SNP Communications

A Novel Single Nucleotide Polymorphism of the Human Methylenetetrahydrofolate Reductase Gene in Japanese Individuals

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Summary: The genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) have been associated with increased toxicity of methotrexate (MTX), a folic acid antagonist that is widely used to treat cancer and immunosuppressive disorders such as rheumatoid arthritis. In this study, we analyzed all the exons and exon/intron junctions of the MTHFR gene from 200 Japanese individuals. We detected a novel single nucleotide polymorphism (SNP) 148C>T (Arg46Trp) in exon 1. The allele frequency of this polymorphism in the Japanese population appears to be extremely low (0.25%).

Key words: methylenetetrahydrofolate reductase; MTHFR; genetic polymorphism; pharmacogenetics; Japanese

Introduction

Methylenetetrahydrofolate reductase (MTHFR) is involved in maintaining folate and homocysteine homeostasis, and deficiencies of MTHFR are implicated in neurological and vascular diseases. To date, many severe mutations and polymorphisms have been identified in MTHFR gene.1–12) Severe MTHFR deficiency results in marked hyperhomocysteinemia and homocystinuria. Milder deficiencies of MTHFR are more common in the general population. Two common genetic polymorphisms in MTHFR, 677C>T (Ala222Val) and 1298A>C (Glu429Ala), are non-synonymous amino acid changes that have been associated with a decreased activity of MTHFR and increased levels of homocysteine.3,6,13) The MTHFR 677C>T variant allele has also been associated with increased toxicity of methotrexate (MTX),14–17) a folic acid antagonist that is widely used to treat cancer and immunosuppressive disorders such as rheumatoid arthritis. Individuals with homozygous 677TT or the heterozygous 677CT genotype present increased risk of side effects following MTX therapy as compared with those having the wild type 677CC genotype.16,18–20) Studies on genetic variations in MTHFR would be useful to reduce the trial-and-error dosing and the risk of adverse drug reaction.

In the present study, we analyzed all the exons and exon/intron junctions of the MTHFR gene from 200 Japanese individuals by using denaturing HPLC (DHPLC). Additionally, we identified a novel non-synonymous SNP of the MTHFR gene located in exon 1.

Materials and Methods

Venous blood was obtained from 200 unrelated healthy Japanese volunteers and patients admitted to Tohoku University Hospital. Written informed consent was obtained from all the blood donors, and the study was approved by the Local Ethics Committee of Tohoku University Hospital and Tohoku Pharmaceutical University. DNA was isolated from K2EDTA-anticoagulated peripheral blood by using QIAamp DNA Mini Kits (Qiagen, Hilden, Germany) in accordance with the manufacturer’s instructions.

Table 1 lists the primer pairs that were used to amplify all exons and exon/intron boundaries of the MTHFR. These primers were designed based on the genomic sequence reported in GenBank (AY338232).
Table 1. Amplification and DHPLC conditions for MTHFR SNP analysis of genomic DNA

<table>
<thead>
<tr>
<th>Exon</th>
<th>Amplified length (bp)</th>
<th>Forward primer (5’ to 3’)</th>
<th>Reverse primer (5’ to 3’)</th>
<th>DHPLC Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>350</td>
<td>GTGGCTGCCTGCCCCCTGATGCTCC</td>
<td>AGTTTGTCCCTCCCCAGGACCACACT</td>
<td>61.5</td>
</tr>
<tr>
<td>2</td>
<td>353</td>
<td>CAGTGACGGATGGTATTTCTCCTGG</td>
<td>TACCAAGTGCCCTCCGGAAGGCCAG</td>
<td>62.7</td>
</tr>
<tr>
<td>3</td>
<td>230</td>
<td>AGAAAGGGTCTCGGAGGTGGTG</td>
<td>TCTGGGATCTCAGGCTCTTGGAGTGT</td>
<td>62.2</td>
</tr>
<tr>
<td>4</td>
<td>310</td>
<td>TCGCCTGACACGGTGGAGGCCACG</td>
<td>GTGCGAGAGCAGTGCGGTAGGTAG</td>
<td>62.3</td>
</tr>
<tr>
<td>5</td>
<td>360</td>
<td>AGGGTGGAGACGGGCTGGCCAGCA</td>
<td>CAGCCGGGCTGCTCTTGGACCCTC</td>
<td>63.4</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>GCTTCCGGCTCTCCCTAGCAATCC</td>
<td>CCCTGCCGCTCCAAGAACAGAAGAT</td>
<td>61.6</td>
</tr>
<tr>
<td>7</td>
<td>316</td>
<td>TGGCAGCTGCCCCTGTGACAGATTGT</td>
<td>ACAGCCCCGAGCTGGCTGCAGCT</td>
<td>61.8</td>
</tr>
<tr>
<td>8</td>
<td>290</td>
<td>ACTCAGGGTGCCAAACTGTAGGTC</td>
<td>GAACCCAGGGTCCGCTGCAAGAGA</td>
<td>64.7</td>
</tr>
<tr>
<td>9</td>
<td>278</td>
<td>GGCCTCCAGTGATCTCTGATACCTTAG</td>
<td>CTTGCACAATGCTCCAGCGCTAG</td>
<td>62.4</td>
</tr>
<tr>
<td>10</td>
<td>231</td>
<td>AGTGGGACTCCAGTTGTTCTTGGCC</td>
<td>TCCCTCCCCAGGTTTCCAGGTTG</td>
<td>62.7</td>
</tr>
<tr>
<td>11</td>
<td>330</td>
<td>TTGCTCTGCTGTGCTGTGCTCAGTG</td>
<td>TGTGAGGAGAAGGCGGCCAGAAGA</td>
<td>62.0</td>
</tr>
</tbody>
</table>

Amplicons were generated with the AmpliTaq Gold PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The thermal profile consisted of denaturation at 95°C for 10 min, followed by 35–40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. Heteroduplexes were then generated by means of a thermal cycler as follows: 95°C for 1 min; 95°C, reducing at 1.5°C per 1 min, for 47 cycles.

The PCR products were denatured using the DHPLC system, WAVE® (Transgenomic Inc., Omaha, NE, USA). Unpurified PCR samples (5 µL) were separated on a heated C18 reverse phase column (DNASep®) using 0.1 M triethylammonium acetate (TEAA) in water and 0.1 M TEAA in 25% acetonitrile at a flow rate of 0.9 mL/min. The software provided with the instrument selected the temperature for the heteroduplex separation in the heterozygous MTHFR fragment. Table 1 summarizes the DHPLC running conditions for each amplicon. The linear acetonitrile gradient was adjusted so that the retention time of the DNA peaks was 4–5 minutes.

Both the strands of samples with variants that were detected using DHPLC were analyzed with a CEQ8000® automated DNA sequencer. We sequenced the PCR products by the fluorescent deoxy termination sequencing using the DTCS DNA Sequencing Kit (Beckman-Coulter Inc.) in accordance with the manufacturer’s instructions.

For the haplotype analysis of MTHFR variant alleles, the PCR products including fragments from exon 1 to exon 4 of the MTHFR genes were subcloned into a pCR®XL-TOPO® vector (Invitrogen Co., CA, USA). The clones inserted into the MTHFR fragments were sequenced using a CEQ8000® automated DNA sequencer.

**Results and Discussion**

We found the following novel SNP:

- SNP: 050611Hiratsuka06; GENE NAME: MTHFR; ACCESSION NUMBER: AY338232; LENGTH: 25 bases; 5’-CATGAGAGACTCC GTGGGAGAAGATTAC-3’.

The DHPLC analysis of the MTHFR gene (11 exons) in the 200 DNA samples from Japanese individuals revealed chromatographic profiles that were distinct from the wild type in exon 1. We tested the specificity of DHPLC in detecting the variant allele in the exon by comparing the results with those of direct sequencing. The DHPLC chromatograms and the electrophoretograms of the novel SNPs are shown in Figs. 1 and 2, respectively. The SNP in exon 1 was 148C>T resulting in an amino acid change of Arg46Trp. Haplotype analysis indicated that other SNPs did not exist in the same allele of the MTHFR gene (data not shown). Among the 200 individuals, one was heterozygous for the 148C>T SNP, suggesting that the allele frequency was 0.0025 in the Japanese population. The sequences for each sample were obtained from at least two different PCR amplifications.

The novel SNP 148C>T is located in exon 1 of the MTHFR gene and results in amino acid substitution. The N-terminal domain of MTHFR contains the flavin binding site and residues necessary to bind a folate substrate and catalyze its reduction. Furthermore, Arg46 is conserved in the MTHFR gene in humans, *Escherichia coli*, and *Salmonella typhimurium*. Thus,
A Novel SNP in MTHFR Gene

SNP37 (389)

A Novel SNP in MTHFR Gene

the amino acid substitution is expected to alter the catalytic properties of the MTHFR gene. To date, many severe mutations and polymorphisms have been identified in the MTHFR gene.\textsuperscript{1-12} Although we could not determine whether the SNP (148C>T) found in this study caused a severe mutation leading to hyperhomocysteinemia or a polymorphism which in turn resulted in mild enzyme activity, further studies are being conducted in our laboratory to establish whether the newly identified SNP (148C>T, Arg46Trp) affect the MTHFR function.

In conclusion, we found a novel nonsynonymous SNP (148C>T) located in exon 1 of MTHFR gene in Japanese individuals. In this mutation, a substitution of Arg to Trp occurs at position 46 in the catalytic domain of MTHFR. The frequency of this mutation in the Japanese population appears to be extremely low (0.25%).

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References

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Fig. 1. DHPLC chromatograms of exon 1 of human MTHFR gene. The elution profiles of heterozygous sequence variants are compared with a reference wild-type DNA chromatogram.

Fig. 2. The nucleotide sequences of the MTHFR gene in exon 1. Although sequences are shown for sense strands, both strands were sequenced. Arrows indicate the variant nucleotide positions.


