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Genetic Variation in the MTHFR Gene Influences Thiopurine Methyltransferase Activity, Monica Arenas, Gemma Simpson, Cathryn M. Lewis, El-Monsor Shobouale-Bakre, Emilía Escuredo, Lynette D. Fairbanks, John A. Duley, Azhar Ansari, Jeremy D. Sanderson, and Anthony M. Marinati (1) (Purine Research Laboratory, Department of Chemical Pathology, and 2 Department of Gastroenterology, Guy’s and St. Thomas’ Hospital NHS Trust, London, United Kingdom; 3 Department of Medical and Molecular Genetics, GKT School of Medicine, King’s College, London, United Kingdom; † current address: School of Microbial and Molecular Sciences, University of Queensland, Australia; * address correspondence to this author at: Purine Research Laboratory, Department of Chemical Pathology, 5th Floor Thomas Guy House, Guy’s Hospital, London SE1 9RT, United Kingdom; fax 44-207-188-1280, e-mail tony.marinati@kcl.ac.uk)

The immunosuppressive drug 6-mercaptopurine (6-MP) and its prodrug azathioprine are used in the treatment of inflammatory bowel disease and other disorders of immune regulation (1). Thiopurine methyltransferase (TPMT) inactivates 6-MP by methylation. The generic variants TPMT*2 to *19 are associated with decreased TPMT activity (2), and TPMT*3A, *3C, and *2 are the most common deficiency-associated variants (1). A heterozygous TPMT genotype (1 in 10 individuals from the general population) is associated with an increased risk of myelosuppression with standard-dose azathioprine therapy (3) and a favorable response to reduced-dose thiopurine therapy (1). Patients with complete TPMT deficiency (1 in 300 individuals from the general population) are at high risk for myelosuppression (4).

The erythrocyte TPMT activity distribution is continuous, and concordance between genotype and phenotype in the carrier range varies, depending on where the cutoff is established between the ranges for carriers and noncarriers. We propose that genetic variation in folate metabolism influences TPMT activity and contributes to the lack of concordance between genotype and phenotype in the carrier range.

TPMT irreversibly transfers a methyl group from S-adenosylmethionine (SAM) to 6-MP, forming 6-methylmercaptopurine (6-MeMP) and S-adenosylhomocysteine (SAH). The adenosyl moieties of SAH is subsequently cleaved, and homocysteine is remethylated to methionine. The methyl donor for this folate-dependent remethylation cycle is 5-methyltetrahydrofolate, which is formed from 5,10-methylene tetrahydrofolate (MTHF) in a reaction catalyzed by 5,10-MTHF reductase (MTHFR). The MTHFR 677C>T (A222V) thermolabile variant (5) and the 1298A>C (E429A) variant (6) are associated with decreased MTHFR activity. The homozygous MTHFR 677TT genotype occurs in 8%–10% of the population (7), shows 30%–50% of wild-type activity in lymphocytes (8, 9), and is associated with hyperhomocysteinemia (10), DNA hypomethylation (11), increased risk of neural tube defects (12), and decreased risk of some cancers (13, 14). The homozygous MTHFR 1298CC genotype (frequency, 10%) is associated with 60% of wild-type activity in lymphocytes (6, 7).

MTHF dehydrogenase/methenyltetrahydrofolate cyclohydrodrolase/formyltetrahydrofolate synthetase (MTHFD1) catalyzes the conversion of tetrahydrofolate to N10-formyltetrahydrofolate, N5,N10-methenyltetrahydrofolate, and 5,10-MTHF. The MTHFD1 1958G>A (R653Q) allele is associated with neural tube defects (15).

The Ethics Committee of Guy’s and St. Thomas’ Hospitals NHS Trust approved this study, which used leftover blood samples. Erythrocyte TPMT activity was measured in EDTA blood as the conversion of 6-MP to 6-MeMP (16) and expressed as picomoles of 6-MeMP formed per hour per milligram of hemoglobin [pmol 6-MeMP·h·1·(mg Hb)−1]. Completely deficient TPMT activity was defined as <2.5 pmol 6-MeMP·h·1·(mg Hb)−1, and the carrier and noncarrier ranges were 2.5–7.5 and >7.5–14.5 pmol 6-MeMP·h·1·(mg Hb)−1, respectively.

Patients were genotyped for the MTHFR 677C>T and 1298A>C variants and the TPMT*3A, *3C, and *2 variants (16). The MTHFD1 1958G>A mutation (R653Q) destroys an HpaII site and was amplified with the primers 1958 for (5′-TTCTTCTATCCCTTCTCACCCTG-3′) and 1958rev (5′-CAATGTCCTCCCAATCTGTC-3′). The thermocycler profile was 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s. The 421-bp fragment digested by HpaII yielded fragments of 253 and 168 bp for the wild-type allele.

All variants were tested for Hardy–Weinberg equilibrium. Genotype frequencies were compared by use of 2 ×
2 contingency tables, with recessive and dominant models applied. Significance was determined by 2-tailed Fisher exact tests. Because the analyses were not independent, no correction was applied for multiple testing of variants or models. The linkage disequilibrium coefficient between the *MTHFR* 677T>C and 1298A>C variants was calculated, and haplotype models were fitted by use of a log-linear model in COCAPHASE (17). Models were also fitted with *MTHFD1* included to determine any multilocus effects across *MTHFR* and *MTHFD1*.

Concordance between *TPMT* genotype and phenotype in the laboratory carrier range [2.5–7.5 pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹] was determined from a series of 1575 predominantly Caucasian patients. Of the 185 (11.7%) patients with a carrier phenotype, 158 were heterozygous for a common *TPMT* mutation, 18 were homozygous for the wild-type *TPMT* allele, and 5 were carriers with a previously identified heterozygous *TPMT* enzyme. We postulated that mutations occurring in folate metabolism play a role in modulating *TPMT* activity in the carrier range (18).

To test the hypothesis that a polymorphism in folate metabolism influences *TPMT* activity, we defined 2 groups in the *TPMT* carrier range: patients with a carrier genotype and phenotype (66 patients), and individuals with homozygous wild-type activity with a wild-type *TPMT* genotype (n = 70). These groups were compared with a well-separated group of patients (n = 83) with *TPMT* activity in the narrow interval of 12.0–14.5 pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹ at the upper limit of the laboratory noncarrier range of >7.5–14.5 pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹. The study populations were enriched, and although the groups were recruited sequentially on the basis of *TPMT* genotype and phenotype, the sizes are not proportional to those found in the general population.

All 83 patients with *TPMT* activity in the noncarrier range [12–14.5 pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹] were wild type for *TPMT* *a*3A, *a*3C, and *a*2 mutations. The median *TPMT* activity of patients with a heterozygous genotype was 6.3 (range, 5.0–7.5) pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹, which was significantly different from the value of 7.1 (range, 4.0–7.5) pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹ obtained for wild-type patients with *TPMT* activity in the carrier range (P < 0.0001, Mann–Whitney rank-sum test).

Table 1. *MTHFR* and *MTHFD1* genotype frequencies in groups with *TPMT* activity in the noncarrier and carrier ranges.

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>Noncarrier <em>TPMT</em> activity, wild-type genotype</th>
<th>Carrier <em>TPMT</em> activity, heterozygous genotype</th>
<th>Carrier <em>TPMT</em> activity, wild-type genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>677CC</td>
<td>35 (0.42)</td>
<td>22 (0.34)</td>
<td>28 (0.4)</td>
</tr>
<tr>
<td>677CT</td>
<td>41 (0.49)</td>
<td>33 (0.48)</td>
<td>26 (0.34)</td>
</tr>
<tr>
<td>677TT</td>
<td>7 (0.08)</td>
<td>11 (0.17)</td>
<td>16 (0.23)</td>
</tr>
<tr>
<td>1298AA</td>
<td>41 (0.49)</td>
<td>37 (0.56)</td>
<td>34 (0.61)</td>
</tr>
<tr>
<td>1298AC</td>
<td>34 (0.41)</td>
<td>23 (0.34)</td>
<td>34 (0.35)</td>
</tr>
<tr>
<td>1298CC</td>
<td>8 (0.09)</td>
<td>6 (0.09)</td>
<td>2 (0.04)</td>
</tr>
<tr>
<td>MTHFD1 genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1958GG</td>
<td>25 (0.30)</td>
<td>21 (0.33)</td>
<td>24 (0.33)</td>
</tr>
<tr>
<td>1958GA</td>
<td>39 (0.47)</td>
<td>28 (0.42)</td>
<td>30 (0.43)</td>
</tr>
<tr>
<td>1958AA</td>
<td>19 (0.23)</td>
<td>17 (0.25)</td>
<td>16 (0.24)</td>
</tr>
</tbody>
</table>

*TPMT* activity = 12–14.5 pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹.

*TPMT* activity = 2.5–7.5 pmol 6-MeMP·h⁻¹·(mg Hb)⁻¹.

P = 0.0216.
Moreover, a recent study of the binding of the SAM analog sinefungin to TPMT from *Pseudomonas syringae* suggested that substrate binding leads to conformational stability of peripheral structural elements of the enzyme as well as an increase in backbone mobility that may protect variant and, by implication, wild-type TPMT enzymes from ubiquitilation and degradation (20). Studies of the MTHFR-deficient mouse model have predicted decreased SAM concentrations and an altered SAM/SAH ratio (21, 22), although recently a slight but significant increase in erythrocyte SAM concentrations in individuals with an MTHFR 677CT heterozygous genotype has been reported (23). Little is known about the interplay between plasma and intracellular concentrations of SAM and SAH and how these are affected by polymorphisms in folate-metabolizing enzymes.

The coinheritance of variant MTHFR and MTHFD1 alleles did not have an additive effect on TPMT activity, consistent with reports concluding that MTHFD1 does not directly influence reactions for which a methyl group is required and does not affect SAM concentrations through 5,10-MTHF pools (15). In a pharmacogenetic context, inheritance of an MTHFR 677T-1298A haplotype and an MTHFD1 1958A allele was associated with a lower probability of event-free survival in children treated with the antifolate drug methotrexate for acute lymphoblastic leukemia, particularly when in combination with the thymidine synthase triple repeat associated with increased thymidylic synthase concentrations (24). Polymorphisms in both genes thus have a demonstrable effect on folate metabolism within the cell, with the potential to affect intracellular SAM concentrations and hence, indirectly, TPMT activity.

TPMT activity in individuals heterozygous for a variant TPMT allele was significantly lower than in those with a wild-type TPMT genotype and activity in the carrier range. Concordance between TPMT genotype and phenotype in the carrier range will thus be determined essentially by the limits of the carrier range. Rare polymorphisms in the TPMT gene may also contribute to the lack of concordance.

The TPMT phenotype is determined in erythrocytes, and it is not known how a MTHFR 677TT genotype will affect TPMT activity or thiopurine metabolism in nucleated cells, which unlike erythrocytes are able to continuously synthesize protein. Methylated thiopurine metabolites have been reported to be immunosuppressive (25), and high concentrations of 6-MeMP have been implicated in hepatotoxicity (26). It is thus possible that patients with an MTHFR 677TT genotype may be poor responders to therapy or, conversely, may be protected against methylated metabolite–mediated toxicity.

In conclusion, polymorphisms in the MTHFR gene may play an important role in determining the erythrocyte TPMT phenotype, but this effect needs to be replicated in further genetic association studies. We speculate that decreased intracellular SAM pools lead to enhanced TPMT enzyme degradation. Further studies should investigate the effect of an MTHFR 677TT genotype on clinical responses to thiopurine drug therapy.

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References
Prepercutaneous Coronary Intervention Plasma Homocysteine Concentration Is a Useful Predictor of Angioplasty-Induced Myocardial Damage, Camille Brasselet,1* Roselyne Garnot,2 Antoine Lafont,3 Sophie Perotin,1 Fabien Vitry,4 Eric Durand,3 Laurent Dicher,1 Jacques Elaerts,1 Damien Metz,5 and Philippe Gillyer5 (1 Department of Cardiology, Hôpital Robert Debré, Reims, France; 2 Department of Biochemistry, American Memorial Hospital, Hôpital Robert Debré, Reims, France; 3 Department of Cardiology, HEGP, Paris, France; 4 Unité d’Aide Méthodologique à la Recherche Clinique, Hôpital Maison Blanche, Reims, France; 5* address correspondence to this author at: Department of Cardiology, Hôpital Robert Debré, Centre Hospitalier Universitaire, 51092 Reims Cedex, France; fax 33-3-2678-4132, e-mail camille.brasselet@wanadoo.fr)

Plasma homocysteine is a modifiable cardiovascular risk factor related to the extent of both coronary and carotid atherosclerosis (1–4). Plasma homocysteine has been shown to predict the occurrence of cardiac events and mortality in patients with coronary atherosclerosis (5–7). The predictive value of homocysteine on restenosis after percutaneous coronary intervention (PCI) has been debated (8–10). Recent evidence, however, indicated that the pre-PCI homocysteine plasma concentration was an independent predictor of death, nonfatal myocardial infarction (MI), and target lesion revascularization (11). Cardiac troponins provide prognostic information in patients with acute coronary syndrome (ACS) (12). Several studies have demonstrated that PCI induces MI as assessed by increases in cardiac troponins, particularly in the case of ACS (13–15). Furthermore, increased cardiac troponin concentrations after PCI are associated with poor clinical outcome (16–19). We therefore hypothesized that the pre-PCI plasma homocysteine concentration could be related to the occurrence of MI after PCI, as assessed by changes in plasma cardiac troponin I (cTnI) concentration.

Consecutive admissions for nonemergency PCI were studied prospectively. All patients had a stenosis >70% in 1 or more coronary arteries. Two groups were examined: patients with stable angina (SA) pain and those with ACS. The SA pain group had myocardial ischemia during exercise stress testing (n = 29). The ACS group included patients admitted with unstable angina without a subsequent increase in troponin concentrations (n = 28) and those with a definite MI with a documented cTnI increase and electrocardiogram changes that had occurred 7–14 days previously (n = 39). Patients with inflammatory diseases, as well as those being treated with corticosteroids or nonsteroidal antiinflammatory or immunosuppressive drugs, were excluded to minimize potential bias. This study complies with the Declaration of Helsinki, and the protocol was approved by the local institutional ethics committee. Informed written consent was obtained from each patient. Pre-PCI medications, including aspirin, heparin, nitrates, calcium channel–blocking agents, and β-adrenergic–blocking drugs, were maintained throughout the study. None of the patients had received long-term vitamin B supplementation before enrollment. No vitamin B supplementation was planned during the study, and none of the patients were supplemented in case of hyperhomocysteinemia at the time of the study. Procedures were performed with standard angioplasty techniques. Almost all of the patients were treated with stent implantation (n = 85).

Immediately before and 24 h after PCI, venous blood was collected under standard conditions into glass tubes containing an anticoagulant (lithium heparin). Plasma cTnI was measured on an AxSYM system (first generation; Abbott Diagnostics; CV <8.0% at 10 μg/L; manufacturer-reported reference values, <0.4 μg/L; detection limit of the assay, 0.4 μg/L). cTnI values <0.4 μg/L were stated as 0. The variation between pre- and post-PCI cTnI was defined as ΔcTnI [(post-PCI cTnI) – (pre-PCI cTnI)] = ΔcTnI]. For homocysteine measurements, samples were collected in plain glass tubes, transported on ice, and assayed within 4 h after venipuncture. Homocysteine was measured by ion-exchange HPLC (Hitachi 8800; Roche Diagnostics; CV <4.3% at 10 μmol/L; reference values, <12 μmol/L). Plasma creatine kinase and creatinine were measured with an Hitachi 911 analyzer (Roche Diagnostics). Post-PCI MI was defined by a post-PCI cTnI value >2 μg/L when pre-PCI cTnI was <0.4 μg/L, a post-PCI cTnI value >3 μg/L when pre-PCI cTnI was 0.4–2 μg/L, or an increase >50% of the initial value when pre-PCI cTnI was >2 μg/L. When MI was defined biologically, the difference between post-PCI non-Q-wave MI and post-PCI Q-wave MI was defined as the occurrence of a new Q-wave on post-PCI electrocardiograms. Acute renal failure was defined by a post-PCI increase in plasma creatinine concentration >2-fold higher than the initial value when the post-PCI plasma creatinine concentration was >120 μmol/L (1.4 mg/dL).