Hilden, Germany). The C452T polymorphism of GGH was determined by a PCR-RFLP reported by Chave et al. with a minor modification. Genomic DNA (100 ng) was amplified in 1×PCR buffer containing 200 mM concentrations of dNTP mixture (Applied Biosystems, Foster City, CA, U.S.A.), 0.5 μM each of forward primer (5′-GTG CCT ATT TGG TTA TGA CA-3′) and reverse primer (5′-CTT ACT AAT CCT GCC CA-3′), 0.5 units HotStar Taq DNA polymerase (Qiagen), and 1.5 mM MgCl2. Amplification was performed by i-Cycler thermal cycler (Bio-Rad, Hercules, CA, U.S.A.). PCR conditions were an initial denaturation/enzyme activation step of 15 min at 95°C, 35 cycles of 15 s at 94°C, 30 s at 55°C, 45 s at 72°C, and a final extension step of 10 min at 72°C. The amplified PCR products (286 bp) were digested with 2.5 U Ase I (New England Bio-Labs, Beverly, MA, U.S.A.) at 37°C. Digested products were separated on 3% agarose gel (agarose S, Nippon Gene, Tokyo, Japan). The genotype was determined by digestion

© 2007 Pharmaceutical Society of Japan
patterns of PCR products by Asel (wild C allele: 286 bp, mutant T allele: 177+109 bp) (Fig. 1).

**Statistical Analysis** The Fisher’s exact test was used for comparison of genotype and allele frequencies among different populations. The CC/CT/TT genotype or C/T allele frequencies were analyzed between a Japanese population and different ethnic populations as reported previously.\(^\text{12}\) The 95% confidence intervals were calculated for all observed genotype and allele frequencies. The value of \(p<0.05\) was considered to be statistically significant. Statistical analysis was carried out using SAS Proprietary Software Release 8.2 (SAS Institute Inc., Cary, NC, U.S.A.).

**RESULTS AND DISCUSSION**

The C452T polymorphism located in exon 5 of the GGH gene involving Thr to Ile amino acid exchange results in decreased catalytic activity of GGH.\(^\text{12}\) We determined the genotype distribution and allele frequency of C452T polymorphism in 269 Japanese healthy individuals by PCR-RFLP. As shown in Table 1, the genotype distribution of C452T polymorphism was well agreed with those expected by Hardy–Weinberg equilibrium. The number of each genotype was as follows: C/C, 240 (89.2%); C/T, 28 (10.4%); T/T, 1 (0.4%). The frequency of C and T allele was 0.944 and 0.056, respectively.

The genotype distribution and allele frequency in a Japanese population obtained in this study was compared with those in Caucasians and in African–Americans as reported previously.\(^\text{12}\) As shown in Table 2, the genotype distribution and allele frequency in a Japanese population was significantly different from Caucasians (genotype distribution: \(p=0.029\), allele frequency: \(p=0.019\)), but was not different from African–Americans (genotype distribution: \(p=0.871\), allele frequency: \(p=0.689\)). Although the reason for the similarity of distribution of C452T between in a Japanese population and in African–Americans remains obscure, these results suggest that the ethnic difference may exist in C452T polymorphism of GGH.

MTX is one of the most widely used anti-inflammatory and anticancer agents. Low dose MTX, oral administered in a weekly pulse, has been extensively used in the treatment of RA.\(^\text{5,6}\) On the other hand, high dose MTX is usually administered as a prolonged i.v. infusion and is an important component in the treatment regimens for a variety of cancers, including ALL, lymphoma, osteosarcoma, breast cancer, and head and neck cancer.\(^\text{15}\) MTX-treated patients often show the side-effects such as hepatitis with raised aminotransferases, hematological abnormalities and central nervous system toxicity.\(^\text{16}\) Therapeutic drug monitoring (TDM) of serum MTX is clinically employed for avoiding side-effects in MTX-treated patients, particularly in high dose MTX-treated patients. As for low dose MTX-treated patients, TDM is not applicable because serum MTX level would be under detection limit after 24 h of MTX administration. In these cases, erythrocyte MTXPG may be applicable as a marker for predicting efficacy and side-effects of MTX.\(^\text{13}\) Since increased intracellular MTXPG may result from low GGH activity caused by variant T allele of C452T polymorphism of GGH, this polymorphism may be helpful for anticipating the efficacy and side-effects of MTX even though the serum MTX is undetectable.

Retrospective study about the correlation between the C452T polymorphism of GGH and the efficacy and side-effects of MTX in Japanese RA patients is now in progress.

**Acknowledgements** We would like to thank Dr. Atsushi Oshio, Department of Psychology, Chubu University, for his statistics expertise. We gratefully acknowledge the participation of all volunteers.

**REFERENCES**

2) Gorlick R., Goker E., Trippett T., Waltham M., Banerjee D., Bertino J.

---

**Table 1. Genotype Distribution and Allele Frequency of C452T Polymorphism of GGH in a Japanese Population**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed freq. ((n))</th>
<th>95% CI a</th>
<th>Expected freq. b</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>89.2% (240)</td>
<td>85.5—92.9</td>
<td>89.1%</td>
</tr>
<tr>
<td>C/T</td>
<td>10.4% (28)</td>
<td>6.7—14.1</td>
<td>10.6%</td>
</tr>
<tr>
<td>T/T</td>
<td>0.4% (1)</td>
<td>0.0—1.1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>94.4% (508)</td>
<td>92.5—96.4</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>5.6% (30)</td>
<td>3.6—7.5</td>
<td></td>
</tr>
</tbody>
</table>

a) 95% confidence interval. b) Expected by Hardy–Weinberg equilibrium.

**Table 2. Comparison of Genotype and Allele Frequency of C452T Polymorphism of GGH in a Japanese Population with Other Ethnic Groups**

<table>
<thead>
<tr>
<th>Population ((n))</th>
<th>Genotype freq.</th>
<th>p value</th>
<th>Allele freq.</th>
<th>p value</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C</td>
<td>C/T</td>
<td>T/T</td>
<td>(p) value</td>
<td>C</td>
</tr>
<tr>
<td>Japanese (269)</td>
<td>0.892</td>
<td>0.104</td>
<td>0.004</td>
<td></td>
<td>0.944</td>
</tr>
<tr>
<td>Caucasian (155)</td>
<td>0.806</td>
<td>0.187</td>
<td>0.006</td>
<td>0.029(^c)</td>
<td>0.900</td>
</tr>
<tr>
<td>African–American (80)</td>
<td>0.913</td>
<td>0.088</td>
<td>0.006</td>
<td>0.871</td>
<td>0.956</td>
</tr>
</tbody>
</table>

\(p\) value was obtained by Fisher’s exact test. a) Significantly different from the Japanese population.