Short communication

The effect of 677C → T and 1298A → C mutations on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase activity in healthy subjects

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We have studied the effect of common mutations (677C → T and 1298A → C) of the methylene-tetrahydrofolate reductase (MTHFR) gene in sixty-six healthy French subjects, aged 27–47 years. Serum folate, vitamin B12, and plasma total homocysteine were measured as well as the specific activity of MTHFR in lymphocytes. The frequency of subjects homozygous for the 677TT genotype was 18 %, and that of those homozygous for the 1298CC genotype was 12.5 %. The frequency of individuals heterozygous for both mutations was 23.5 %. The 1298A → C mutation was associated with decreased MTHFR specific activity in subjects with both 677CC and 677CT genotypes. This activity was 60 % for the 677CC/1298AC genotype and 52 % for the 677CC/1298CC genotype when compared with the MTHFR specific activity of the 677CC/1298AA genotype. Heterozygotes for both mutations (677CT/1298AC genotype) had 36 % of the reference specific activity. Although homocysteine levels in 677TT and 1298CC genotype subjects were higher than for other genotypes, no significant differences were observed among different genotypes. This may be due to high serum folate level in our samples, and suggests that folate therapy may be useful to prevent hyperhomocysteinaemia in homozygous mutant subjects.

Folate: Homocysteine: Methylenetetrahydrofolate reductase: Polymorphism

Abbreviations: MTHFR, 5,10-methylenetetrahydrofolate reductase; tHcy, total homocysteine.
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Subjects, materials and methods

The protocol was approved by the ethics committee of CHU Nancy, France and subjects gave their informed consent. Fasting blood was collected from sixty-six subjects (thirty males, thirty-six females) aged 27–47 years, and placed on ice immediately. All samples were processed within 2 h by centrifugation for 20 min at 2000 g and portions were stored at −70 °C until analysis. Lymphocytes were isolated from heparinized blood samples at room temperature by centrifugation through a Ficoll gradient with Ficoll-Hypack solution (Pharmacia, Uppsala, Sweden). Plasma tHcy and the total amount of protein- and non-protein-bound homocysteine were determined by HPLC according to Araki & Sako (1989). Serum folate and vitamin B12 were determined using a kit from Ciba-Corning (Medfeld, MA, USA). Lymphocytes for MTHFR measurements were available for forty-two subjects (nineteen females and twenty-three males) and digestion with the specific restriction enzyme. The genotype activities expressed as nmol formaldehyde/mg protein per h. Protein concentrations were determined using the Bradford dye-binding procedure with a kit from Bio-Rad Laboratories, Hercules, CA, USA). Genomic DNA was isolated from peripheral blood leukocytes using a Qiagen kit (Chatsworth, CA, USA) according to the manufacturer’s instructions. As the 677C→T mutation creates a HinfI restriction site (Frosst et al., 1995), and 1298A→C abolishes a Mbo II restriction site (van der Put et al., 1998), mutations were investigated by polymerase chain reaction of genomic DNA and digestion with the specific restriction enzyme. The polymerase chain reaction for the 1298A→C was carried out according to van der Put et al. (1998) in a total volume of 50 µl containing 1 µM of the forward primer 5 ’-CTTG GGA GCT GAA GCA CTA CTA C and 1 µM of the reverse primer 5 ’-CAC TTT GTG ACC ATT CCC GTT TG, 200 µM each dNTP, 1.5 Unit Taq polymerase (Applied Biosystems, Illkirsh, France) and 1× of the Taq buffer. The polymerase chain reaction program on GeneAmp (Perkin Elmer, Foster, CA, USA) consisted of an initial denaturation step of 2 min at 94 °C, followed by thirty-five cycles of 94° (60 s), 51° (30 s), and a final extension of 72° for 7 min. The polymerase chain reaction for the 677C→T was carried out using the forward primer 5’-TGA AGA AGG TGT CGG CG and the reverse primer 5’-AGG ACG GTG CGG TGA GAG TG.

Statistical analyses were performed using StatView-5 (SAS Inst. Inc, Cary, NC, USA) on a Macintosh computer. Results are expressed as the mean value and standard deviation. Comparisons were analysed by ANOVA followed by post-ANOVA Bonferroni/Dunn test. The distributions of serum vitamin B12 and folate were skewed and logarithmically transformed.

Results

The mean fasting plasma tHcy for all samples was 9.3 (SD 3.3) µmol/l. The mean serum folate and vitamin B12 concentrations were 15.6 (SD 8.7) (normal range 6–30) nmol/l and 359.9 (SD 158.9) (normal range 156–674) pmol/l respectively. The mean MTHFR activity was 16.7 (SD 8.2) (range 4.8–41.0) nmol formalddehyde/mg protein per h. Subjects were divided into three groups based on either their 677C→T or their 1298A→C genotype without taking into account their status at the second polymorphic site (Table 1). In this present sample, 18% of the subjects were homozygous for the 677T allele and 12.5% were homozygous for the 1298C allele. Even when the status at the 1298 position was not taken into account, MTHFR specific activity in homozygous 677TT genotype individuals was 32% and MTHFR specific activity in 677CT heterozygotes was 64% of that seen in 677CC subjects. MTHFR specific activity in 1298CC subjects was not significantly different from that seen in 1298AA subjects or in 1298AC subjects when the allele status at position 677 was not taken into account.

When the sample was analysed to look at the effect of the two mutations in combination, 23.5% of the subjects were 677CT/1298AC (Table 2). The 677CC/1298AC MTHFR activity was 60% and the 677CC/1298CC MTHFR activity was 52% of the 677CC/1298AA MTHFR activity used as a reference. Heterozygotes for both mutations (677CT/1298AC genotype) had 36% of the reference activity. Results in Table 2 show that as has been proposed previously, the 1298A→C mutation is associated with decreased MTHFR activity (van der Put et al., 1998; Weisberg et al., 1998). The reduced MTHFR activity due to the 1298A→C mutation is less than that due to the 677C→T mutation and can be

Table 1. Plasma homocysteine, serum folate and B12 and methylenetetrahydrofolate reductase (MTHFR) specific activity according to genotype (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>677CC</th>
<th>677CT</th>
<th>677TT</th>
<th>1298AA</th>
<th>1298AC</th>
<th>1298CC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/l)*</td>
<td>29.0</td>
<td>44.5%</td>
<td>25.0</td>
<td>37.5%</td>
<td>12.0</td>
<td>18.0%</td>
</tr>
<tr>
<td>Serum folate (nmol/l)*</td>
<td>9.0</td>
<td>2.7</td>
<td>9.3</td>
<td>3.3</td>
<td>10.7</td>
<td>5.3</td>
</tr>
<tr>
<td>Serum B12 (pmol/l)*</td>
<td>349.8</td>
<td>113.2</td>
<td>370.8</td>
<td>154.2</td>
<td>299.4</td>
<td>88.2</td>
</tr>
<tr>
<td>MTHFR specific activity</td>
<td>22.5*</td>
<td>8.2</td>
<td>14.5*</td>
<td>4.8*</td>
<td>7.3</td>
<td>1.4*</td>
</tr>
</tbody>
</table>

* Plasma homocysteine, serum folate and B12 were not significantly different according to genotype (post-ANOVA Bonferroni/Dunn test).

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Table 2. Effect of the 1298A→T genotype on plasma homocysteine, serum folate and \(B_12\) and methylenetetrahydrofolate reductase (MTHFR) specific activity according to 677CC→T genotype (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>677CC</th>
<th>677TT</th>
<th>1198AA</th>
<th>1198AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>677TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.4</td>
<td>11.0</td>
<td>12.34</td>
<td>13.48</td>
</tr>
<tr>
<td>SD</td>
<td>1.6</td>
<td>2.7</td>
<td>2.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Discussion

It is now established that the 677C→T mutation results in a thermolaible MTHFR variant with reduced activity, but the exact physiological consequences of MTHFR thermolability are not yet known. As the alanine residue is involved in the folate-dependent stabilization of MTHFR (Frosst et al. 1995), replacement of the alanine by valine may alter the interaction between MTHFR and folate, leading to perturbation in conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulating form of folate.

Subjects who are homozygous for 677C→T are reported to have high plasma tHcy levels in the presence of folate insufficiency (Jacques et al. 1996). In this study, plasma tHcy levels were not significantly increased by either the 677C→T nor by the 1298A→C mutation. However, there was a trend to higher tHcy levels in the homozgyote 677TT or 1298CC individuals. Only two of the sixty-six subjects in our study had clearly elevated plasma tHcy levels. These two subjects were men with the 677TT genotype, without the 1298A→C mutation. The first had a serum folate concentration of 5.5 nmol/l, a serum vitamin \(B_12\) of 307 pmol/l, and a plasma homocysteine of 18.1 \(\mu\)mol/l. The second had a serum folate of 9 nmol/l, a serum vitamin \(B_12\) of 194 pmol/l, and a plasma homocysteine of 19.1 \(\mu\)mol/l. The lack of a significant difference in tHcy levels may be explained by the high serum folate level in the homozygous 677TT individuals, and suggests that folate therapy may be useful to prevent hyperhomocysteinaemia in homozygous mutant subjects. For the novel variant, as suggested by Weisberg et al. (1998), the 1298A→C polymorphism could affect enzyme regulation, possibly by \(S\)-adenosylmethionine, an allosteric inhibitor of MTHFR, which is known to bind in the C-terminal region.

The allele frequency of the 677TT allele in this present group was 37% and the frequency of 677TT homozygotes was 18%. In previous studies from France, homozygote frequencies of 10–18.5% have been quoted for control subjects (Chadefaux-Vekemans et al. 1996; Faure-Delanef et al. 1997; Mornet et al. 1997). The allele frequency of the 1298C allele was 33% and the frequency of 1298CC homozygotes was 12.5%. This study in French subjects is a confirmation that the second mutation 1298A→C is a genetic polymorphism. It is interesting that to date, there has been only one reported instance where an individual has been 677TT/1298AC (Weisberg et al. 1998). The present study confirms that the two mutant phenotypes rarely occur together in cis (van der Put et al. 1998; Weisberg et al. 1998).
Acknowledgements
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References

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