The Plasma Homocysteine/Creatinine Ratio Can Be Used to Study the Implication of (C677T) MTHFR Genetic Variants in Homocysteine Homeostasis

To the Editor:
As discussed in a recent review (1), it is accepted that the determinants of total plasma homocysteine are numerous. The usual factors involved are age, sex, nutritional status, renal function, and genetic status. In this study, we sought to discriminate the influence of the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene on plasma total homocysteine. We studied a healthy French population (n = 78) with serum folate (9.4 ± 3.2 μg/L, mean ± SD) and plasma creatinine (79.7 ± 12.8 μmol/L) values within the references intervals and of known age, sex, and MTHFR genetic status (41 women, ages 38.2 ± 9.9 years, TT variant = 6; 37 men, ages 38.4 ± 9.8 years, TT variant = 5). Before entering the study, participants gave written informed consent; they also reported no intake of vitamin B<sub>b</sub>, B<sub>12</sub>, or folate supplements.

We measured the total plasma homocysteine concentration with an HPLC assay that used fluorometric detection [ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBDF)] and 3-mercapto-1,2-propanediol as an internal standard, using a method derived from Vester and Rasmussen (2). Using this method, we measured the sum of homocysteine, homocysteine, and homocysteine-cysteine mixed disulfides, free and protein-bound. Serum folate was measured using a commercially available kit (Magic-Lite folate; Ciba-Corning). Serum creatinine was assayed with an alkaline picrate method on a Hitachi 747 (crea-sys3; Boehringer-Mannheim). MTHFR genotypes were determined by PCR amplification followed by digestion of the amplification products by the restriction enzyme Hinfl, as described by Frost et al. (3). Stat-View software for the Macintosh was used for the statistical calculations (ANOVA, Fisher test, and linear regression). All statistical tests were two-tailed at the 5% level. The results are presented in Table 1 and expressed as the mean ± SD.

We confirmed that total plasma homocysteine and creatinine concentrations are higher in men than in women (21.7%, P < 0.0001; and 21.3%, P < 0.0001, respectively). However, this sex difference disappeared when the homocysteine/creatinine ratio was evaluated. The homocysteine/creatinine ratio was also independent of age (P = 0.215) and serum folate concentration (P = 0.095).

Our results indicated that total plasma homocysteine (22%, P = 0.006) and the homocysteine/creatinine ratio (22.6%, P = 0.001) were higher in healthy individuals homozygous for the TT variant than in heterozygous CT individuals and CC wild-type individuals. The plasma homocysteine differences observed were independent of plasma creatinine concentration. Moreover, the folate status in the homozgyous TT group was not different from the other groups (CT and CC).

These results in a healthy population homogeneous for the usual homocysteine determinants (age, sex, and folate status) suggest that the C677T polymorphism of the MTHFR gene is an independent variable in total plasma homocysteine determination normalized to the creatinine concentration.

Clinical studies usually include unbalanced series of patients who differ in gender and/or age. Thus, we propose the use of the homocysteine/creatinine ratio to isolate from these two variables the involvement of the C677T variant in hyperhomocysteinemia observed among patient groups.

The homocysteine/creatinine ratio could be a tool to study homocysteine homeostasis in some pathological conditions such as neural tube defects and vascular diseases.

References

Maryvonne Cuer *
Sandrine Barrot
Françoise Jaureguy
Dieudonné Manéné
Geneviève Durand
Gisèle Le Moël

Biochemical A Laboratory
46 rue Huchard
Bichat-Claude Bernard Hospital
75877 Paris cédex 18, France

*Author for correspondence. Fax 33-1-40258821; e-mail labo.bioa@bch.ap-hop-paris.fr.

Table 1. Plasma homocysteine determinants in a healthy French population.*

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Men</th>
<th>Women</th>
<th>Variant TT genotype</th>
<th>CT + CC genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>78</td>
<td>37</td>
<td>41</td>
<td>11</td>
<td>67</td>
</tr>
<tr>
<td>Age, years</td>
<td>38.3 ± 9.8</td>
<td>38.4 ± 9.8</td>
<td>38.2 ± 9.9</td>
<td>42.2 ± 12.1</td>
<td>37.6 ± 9.3</td>
</tr>
<tr>
<td>Serum folate, μg/L</td>
<td>9.4 ± 3.2</td>
<td>8.3 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.3 ± 3.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3 ± 2.6</td>
<td>9.6 ± 3.2</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>79.7 ± 12.8</td>
<td>89.7 ± 10.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.6 ± 6.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>79.1 ± 13.3</td>
<td>79.8 ± 12.8</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>7.4 ± 2.2</td>
<td>8.3 ± 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.5 ± 2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1 ± 2.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.1 ± 2.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homocysteine/creatinine ratio, μmol/μmol</td>
<td>0.093 ± 0.026</td>
<td>0.094 ± 0.022</td>
<td>0.092 ± 0.029</td>
<td>0.115 ± 0.038&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.089 ± 0.021&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup>All results are presented as mean ± SD.
<sup>b</sup>Men vs women: <sup>b</sup>P < 0.005; <sup>c</sup>P < 0.0001.
<sup>d</sup>TT variants vs CT + CC: <sup>d</sup>P = 0.006; <sup>e</sup>P = 0.001.