Opposite Effects of Plasma Homocysteine and the Methylenetetrahydrofolate Reductase C677T Mutation on Carotid Artery Geometry in Asymptomatic Adults

Karine Demuth, Nicole Moatti, Olivier Hanon, Marie Odile Benoit, Michel Safar, Xavier Girerd

Abstract—Studies of symptomatic patients have identified hyperhomocysteinemia as an independent risk factor for vascular disease. In case-control studies, a point mutation (C677T) in the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR) has also been linked to an increased risk of vascular disease through its effect on homocysteinemia. Our aim was to extend these observations to asymptomatic subjects by studying the influence of both homocysteinemia and its mutation on carotid artery geometry. We examined 144 subjects free of atherosclerotic lesions. Fasting homocysteinemia was measured by high-performance liquid chromatography with fluorometric detection. MTHFR genotype was analyzed by polymerase chain reaction followed by Hinfl digestion. Carotid artery geometry was characterized by internal diameter and intima-media thickness, as assessed by a high-resolution echo-tracking system. Subjects in the upper homocysteine tertile had a greater carotid internal diameter than did subjects in the middle and lower tertiles (6516±770 vs 6206±641 and 5985±558 μm, respectively; P<0.001). Subjects homozygous for the mutation had a smaller carotid artery internal diameter than did subjects heterozygous or homozygous for the wild-type allele (5846±785 vs 6345±673 and 6199±671 μm, respectively; P<0.05). Homocysteinemia was not significantly increased in subjects homozygous for the mutation. In multivariate regression analysis, homocysteinemia was independently and positively associated with lumen diameter (P=0.0008) and wall thickness (P=0.020). Conversely, homozygosity for the mutation was negatively associated with internal diameter (P=0.009). These preliminary data suggest that mildly elevated homocysteinemia and homozygosity for the MTHFR C677T mutation are associated with opposite preclinical modifications of carotid artery geometry. If confirmed, these results may have important implications for new treatment strategies for vascular disease before the onset of clinical manifestations. (Arterioscler Thromb Vasc Biol. 1998;18:1838-1843.)

Key Words: homocysteine ■ methylenetetrahydrofolate gene ■ artery ■ carotid ■ remodeling

Several teams have reported hyperhomocysteinemia in a large proportion of patients with cerebrovascular or peripheral arterial diseases,1-5 and hyperhomocysteinemia is now recognized as an important determining factor in vascular disease.6 Except for the study by Malinow et al,7 which focused on the relation between homocysteinemia and carotid artery intimal-medial wall thickening in asymptomatic adults, almost all published data concerning the influence of homocysteinemia on cardiovascular disease have been obtained in subjects with clinical atherosclerosis. However, the possibility of transient changes in homocysteinemia due to the arterial occlusive disease itself or associated medications was seldom considered in these reports. In a recent nested case-control study, Evans et al8 suggested that the higher homocysteine concentrations among case patients than control subjects could be an effect rather than a cause of the disease itself (ie, myocardial infarction or stroke) or an underlying vascular disease (eg, atherosclerosis). For these reasons, studies on asymptomatic subjects are required to establish meaningful relations between plasma homocysteine levels and other parameters. Hyperhomocysteinemia can result from genetic or nutrient-related disturbances of homocysteine metabolism. 5,10-Methylenetetrahydrofolate reductase (MTHFR) is a folic acid-related enzyme involved in the remethylation of homocysteine to methionine. A common mutation in the MTHFR gene, involving a C-to-T substitution at nucleotide 677 (C677T), results in the conversion of alanine (Ala) to valine (Val) at position 226 of the protein.9 The C677T mutation reduces the specific activity of MTHFR, increases its thermolability, and has been reported to induce hyperhomocysteinemia.9 The C677T mutation was thus proposed as a candidate genetic risk factor for vascular disease through its influence on plasma homocysteine levels.10-12 However, the association between homozygosity for the mutation and increased plasma homocysteine levels is inconsistent,13 and some investigations have failed to show an...
increased risk of vascular disease among persons homozygous for the mutation. 14–16

In this report, we extended such observations to asymptomatic adults free of atherosclerotic lesions and examined the influence of both the MTHFR genotype and homocysteine on preclinical modifications of carotid artery geometry as predictors of subsequent cardiovascular disease. Our aim was to assess mild hyperhomocysteinemia and the MTHFR C677T mutation as markers of preclinical arterial disease.

Methods

Population

Between January and December 1996, a cohort of 1450 ambulatory subjects referred for a cardiovascular risk factor screening program at Broussais Hospital underwent ultrasound examination of the carotid arteries. Subjects were classified in 3 strata on the basis of ultrasound morphology: stratum I, subjects with a carotid stenosis; stratum II, subjects with at least 1 plaque in the right or left common carotid artery (CCA) (defined as an intima-media thickness >1.0 mm); and stratum III, subjects with a normal carotid wall (defined as the absence of plaque). We selected 144 subjects, aged 19 to 86 years, on the basis of 3 criteria: (1) Subjects in stratum III of the classification. Their inclusion ensured that we studied only subjects with no evidence of preclinical arterial lesions and provided optimal characterization of arterial geometry. (2) Subjects with no history or clinical evidence of cardiovascular disease, to avoid transient changes in homocysteinemia due to symptomatic arterial disease or associated medications. (3) Subjects never having received antihypertensive drugs. The selected population was thus asymptomatic and had a low cardiovascular risk profile. 17 Eighteen percent of the subjects had essential hypertension, 18% had a total cholesterol value >6.4 mmol/L; and 22% were current or past smokers. The study protocol was approved by the human research committee of our institution, and informed consent was obtained from each subject.

Blood Sampling

Venous blood was obtained after an overnight fast. Plasma or serum was immediately separated at 4°C in a refrigerated centrifuge and stored at either 4°C (for determination of serum lipids, glucose, and creatinine) or −80°C (for total plasma homocysteine, serum vitamin B12, and folate). Cells were stored separately at −80°C.

Biochemical Measurements

Total homocysteine was determined in plasma by the fluorometric high-performance liquid chromatography method described by Fortin and Genest. 18 Serum vitamin B12, and folate were determined with commercial radioimmunoassay kits. 19, 20 Serum lipids, glucose, and creatinine were routinely determined as previously described. 21

Detection of the C-to-T Substitution at the MTHFR Locus

DNA was extracted from frozen blood cells by a salting-out method adapted from that described by Miller et al 22 for frozen whole blood. The DNA samples were subjected to amplification by the polymerase chain reaction and with the primers for amplification of the mutation region of the MTHFR gene described elsewhere. 9 The primers generate a 198-bp fragment. Because the C-to-T substitution at bp 677 creates an Hinfl recognition sequence, the restriction enzyme Hinfl was used to identify those subjects bearing the mutation. If the mutation is present, Hinfl digests the 198-bp fragment into a 175-bp and a 23-bp fragment. The fragments were analyzed by polyacrylamide gel electrophoresis. The mutant allele was designated “Val” and the wild-type allele “Ala.”

Carotid Artery Measurements

Ultrasound examination of the cervical arteries was performed with the patient in the recumbent position by using a Sigma 44 KONTRON with a transducer frequency of 7.5 MHz. The CCA, carotid bifurcation, and the origin (first 2 cm) of the internal carotid artery were scanned on both sides. Vessel wall properties of the right CCA were assessed with a pulsed ultrasound echo-tracking system (Wall-Track system, Neurodata) developed to measure the wall motion of superficial large arteries after echographic location. A detailed description of this system and its reproducibility have been published previously. 23 The following parameters were determined at the right CCA, 2 cm beneath the carotid bifurcation: the internal diameter (Di), the intima-media thickness of the posterior wall (h), and the end-diastolic wall cross-sectional area (WCSA). Circumferential stress was calculated from the previous parameters as mean blood pressure (BP)×(Di/2×h).

Statistical Analysis

For the distribution of MTHFR genotypes, Hardy-Weinberg equilibrium was assessed by \( \chi^2 \) analysis as described by Emery. 24 Univariate comparisons of plasma homocysteine tertiles and of MTHFR genotype groups were performed with ANOVA for quantitative variables and a \( \chi^2 \) test for qualitative variables. We also used univariate and multivariate linear regression analyses to identify links between arterial parameters and plasma homocysteine levels as well as MTHFR genotype. Multivariate linear regression analysis studies the relationship between 1 dependent variable (internal diameter or wall thickness) and \( p \) independent variables (age, sex, diastolic BP, etc.) that are called predictors. Multivariate linear regression estimates the \( \beta \)’s in the equation \( y = b_0 + b_1x_1 + b_2x_2 + \ldots + b_px_p + e \), where the \( x \)’s are independent variables, \( y \) is the dependent variable, and the \( b \)’s are the unknown regression coefficients. The \( \beta \) coefficients (partial regression coefficients) represent the net effect each independent variable has on the dependent variable while the remaining independent variables in the equation are held constant. The multivariate \( r^2 \) (coefficient of determination) represents the percentage of the variation in the dependent variable explained by the independent variables in the model. The \( P \) values (probability levels) give the significance of the regression coefficient. Calculations were done on a Macintosh PowerBook 1400 cs computer (Apple Computer) with statistical software (Stat-View II, Abacus Concepts Inc). The results are expressed as mean±SD. A value of \( P<0.05 \) was considered significant.

Results

Relation Between Plasma Homocysteine Concentrations and Clinical and Biological Parameters

The mean plasma homocysteine level in the study population was 9.7±2.8 \( \mu \text{mol/L} \), and there was a significant difference between the sexes (10.7±2.8 \( \mu \text{mol/L} \) in men versus 8.9±2.6 \( \mu \text{mol/L} \) in women; \( P<0.001 \)). Only 4% of the subjects had moderate hyperhomocysteinemia (between 16 and 30 \( \mu \text{mol/L} \)), as defined by Kang and Wong. 25 To describe the relation between plasma homocysteine and clinical or biological parameters, the population was classified into 3 tertiles according to homocysteine concentration: tertile I, 4.4 to 8.1 \( \mu \text{mol/L} \); II, 8.2 to 10.2 \( \mu \text{mol/L} \); and III, 10.3 to 20 \( \mu \text{mol/L} \). Characteristics of the study subjects according to tertiles of plasma homocysteine are presented in Table 1. The proportion of males, height, and weight were significantly higher in subjects in the upper tertile. There was a higher proportion of smokers in the upper tertile of homocysteine, and the effect of smoking on homocysteine reached statistical significance when the population was considered as a whole (10.8±8.3 \( \mu \text{mol/L} \) for smokers; 9.5±2.6 \( \mu \text{mol/L} \) for...
TABLE 1. Clinical and Biological Parameters According to Tertiles of Total Plasma Homocysteine Concentration

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<th>Tertile</th>
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<tr>
<td></td>
<td>I (n=48)</td>
<td>II (n=48)</td>
<td>III (n=48)</td>
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<tr>
<td>Clinical parameters</td>
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<tr>
<td>Age, y</td>
<td>46±12</td>
<td>49±12</td>
<td>49±14</td>
<td></td>
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<tr>
<td>Sex ratio, M/F</td>
<td>11/37</td>
<td>21/27*</td>
<td>34/14†</td>
<td></td>
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<tr>
<td>Height, cm</td>
<td>164±10</td>
<td>164±10</td>
<td>172±8‡$</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68±12</td>
<td>70±14</td>
<td>77±17§</td>
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<tr>
<td>Systolic BP, mm Hg</td>
<td>139±16</td>
<td>142±14</td>
<td>145±21</td>
<td></td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>81±12</td>
<td>82±9</td>
<td>84±12</td>
<td></td>
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<tr>
<td>Heart rate, bpm</td>
<td>73±13</td>
<td>70±11</td>
<td>71±11</td>
<td></td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>11</td>
<td>23</td>
<td>31</td>
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<tr>
<td>Biological parameters</td>
<td></td>
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<tr>
<td>Homocysteine, μmol/L</td>
<td>7.1±0.8</td>
<td>9.2±0.7†</td>
<td>12.9±2.4§</td>
<td></td>
</tr>
<tr>
<td>Folate, ng/mL</td>
<td>8.3±2.9</td>
<td>6.3±1.9</td>
<td>5.6±1.5‡</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₁₂, pg/mL</td>
<td>524±208</td>
<td>493±133</td>
<td>366±165</td>
<td></td>
</tr>
<tr>
<td>Fasting glycemia, mmol/L</td>
<td>6.1±2.0</td>
<td>6.0±1.5</td>
<td>6.1±0.8</td>
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</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>76±16</td>
<td>77±13</td>
<td>87±14§$</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.3±1.0</td>
<td>5.8±1.0</td>
<td>5.5±0.9</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.0±1.3</td>
<td>0.9±0.4</td>
<td>0.9±0.4</td>
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</tr>
<tr>
<td>MTHFR C677T genotype</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ala/Ala, Ala/Val, Val/Val</td>
<td>18/25/5</td>
<td>26/19/3</td>
<td>19/24/5</td>
<td></td>
</tr>
</tbody>
</table>

The results are expressed as mean±SD.

*P<0.05 for medium tertile vs low tertile.
†P<0.001 for high tertile vs low tertile.
‡P<0.05 for high tertile vs medium tertile.
§P<0.001 for high tertile vs medium tertile.

The allele frequency of the mutant variant of the MTHFR gene was 0.33, and the genotype frequencies were consistent with Hardy-Weinberg equilibrium ($\chi^2=0.8, df=1, P=0.5$). None of the clinical or biological parameters listed in Table 1 were significantly different between subjects with the 3 different genotypes. In particular, plasma homocysteine levels were not statistically higher in subjects with the Val/Val genotype than in subjects with the Ala/Ala and Ala/Val genotypes (10.2±3.8, 9.8±2.8, and 9.5±2.7 μmol/L, respectively), and serum folate levels were not significantly lower in subjects with the Val/Val genotype than in subjects with the Ala/Ala and Ala/Val genotypes (4.6±1.6, 7.3±2.5, and 6.7±2.4 ng/mL, respectively).

TABLE 2. Cartoid Artery Parameters According to Tertiles of Total Plasma Homocysteine Concentration

<table>
<thead>
<tr>
<th></th>
<th>Tertile</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>I (n=48)</td>
<td>II (n=48)</td>
<td>III (n=48)</td>
<td></td>
</tr>
<tr>
<td>Internal diameter, μm (Di)</td>
<td>5985±558</td>
<td>6206±641</td>
<td>6516±770†</td>
<td></td>
</tr>
<tr>
<td>Wall thickness, μm (h)</td>
<td>510±88</td>
<td>531±88</td>
<td>544±125</td>
<td></td>
</tr>
<tr>
<td>WCSA, mm²</td>
<td>10.7±2.9</td>
<td>11.1±2.3</td>
<td>12.0±4.4</td>
<td></td>
</tr>
<tr>
<td>Circumferential stress, kPa</td>
<td>83±14</td>
<td>83±16</td>
<td>84±18</td>
<td></td>
</tr>
</tbody>
</table>

The results are expressed as mean±SD.

*P<0.001 for high tertile vs low tertile.
†P<0.05 for high tertile vs medium tertile.

There was a trend toward intima-media thickening and WCSA extension with increasing homocysteine concentrations, but ANOVA did not show statistical significance. However, univariate regression analysis indicated a positive and significant relationship between the homocysteine concentration and wall thickness ($r^2=0.078; P=0.0008$) as well as between the homocysteine concentration and WCSA ($r^2=0.095; P=0.0002$). Circumferential stress was not statistically different between the 3 tertiles.

Distribution of MTHFR C677T Genotypes and Relation With Clinical and Biological Parameters

Among the 144 asymptomatic white subjects studied, the distribution of the 3 MTHFR genotypes was as follows: Ala/Ala, 43.8% (n=63); Ala/Val, 47.2% (n=68); and Val/Val, 9.0% (n=13). The allele frequency of the mutant variant of the MTHFR gene was 0.33, and the genotype frequencies were consistent with Hardy-Weinberg equilibrium ($\chi^2=0.8, df=1, P=0.5$). None of the clinical or biological parameters listed in Table 1 were significantly different between subjects with the 3 different genotypes. In particular, plasma homocysteine levels were not statistically higher in subjects with the Val/Val genotype than in subjects with the Ala/Ala and Ala/Val genotypes (10.2±3.8, 9.8±2.8, and 9.5±2.7 μmol/L, respectively), and serum folate levels were not significantly lower in subjects with the Val/Val genotype than in subjects with the Ala/Ala and Ala/Val genotypes (4.6±1.6, 7.3±2.5, and 6.7±2.4 ng/mL, respectively).

MTHFR C677T Genotype and Carotid Artery Parameters

Arterial parameters according to MTHFR C677T genotype were the following. Subjects with the Val/Val genotype had a significantly lower carotid artery internal diameter than did subjects with the Ala/Ala and Ala/Val genotypes (5846±785 μm for subjects with the Val/Val genotype versus 6199±671 and 6345±673 μm for subjects with the Ala/Ala and Ala/Val genotypes, respectively; P<0.05, Student’s t test after significant ANOVA). However, carotid artery wall thickness, WCSA, and circumferential stress were similar in the 3 MTHFR genotypes.

Multivariate Analysis of the Relation Between Carotid Artery Parameters and Clinical or Biological Parameters

All variables considered as candidates for a link with carotid artery parameters, plus homocysteinemia and the MTHFR C677T genotype, were studied in a multivariate regression analysis. Table 3 indicates the results of these analyses for internal diameter and wall thickness. With carotid artery lumen diameter as the dependent variable, age, male sex, and total cholesterol were indepen-
that hyperhomocysteinemia may predispose subjects to vascular defects through altered elastin metabolism, Jackson demonstrated that homocysteine blocks aldehyde groups in elastin, thus inhibiting the cross-linking required to form stable elastin. Although we have no histological evidence that mildly elevated homocysteine levels are toxic to the wall matrix of asymptomatic subjects, elastic lamina fragmentation might explain the carotid artery luminal enlargement observed here in normal subjects with the highest plasma homocysteine concentrations. The results obtained here with the CCA were not observed by Tawakol et al with the brachial artery. The internal diameter of the brachial artery, assessed by high-resolution ultrasonography, was not increased in a group of hyperhomocysteinemic elderly subjects compared with age- and sex-matched controls. This apparent discrepancy between our results and those reported by Tawakol et al could be explained by the histological composition of the 2 arteries. Indeed, the CCA is an elastic artery with a media containing numerous elastic laminas, whereas the brachial artery is muscular, with a large amount of smooth muscle cells composing the media. Given the toxicity of hyperhomocysteinemia to the elastic lamina, it is conceivable that a positive correlation between homocysteine and arterial diameter would be observed for the carotid artery and not for the brachial artery. The carotid artery intimal-medial wall thickening in subjects with higher plasma homocysteine levels has already been observed in asymptomatic subjects, but the reasons were unclear. On the basis of the relations obtained between plasma homocysteine concentration and carotid artery parameters in the present study, we suggest that an increase in plasma homocysteine concentration initially induces luminal enlargement through its toxicity to the arterial wall matrix and that wall thickening may represent an adaptive compensatory mechanism in response to diameter enlargement, thereby maintaining a constant circumferential wall stress of the artery. However, although our results provide new evidence that mild hyperhomocysteinemia is a risk factor for cardiovascular disease, as carotid wall thickening is a predictor of subsequent cardiovascular disease, longitudinal studies will be necessary to confirm this sequence of events.

The second original finding in this study is that homozygosity for the C677T mutation (Val/Val genotype) in the MTHFR gene is another independent determinant of carotid artery diameter. Moreover, this mutation is a determinant of carotid internal diameter per se, ie, not through its influence on plasma homocysteine. Indeed, the Val/Val genotype was independently associated with smaller lumen diameter in a multivariate regression analysis that included candidate variables linked to lumen diameter (including homocysteine), and plasma homocysteine and the Val/Val genotype had opposite effects on arterial diameter. Last, no significant difference was found in homocysteine levels between the 3 MTHFR genotypes. It has often been reported that the Val/Val MTHFR genotype is associated with increased plasma homocysteine levels as a result of the reduced activity and increased thermolability of this enzyme. However, some authors failed to find this direct relation between the MTHFR genotype and homocysteinemian. In the present

### Table 3: Multivariate Relation Between Carotid Artery Parameters and Clinical or Biological Parameters

<table>
<thead>
<tr>
<th>Carotid artery parameter</th>
<th>Multivariate r²</th>
<th>Coefficient</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery diameter</td>
<td>0.365</td>
<td>0.0008</td>
<td></td>
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<tr>
<td>Homocysteine</td>
<td>0.88</td>
<td>0.0008</td>
<td></td>
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<tr>
<td>Age</td>
<td>14.9</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>-387.0</td>
<td>0.004</td>
<td></td>
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<tr>
<td>Diastolic BP</td>
<td>10.4</td>
<td>0.032</td>
<td></td>
<td></td>
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<tr>
<td>Total cholesterol</td>
<td>52.2</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>132.9</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>-1.5</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTHFR C677T genotype</td>
<td>-472.7</td>
<td>0.009</td>
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</tbody>
</table>

MTHFR C677T genotype was entered as a categorical variable with Val/Val coded as +0.5 and Ala/Ala and Ala/Val both coded as -0.5. Multivariate r² is the coefficient of determination; β coefficients, partial regression coefficients.

Discussion

In a population of selected asymptomatic subjects free of atherosclerotic lesions in the CCA, we observed geometric modifications of the carotid artery related both to plasma homocysteine levels and to the C677T mutation in the MTHFR gene. A higher homocysteine level, even in the range of normal values (<16 μmol/L), was associated with lumen enlargement, wall thickening, and an increase in WCSA. Conversely, homozygosity for the MTHFR C677T mutation (Val/Val genotype) was associated with a smaller internal diameter of the CCA.

Enlargement of the CCA has been associated with age, BP, body weight, height, and sex. However, in a multivariate analysis, Bonithon-Kopp et al demonstrated that these parameters explained only 32% of the variance of carotid artery diameter in asymptomatic subjects. We found that homocysteine is a new independent determinant of carotid artery internal diameter in humans, because it was significantly associated in multivariate analyses that included known determinants of carotid artery diameter. In an experimental study of minipigs, it was found that hyperhomocysteinemia induced arterial geometry modifications, such as diameter enlargement of the abdominal aorta, and that these geometric modifications were associated with fragmentation of the arterial wall elastic lamina. Supposing the hypothesis...
Homocysteine, MTHFR, and Carotid Artery Geometry

study, we found no association between C677T genotypes and plasma homocysteine concentrations. The discrepancy between these data may be due to ethnic differences, inclusion of 1 or both sexes, and age differences. In addition, Jacques et al reported that homozgyosity for the C677T mutation predisposes subjects to high homocysteine values only in cases of poor plasma folate status, suggesting the involvement of a genetic-environmental interaction in the onset of hyperhomocysteinemia. In our population, serum folate levels were normal (3.1 to 12.4 ng/mL) in all the subjects studied, and there was no significant difference in serum folate levels between the 3 MTHFR genotypes. The absence of subjects with low folate levels in our population prevented us from examining the interaction between homocysteinemia and the MTHFR genotype in such subjects. On the other hand, homocysteinemia was negatively correlated with serum folate levels. The observation that mild hyperhomocysteinemia and the Val/Val MTHFR genotype have opposite effects on carotid artery internal diameter (lumen enlargement and narrowing, respectively) may explain in part the reported involvement of hyperhomocysteinemia in cardiovascular disease and the lack of involvement of the MTHFR C677T mutation.34–36 Indeed, our results suggest that pathophysiological processes associated with hyperhomocysteinemia and the Val/Val MTHFR genotype are different. Homocysteine may act through direct toxicity on matrix components, such as collagen, elastin, and proteoglycans,27,28,37 whereas the Val/Val MTHFR genotype may act on multiple pathways affecting cellular methylation through its ability to decrease S-methyltetrahydrofolate levels and probably decrease cellular methionine and S-adenosylmethionine levels as well, as previously suggested by Chen et al.38 Whereas CCA internal diameter was lower in subjects with the Val/Val genotype, CCA wall thickness and WCSA were not different between homozygotes for the mutation and the other subjects. A decreased internal diameter with no changes in WCSA may represent eutrophic inward remodeling, by analogy with the classification proposed by Heagerty et al for the description of changes in resistance arteries. It thus seems that the Val/Val genotype is associated with remodeling of the CCA. Remodeling is a rearrangement of preexisting tissue elements in the vessel wall, without the need for a growth response or a change in media-cross-sectional area (contrary to hypertrophy). Most often, arterial remodeling is associated with hypertensive vascular disease, vascular injury, or a long-term decrease in arterial flow.40 We can eliminate the first 2 causes of remodeling in our population, because homozygous Val/Val subjects had BP values comparable to those of the other subjects and no carotid artery hypertrophy, and only individuals without atherosclerotic plaque were included in the study. Regarding modifications of arterial flow in the CCA, we did not record this parameter and were thus unable to study it. However, the relationship between variations in arterial flow and arterial diameter modifications has been extensively studied in humans. Joannides et al indicated that a 200% increase in blood flow induced a 4% increase in diameter. In our study, we observed a 7% decrease in CCA internal diameter in subjects with the Val/Val MTHFR genotype compared with the other subjects. To explain this diameter variation by a change in mean blood flow alone, the latter should be decreased by 350%, which is highly unlikely. Therefore, the Val/Val MTHFR genotype appears to be an independent determinant in carotid arterial wall remodeling.

In conclusion, this study suggests that in asymptomatic adults, a mildly elevated plasma homocysteine concentration is associated with lumen enlargement and wall thickening of the carotid artery, both of which are involved in the development of cardiovascular disease. If these preliminary results are confirmed, they may stimulate interest in clinical trials of folic acid supplementation as primary preventive therapy for the homocysteinemia form of vascular disease. Indeed, normalization of hyperhomocysteinemia by folic acid has been demonstrated, but the clinical benefit of this biochemical effect in symptomatic subjects has not. This study also describes for the first time to our knowledge an arterial phenotype associated with homozgyosity for the MTHFR C677T mutation. Moreover, contrary to elevated homocysteinemia, homozgyosity for the C677T mutation was associated with a decreased internal diameter of the CCA, independent of homocysteinemia. Based on the opposite effects of plasma homocysteine and the MTHFR C677T mutation on carotid artery geometry, which also must be confirmed in larger studies, the links between hyperhomocysteinemia, the MTHFR mutation, and cardiovascular risk require further investigation. Indeed, homocysteinemia is not directly dependent on the MTHFR genotype, and we postulate that the pathophysiological processes associated with hyperhomocysteinemia and the MTHFR genotype are probably different, even if the process explaining the association of the MTHFR C677T mutation with carotid artery geometry is not at present elucidated.

Acknowledgments

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