The Methionine Synthase Polymorphism c.2756A>G Alters Susceptibility to Glioblastoma Multiforme

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Abstract

Genetic polymorphisms of methionine metabolism, in particular methionine synthase (MTR) c.2756A>G (D919G) and methylenetetrahydrofolate reductase (MTHFR) c.677C>T (A222V), have been associated with various human cancers. We investigated MTR c.2756A>G, MTHFR c.677C>T, and a third polymorphism, transcobalamin 2 c.776C>G (P259R), for a potential association with the formation of glioblastoma multiforme. The MTR c.2756G allele was significantly underrepresented among 328 glioblastoma multiforme patients of Caucasian origin when compared with 400 population controls [patients AA/AG/GG: 0.72/0.26/0.02 and controls AA/AG/GG: 0.57/0.38/0.05, degrees of freedom = 2; χ² = 17.86 (Pearson); P < 0.001]. No association between glioblastoma multiforme and the two other polymorphisms was observed. (Cancer Epidemiol Biomarkers Prev 2006; 15(11):2314–6)

Introduction

Glioblastoma multiforme is the most common glial tumor. The annual incidence of malignant astrocytomas (i.e., anaplastic astrocytoma and glioblastoma multiforme) is 3 to 4 per 100,000 (1). Glioblastoma multiformes account for up to 80% of malignant gliomas (2). Patients diagnosed with glioblastoma multiforme typically undergo microsurgical resection of their tumor followed by radiation. Adjuvant chemotherapy confers a modest survival benefit. However, median survival usually does not exceed 12 to 15 months even in selected patient series (3).

Great progress has been made in delineating characteristic patterns of somatic mutations in glioblastoma multiformes (4). However, relatively little is known with respect to germ-line genetic factors influencing an individual’s risk to develop a malignant glioma. The genetic pathways involved in glioblastoma multiforme formation may vary with ethnicity (5). Well-characterized cancer predisposition syndromes such as the Li-Fraumeni syndrome or Turcot syndrome are rarely defined as a potential association with the formation of glioblastoma multiforme (6). Some studies suggested that polymorphisms of the p53 gene and of genes coding for DNA repair enzymes, glutathione S-transferase and CYP2E1, may be associated with glioblastoma multiforme formation (8-10). Genetic variants associated with asthma may protect against glioblastoma multiforme and gliomas in general (11).

The 5-methyltetrahydrofolate-homocysteine S-methyltransferase (MTR; also called methionine synthase) catalyzes the remethylation of homocysteine to methionine and has influence on DNA methylation as well as on nucleic acid synthesis (12). The missense polymorphism MTR c.2756A>G (D919G) has been reported to alter the susceptibility to various cancers (12-17). Methyltetrahydrofolate reductase (MTHFR) catalyzes the synthesis of 5-methyltetrahydrofolate, a cofactor for MTR. Some evidence suggests that the MTHFR c.677C>T (A222V) polymorphism as well might be associated with a variety of cancers (18). Transcobalamin 2 (Tc2) is the transporter of a vitamin B12 derivate, which serves as the second cofactor for MTR. Synergistic effects between the Tc2 c.776C>G (P259R) and MTR c.2756A>G polymorphisms on the development of white matter in patients with primary central nervous system lymphoma treated with methotrexate have been described (19).

In the present study, we investigated the three missense polymorphisms, MTR c.2756A>G (D919G), MTHFR c.677C>T (A222V), and Tc2 c.776C>G (P259R), for a potential association with glioblastoma multiforme formation.

Materials and Methods

We investigated 328 consecutive glioblastoma multiforme patients (59% male; average age ±1 SD, 58.8 ± 12.7 years) of Caucasian origin recruited for the tumor library of the Department of Neurosurgery of the University Hospital of Bonn between September 1994 and May 2006 (response rate, 75%). The Department of Neurosurgery is a tertiary referral center serving a population of 1,000,000 in the greater Bonn area. All histologic diagnoses were made at the Institute for Neuropathology/German Brain Tumor Reference Center at the University of Bonn. In 12 of the patients, the histologic subtype was gliosarcoma; in 6 patients, giant cell glioblastoma multiforme; in all others, glioblastoma multiforme. Four hundred apparently healthy white Caucasian Bonn area residents (52% female; average age ±1 SD, 52.9 ± 15.7 years) without a history of cancer served as controls (Table 1).

Genomic DNA prepared from peripheral leukocytes was used for allelotyping by PCR amplification and restriction analysis as previously published (12). The two-sided Pearson’s χ² test was employed to analyze the distribution of the respective allelotype in the patient and the control group. The Hardy-Weinberg equation was tested with a χ² test (a) = 0.05) was additionally used to test the
Table 1. Genetic variants of methionine metabolism in glioblastoma multiforme patients and controls

<table>
<thead>
<tr>
<th>MTHFR c.677C&gt;T</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Pearson: $\chi^2$; $P$</th>
<th>Regression: $\chi^2$; $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 328)</td>
<td>0.42</td>
<td>0.46</td>
<td>0.12</td>
<td>$\chi^2 = 2.30$; $P = 0.316$</td>
<td>$\chi^2 = 3.55$; $P = 0.169$</td>
</tr>
<tr>
<td>Controls (n = 400)</td>
<td>0.46</td>
<td>0.46</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTR c.2756A&gt;G</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>$\chi^2 = 2.0$; $P = 0.154$</td>
<td>$\chi^2 = 17.86$; $P &lt; 0.001$</td>
</tr>
<tr>
<td>Patients (n = 328)</td>
<td>0.72</td>
<td>0.26</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 400)</td>
<td>0.57</td>
<td>0.38</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tc2 c.776C&gt;G</td>
<td>CC</td>
<td>CG</td>
<td>GG</td>
<td>$\chi^2 = 0.18$; $P = 0.912$</td>
<td>$\chi^2 = 0.735$; $P = 0.693$</td>
</tr>
<tr>
<td>Patients (n = 328)</td>
<td>0.28</td>
<td>0.46</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 400)</td>
<td>0.28</td>
<td>0.45</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: The rate of carriers of the different allelotypes is given as relative amount. The $\chi^2$ test values for Pearson’s $\chi^2$ test for all three allelotypes ($= 2$ degrees of freedom) as well as for multiple nominal regression analysis (with simultaneous analysis of all three polymorphisms together with age and gender as covariables) for the differences between patients and controls are shown. The allelic frequencies in the control group are similar to those we reported for another (independent) healthy German population (12).

Results

The Hardy-Weinberg equilibrium was fulfilled for the three polymorphisms among patients and controls. The MTR c.2756G allele (i.e., the MTR c.2756AG and GG allelotypes) was significantly less frequently found among glioblastoma multiforme patients when compared with controls (28% versus 43%; odds ratio, 0.52; 95% confidence interval, 0.37-0.71; $\chi^2 = 17.4$; $P = 0.0003$; Table 1). The underrepresentation of the G-allele of MTR c.2756A>G in glioblastoma multiforme patients suggests that it protects against the incidence of glioblastoma multiforme. Multiple nominal regression analysis with age and gender as covariables proved that the MTR c.2756A>G (D919G) genotype was an independent predictive factor for glioblastoma multiforme formation ($\chi^2 = 14.82$; $P = 0.001$). If the 18 samples of patients with glioblastoma of the giant cell or gliosarcoma subtype were left out of analysis, significance was $\chi^2 = 12.73$ ($P = 0.002$) in nominal regression analysis.

There was no significant association of the two other polymorphisms (MTHFR c.677C>T and Tc2 c.776C>G) with the incidence of glioblastoma multiforme in our study sample.

Discussion

We have analyzed three polymorphisms of methionine metabolism in 328 glioblastoma multiforme patients and 400 controls. We observed a significant association between the G-allele of MTR c.2756A>G (D919G) and glioblastoma multiforme. A 28% prevalence of the G-allele among glioblastoma multiforme patients versus 43% in the control series suggests an epidemiologically relevant protective effect of the G-allele (odds ratio, 0.52).

As our data were created in a case-control design, the observed protective effect of the G-allele of MTR c.2756A>G should be independent of an independent sample. Nevertheless, the G-allele of MTR c.2756A>G (D919G) has been reported to reduce the susceptibility to a number of other cancers, colorectal cancer [protective in combination with low alcohol consumption (13); protective (14)], cervical intraepithelial neoplasia [protective in women when in combination with the MTHFR c.677C>T variant (15)], primary central nervous system non-Hodgkin lymphoma [protective (12)], and acute lymphoblastic leukemia and systemic non-Hodgkin’s lymphoma [protective (16, 17)]. Our study suggests that this allele also protects against glioblastoma multiforme. The functional consequences of this missense polymorphism about MTR expression and activity have not been studied in detail. MTR catalyzes the remethylation of homocysteine to methionine and is therefore involved in methyl, folate, and nucleic acid metabolism (12). Impairment of these pathways can lead to uracil incorporation into DNA, hypomethylation or hypermethylation of DNA, inefficient DNA repair, and increased chromosome malsegregation and chromosomal breakage (20). In addition, the MTR c.2756A>G variant has been reported to influence the methylation of CpG islands in tumor suppressor genes (21). Chromosomal instability and modified DNA methylation are important mechanisms involved in the pathogenesis of most malignant tumors including glioblastoma multiforme (22-25). These findings suggest explanations for the association of the MTR c.2756A>G (D919G) polymorphism with glioblastoma multiforme and other cancers. Interestingly, the presence of the protective G-allele has been observed to promote disease-free longevity in the general German population (26).

The MTHFR c.677C>T polymorphism was suggested to influence the susceptibility to various extracranial tumors, but not to primary central nervous system lymphoma, which was found to be also influenced by the MTR c.2756A>G polymorphism (12). We did not observe any significant associations of MTHFR c.677C>T or Tc2 c.776C>G with glioblastoma multiforme in our study sample.

Finally, it is tempting to speculate about possible clinical implications of the data presented. MTR activity depends on folate and vitamin B12 (27), which might provide a link of nutritional factors and incidence of glioblastoma multiforme and other malignant tumors. Accordingly, an inverse relation between maternal folate intake, the use of various vitamins, and the risk to develop malignant brain tumors in young children has been reported (28). Additional studies on the functional consequences of the MTR c.2756A>G polymorphism and on the detailed influence of this polymorphism and of folate and vitamin B12 on the incidence of different types of cancer are warranted.

References

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