The H475Y Polymorphism in the Glutamate Carboxypeptidase II Gene Increases Plasma Folate without Affecting the Risk for Neural Tube Defects in Humans

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ABSTRACT In the diet, folate exists predominantly in the form of polyglutamates. Before absorption, these polyglutamates must be deconjugated to monoglutamates by the enzyme folylpoly-γ-glutamate carboxypeptidase (FGCP), which is located in the jejunum. Recently, a H475Y polymorphism in the glutamate carboxypeptidase II (GCPII) gene, encoding the FGCP enzyme, was reported to be associated with decreased plasma folate and increased plasma homocysteine (tHcy) levels. Low folate and elevated tHcy levels are risk factors for neural tube defects (NTD). Therefore, we examined whether this polymorphism is associated with NTD risk and plasma folate, erythrocyte folate, plasma homocysteine (tHcy) levels in 96 NTD patients, 113 mothers, 97 fathers and 101 controls. This variation was associated with increased plasma folate and plasma homocysteine (tHcy) levels in 96 NTD patients, 113 mothers, 97 fathers and 101 controls. It was not associated with erythrocyte folate or the risk for NTD. The H475Y polymorphism in the GCPII gene may increase the deconjugation activity of the FGCP enzyme, resulting in an increased absorption of folate in the body, as reflected by the increased plasma folate and decreased plasma homocysteine concentrations. J. Nutr. 133: 75–77, 2003.

KEY WORDS: – glutamate carboxypeptidase II – H475Y polymorphism – folate – homocysteine – neural tube defects

Folate is an essential vitamin for humans and is obtained from the diet. Dietary folates exist mainly as polyglutamates (1). Because the uptake and transport of folate in the body occurs as monoglutamates, the dietary polyglutamated folates have to be hydrolyzed to monoglutamates before absorption. The enzyme responsible for this deconjugation is folylpoly-γ-glutamate carboxypeptidase (FGCP), which is anchored to the intestinal apical brush border (2). After the deconjugation process, the folate monoglutamates are absorbed in the proximal small intestine by the intestinal folate carrier.

A low folate status has been associated with increased risk for neural tube defects (NTD) (3). Several studies established that periconceptional folic acid supplementation reduces NTD risk by 60% (4–6). The exact mechanism of this preventive effect of folic acid on NTD is unknown. In addition to its involvement in the synthesis of purines and thymidine, folate is also required for the remethylation of homocysteine to methionine. Several papers reported elevated plasma homocysteine levels in mothers of children with NTD (7–9), which can be reduced by folic acid intake (8,10,11). Part of the preventive effect of folate is explained by the methylenetetrahydrofolate reductase (MTHFR) 677 C>T polymorphism; women with this polymorphism in the homoyoxous form have low plasma folate and elevated plasma homocysteine levels and have about a twofold higher risk of having a child with NTD (12,13). This demonstrates that genetic defects in enzymes involved in the folate-homocysteine pathway can affect the risk for NTD.

Recently, a H475Y polymorphism (1561 C>T) in the glutamate carboxypeptidase II (GCPII) gene, encoding the above-described FGCP enzyme, was reported to decrease the enzyme’s activity and was associated with decreased plasma folate levels and increased plasma total homocysteine (tHcy) levels (14). Thus, this variant may be associated with increased NTD risk. It is worth mentioning that the supplemented form of folate, folic acid, is a synthetic monoglutamate and does not require FGCP before absorption.

The aim of our study was to investigate whether the H475Y polymorphism in the GCPII gene is associated with NTD risk and whether it influences plasma and red cell folate levels and plasma tHcy in patients with NTD, their parents and in a control population.

SUBJECTS AND METHODS

Study population. Samples of patients with spina bifida and their parents were collected from two study populations. All individuals were Dutch Caucasians. One group was recruited in collaboration with the Dutch society for patients with central nervous system defects (BOSK). The second study population consisted of patients and their parents recruited by participation of the department of Child Neurology of the University Medical Center Nijmegen. The control groups consisted of healthy employees of our University Medical Center with no history of NTD. The protocol was approved by the local ethics committee and written informed consent was obtained. The final study population consisted of 96 patients (mean age 16 ± 11 y), 113 mothers (mean age 43 ± 11 y), 97 fathers (mean age 45 ± 10 y) and 101 controls (mean age 35 ± 8 y). The percentage supplement users in our study population is unknown. However,
samples of the study population were collected in the early 1990s when supplement use was uncommon in the Netherlands.

**Method.** Erythrocyte and plasma folate and plasma vitamin B-12 levels were determined using the Dualcount Solid Phase Boil Radioassay (Diagnostic Products Corporation, Los Angeles, CA). Homocysteine concentrations were determined in EDTA plasma by HPLC with fluorescence detection (15).

The H475Y polymorphism was determined by restriction fragment length polymorphism-polymerase chain reaction on genomic DNA as described by Devlin et al. (14) and the prevalence of the MTHFR 677C>T mutation was investigated as described by Froost et al. (16).

**Statistics.** Odds ratios (OR) and 95% confidence interval (CI) for patients, mothers and fathers compared with controls were calculated by logistic regression analyses. Plasma and erythrocyte folate and plasma homocysteine concentrations showed a nonnormal distribution. Therefore, natural logarithmically transformed values were used for all calculations and mean values were expressed as geometric means. Comparison of biochemical concentrations between the genotypes was performed with general linear model analysis and adjusted for age, gender and the MTHFR 677 C>T polymorphism. All analyses were performed with the SPSS 10.0 software package (Chicago, IL) and statistical significance was accepted at \( P < 0.05 \).

**RESULTS**

The GCPII H475Y polymorphism was analyzed on genomic DNA of patients, their parents and controls. The prevalence of this polymorphism and other characteristics of patients, their parents and controls are summarized in Table 1. The distribution of the H475Y polymorphism in the total study population was in Hardy-Weinberg equilibrium (\( \chi^2 = 0.42, P = 0.52 \)). The allele frequency of the Y allele was 6.9% in the control population and 5.7, 4.4 and 5.7% in patients, mothers and fathers, respectively. Because the homozygous YY genotype was present in two controls only, HY and YY genotypes were combined for both the calculation of OR (Table 1) and the examination of possible associations with metabolic compounds. The metabolite concentrations in the two individuals with the YY genotype were 13 and 21.0 mmol/L for plasma folate, 490 and 790 mmol/L for erythrocyte folate and 14.7 and 19.8 \( \mu \)mol/L for plasma \( \text{tHcy} \), respectively. The high plasma homocysteine levels of the second subject may have been due to low vitamin B-12 levels (130 pmol/L).

Because the genotype distribution did not differ among patients, mothers, fathers and controls, we combined parents of NTD patients and controls to increase statistical power for the examination of possible associations of this polymorphism with plasma folate, erythrocyte folate and plasma \( \text{tHcy} \) levels. Because homocysteine concentrations are age dependent and appropriate controls for the much younger NTD patients were not available, NTD patients were excluded from this analysis. Plasma folate levels of the GCPII genotypes are depicted in Figure 1. Analyses for a possible association between the GCPII polymorphism and plasma folate levels were performed in 297 individuals. Individuals with the HY/YY genotypes had greater plasma folate concentrations than their HH peers (\( P = 0.043 \)). Erythrocyte folate levels in 294 subjects did not differ between HH (mean 555 nmol/L, 95% CI 533–578) and HY/YY (mean 582 nmol/L, 95% CI 518–654) genotypes (\( P = 0.45 \)). The possible association of the GCPII H475Y polymorphism with plasma homocysteine levels was examined in 296 individuals; plasma homocysteine levels tended to be lower (\( P = 0.087 \)) in individuals with the HY/YY (mean 12.3 \( \mu \)mol/L, 95% CI 11.1–13.5) genotype compared with HH (mean 13.4 \( \mu \)mol/L, 95% CI 13.0–13.9) subjects. Analyses of plasma and erythrocyte folate and plasma \( \text{tHcy} \) levels for mothers, fathers and controls separately did not reveal any significant differences.

**DISCUSSION**

We observed an association of the H475Y polymorphism in the GCPII gene with increased plasma folate levels and decreased plasma \( \text{tHcy} \) concentrations. Erythrocyte folate levels were not associated with this polymorphism and we did not observe an association of the H475Y variation with NTD risk in our study population.

Despite our relatively large study group, the low prevalence of the H475Y polymorphism could have precluded the finding

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**TABLE 1**

**Characteristics and distribution of the H475Y polymorphism in the glutamate carboxypeptidase II (GCPII) gene in children with neural tube defects, their parents and controls**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
<th>Mothers</th>
<th>Fathers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y(^1)</td>
<td>16.4 ± 11.3</td>
<td>42.9 ± 10.9</td>
<td>44.9 ± 10.2</td>
<td>35.6 ± 8.3</td>
</tr>
<tr>
<td>Females, ( n ) (%)</td>
<td>55 (56%)</td>
<td>113 (100%)</td>
<td>97 (0%)</td>
<td>73 (100%)</td>
</tr>
<tr>
<td>Plasma folate, ( \mu )mol/L</td>
<td>11.7 (10.5–12.9)</td>
<td>12.7 (11.6–13.8)</td>
<td>12.2 (11.2–13.3)</td>
<td>14.7 (13.4–16.1)</td>
</tr>
<tr>
<td>Erythrocyte folate, ( \mu )mol/L</td>
<td>476 (442–512)</td>
<td>542 (504–582)</td>
<td>574 (539–610)</td>
<td>560 (517–607)</td>
</tr>
<tr>
<td>Plasma ( \text{tHcy} ), ( \mu )mol/L</td>
<td>11.8 (11.0–12.5)</td>
<td>12.5 (11.7–13.2)</td>
<td>14.2 (13.4–15.1)</td>
<td>12.3 (11.7–12.9)</td>
</tr>
<tr>
<td>Plasma vitamin B-12, ( \mu )mol/L</td>
<td>282 (252–315)</td>
<td>209 (190–231)</td>
<td>233 (218–249)</td>
<td>223 (204–245)</td>
</tr>
<tr>
<td>GCPII H475Y, ( n ) (%)</td>
<td>85 (88.5%)</td>
<td>103 (91%)</td>
<td>86 (89%)</td>
<td>88 (88%)</td>
</tr>
<tr>
<td>( \text{HY} )</td>
<td>11 (11.5%)</td>
<td>10 (9%)</td>
<td>11 (11%)</td>
<td>10 (10%)</td>
</tr>
<tr>
<td>( \text{YY} )</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( \text{HY/YY vs. HH} )</td>
<td>1.0 (0.4–2.3)</td>
<td>0.7 (0.3–1.8)</td>
<td>0.9 (0.4–2.3)</td>
<td>1.0 (0.4–2.3)</td>
</tr>
</tbody>
</table>

\(^1\) Values are mean ± SD (\( n \)).

\(^2\) Values are geometric mean (95% confidence interval) (\( n \)) P-values are age-adjusted.

\(^3\) \( \chi^2 = 0.42, P = 0.52 \).

\(^4\) Values are odds ratio (95% confidence interval).

\(^5\) Reference category.

\(*\) Different from female controls \( P < 0.05 \).

\(**\) Different from male controls \( P < 0.05 \).
of a risk factor for NTD; therefore, larger study populations are necessary to investigate this association further.

Folate is required for the remethylation of homocysteine, and the increased plasma folate levels observed in individuals with the 475HY/YY genotype in the GCPII gene are therefore in line with the decreased plasma tHcy concentrations in our population. This finding indicates that the H475Y polymorphism in the GCPII gene may increase activity of the FGCP enzyme, resulting in a greater availability of monoglutamates from the small intestine, which subsequently leads to increased plasma folate and decreased plasma tHcy concentrations.

A recent study by our group in a different population of controls and patients with cardiovascular disease supports this observation because increased plasma and erythrocyte folate levels were observed in individuals with the 475HY/YY genotype in the GCPII gene (17). In a study published very recently, no association of this variant with plasma folate and tHcy levels was observed in 644 women and 680 men as a group. When the association of this variant with plasma folate and tHcy levels was observed in individuals with the GCPII 475HY/YY genotype, it was in line with the decreased plasma tHcy concentrations in our population. This is in line with our previous reported results of Lievers et al. (17) point instead toward increased conjugation activity of the FGCP enzyme, resulting in increased folate absorption in the body and therefore increased plasma folate, rather than toward decreased GCP II activity and decreased plasma folate levels.

**FIGURE 1** Plasma folate levels in the study participants, excluding the neural tube defect patients, analyzed by the H475Y polymorphism in the glutamate carboxypeptidase (GCPII) gene. Values are geometric means and 95% confidence intervals. Geometric means were adjusted for age, gender, plasma vitamin B12 and the methyltetrahydrofolate reductase 677 C→T polymorphism.

In summary, the H475Y polymorphism in the GCPII gene is associated with increased plasma folate levels and decreased plasma tHcy levels. An association with NTD risk was not observed. To elucidate whether this polymorphism in the GCPII gene influences the function of the FGCP enzyme, other types of studies are required, e.g., intervention studies in which the influence of controlled dietary folate on folate levels is observed in individuals with different genotypes for the GCPII polymorphism. Furthermore, the activity of the FGCP enzyme seems to depend on the content of other compounds in the food consumed, which should therefore also be controlled (20).

Our results and the previously reported results of Lievers et al. (17) point instead toward increased conjugation activity of the FGCP enzyme, resulting in increased folate absorption in the body and therefore increased plasma folate, rather than toward decreased GCP II activity and decreased plasma folate levels.

**LITERATURE CITED**