Thiopurine methyltransferase alleles in British and Ghanaian populations

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INTRODUCTION

Thiopurine methyltransferase (TPMT) catalyses the S-methylation of thiopurine drugs such as 6-mercaptopurine, 6-thioguanine and azathioprine. TPMT activity is inherited as an autosomal co-dominant trait, and several mutations in the TPMT gene have been identified which correlate with a low activity phenotype. Although ethnic differences in TPMT activity have been described, population frequency analysis of TPMT alleles has not been well defined in different ethnic groups. The frequency of four allelic variants of the TPMT gene, TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C were compared in British Caucasian (n = 199) and Ghanaian (n = 217) populations using PCR–RFLP and allele-specific PCR-based assays. TPMT*3C was found in 14.8% of Ghanaians (31 heterozygotes, one homozygote). The TPMT*2, TPMT*3A and TPMT*3B alleles were not detected in any of the Ghanaian samples analysed. In contrast, 10.1% of British subjects had variant alleles, consisting of TPMT*2 (n = 2), TPMT*3A (n = 17) and TPMT*3C (n = 1) alleles. The frequencies of mutant alleles in this study were 5.3 and 7.6% in British Caucasians and Ghanaians, respectively. Among Ghanaian tribes, Ewe subjects had a lower frequency of mutant alleles (5.9%) than Ga (13.2%) or Fanti (11.6%), although this did not reach statistical significance. This study provides the first analysis of TPMT mutant allele frequency in an African population and indicates that, unlike Caucasians, TPMT*3C is the most common allele in African subjects.

RESULTS

Mutant TPMT alleles were found in 14.8% (32/217) of Ghanaian subjects and 10.1% (19/199) of British Caucasian subjects (P = 0.33). From these data, the Hardy Weinberg equation predicts a homozygous mutant genotype frequency of 0.6% in Ghanaians, compared with 0.3% in Caucasians. Four alleles of the TPMT gene, TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C, which account for ~80% of Caucasians with low or intermediate TPMT activity, TPMT*2 contains a G→C substitution at nucleotide 238, while TPMT*3A contains two nucleotide transition mutations (G460A and A719G). TPMT*3B has only G460A, while TPMT*3C contains only A719G (15–19). The population frequency of these alleles has been described recently in British Caucasian, Southwest Asian and Chinese subjects, but has not been evaluated in African populations (20).

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were evaluated in the two populations. TPMT*3C was found in 32 Ghanaian subjects (31 heterozygotes, one homozygote) (allele frequency 7.6%; 95% CI: 5–10%). The TPMT*2, TPMT*3A and TPMT*3B alleles were not detected in any of the Ghanaian individuals (Table 1). In the British subjects, two were heterozygous for the TPMT*2 mutation (0.5%; 95% CI: 0–1.2%), while 16 were heterozygous and one homozygous for TPMT*3A (4.5%; 95% CI: 2.5–6.5%). None of the British had the TPMT*3B allele (Tables 1 and 2). One British subject was heterozygous for TPMT*3C (0.3%; 95% CI: 0–0.7%). Neither total mutant genotype nor allele frequency were significantly different between the two populations (P = 0.33 and P = 0.14, respectively). However, the frequency of TPMT*3C alleles was significantly different between the two populations (0.3% versus 7.6%; P = 0.005).

Among the 217 African subjects, 130 were from the three largest Ghanaian tribes (Ga, n = 53; Fanti, n = 43; Ewe, n = 34). However, there was no significant difference in allele frequency among these subjects (Ga wild-type = 86.8%, mutant = 13.2%; Fanti wild-type = 88.4%, mutant = 11.6%; Ewe wild-type = 94.1%, mutant = 5.9%; P = 0.30). Although this study was not designed to determine differences in allele frequency between the different Ghanaian tribes, analysis of the data suggests higher mutant allele frequencies in the Ga and Fanti tribes compared with the Ewe group. The differences, however, were not statistically significant (P = 0.22 and 0.12 respectively), and more studies are required as this may have important therapeutic implications.

**DISCUSSION**

TPMT*3C accounted for 100% of the mutant alleles observed in the African population. This contrasts with the British Caucasian subjects where 5.7% of variant alleles were TPMT*3C. The TPMT*3A allele was not detected in the Ghanaians studied, but accounted for 84.9% of variant alleles in the Caucasian subjects. Therefore, mutation at nucleotide 719 was common in both populations, but occurred most often in the presence of a simultaneous mutation at nucleotide 460 in the Caucasian subjects. Yates et al. suggested that TPMT*3C may be more prevalent in black subjects than white subjects, in that four of nine African Americans with a heterozygous phenotype had the TPMT*3C allele (18). In that study, the TPMT*3C allele was associated with loss of RBC TPMT activity, and subsequently was shown to be associated with loss of immunodetectable TPMT protein in RBC of humans inheriting this allele (18,21). More extensive analysis in African American subjects with an intermediate or low RBC TPMT activity phenotype found TPMT*3C in 52.2% of variant alleles, with the remaining alleles being TPMT*3A, TPMT*2 and TPMT*8 (22). The presence of TPMT*3A alleles in the African American population is consistent with the genetic mixing which has been identified through historical and molecular analysis (23,24).

Together, our data and those of Hon et al. (22) provide complementary evidence that the pattern of variant TPMT alleles differs significantly between ethnic groups. The TPMT*2 allele (G238C) accounted for 9.4% of the mutant alleles in the British population. However, this allele was not found in any of the Ghanaian subjects. This is consistent with a recent study comparing the same group of Caucasians with Chinese and Southwest Asian subjects, in which the TPMT*2 allele was unique to the Caucasians (20). This suggests that TPMT*2 is either very rare in non-Caucasian populations or is specific to Caucasians. The TPMT*3B (G460A alone) was not detected in any of the samples from the two populations. Additional TPMT mutant alleles recently have been identified, in a Korean subject (TPMT*6), a European Caucasian (TPMT*7) and an African American (TPMT*8) (19,22,25). These alleles appear to be relatively rare in Caucasian subjects and their contribution to variant alleles in other ethnic groups has yet to be defined (19,22).

**Table 1. Allele frequencies in Caucasian and Ghanaian subjects**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Caucasian % of allele (95% CI)</th>
<th>N</th>
<th>Ghanaian % of allele (95% CI)</th>
<th>N</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of alleles</td>
<td>–</td>
<td>398</td>
<td>–</td>
<td>434</td>
<td>–</td>
</tr>
<tr>
<td>Wild-type</td>
<td>94.7 (92.5–96.9)</td>
<td>377</td>
<td>92.4 (89.9–94.9)</td>
<td>401</td>
<td>–</td>
</tr>
<tr>
<td>TPMT*2</td>
<td>0.5 (0–1.8)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>4.5 (3.5–6.5)</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>0.3 (0–1.4)</td>
<td>1</td>
<td>7.6 (5–10)</td>
<td>33</td>
<td>0.005</td>
</tr>
<tr>
<td>Total mutant alleles</td>
<td>5.3 (3.1–7.5)</td>
<td>21</td>
<td>7.6 (5–10)</td>
<td>33</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*95% confidence interval for each proportion (%).

**Table 2. Genotype frequencies in Caucasian and Ghanaian subjects**

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>W/W (95% CI)</th>
<th>W/M (95% CI)</th>
<th>M/M (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>199</td>
<td>89.9 (87.8–92.0)</td>
<td>9.6 (5.5–13.6)</td>
<td>0.5 (0–1.5)</td>
</tr>
<tr>
<td>Ghanaian</td>
<td>217</td>
<td>85.3 (80.5–90.0)</td>
<td>14.3 (9.6–18.9)</td>
<td>0.5 (0–1.4)</td>
</tr>
</tbody>
</table>

*Frequencies are expressed as a percentage of all subjects. N: actual number of subjects; W/W, homozygous wild-type; W/M, heterozygote; M/M, homozygous mutant at nucleotides 238, 460 or 719. *95% confidence interval (%).
Phylogenetic analysis estimates the divergence of Africans and non-Africans to be at least 100 000 years ago (24). From gene evolution studies, it is considered that the most common allele in all populations is usually the ancestral allele. Mutation and recombination then give rise to the other genotypes. Genotype analysis in this study suggests that the A719G mutation may be the ancestral TPMT mutant allele, as it was present in both Caucasian and African subjects and has been described in Southwest Asian and Chinese populations (20). This further indicates that the G460A allele was then acquired and added to form TPMT*3A. Since the TPMT*2 allele appears to be confined to Caucasians, it may be a more recent allele of this polymorphic enzyme.

MATERIALS AND METHODS

Study population

British subjects (n = 199; 100 female, 99 male; mean age 37.7 years) were healthy, unrelated blood donors from Aberdeen and Glasgow, UK, as previously described (21). Ghanaian subjects (n = 217; 105 female, 112 male; mean age 25.7 years) were healthy unrelated blood donors from Accra, Ghana and included subjects from 26 different Ghanaian tribes. Ethical approval for this study was obtained from the West of Glasgow Hospitals Ethical Committee, the Joint Ethical Committee of the Gramian Health Board and the University of Aberdeen, and the University of Ghana Medical School Ethical and Protocol Review Committee. Written informed consent was obtained from all subjects.

Molecular studies

DNA was extracted from 5–10 ml of whole blood using a sodium perchlorate–chloroform extraction method (Nucleon, Coatbridge, UK). The genotyping of each subject at the G238C (TPMT*2), G460A (TPMT*3B) and A719G (TPMT*3C) loci was performed using previously described PCR-based assays, with minor modifications (18,20). The TPMT*3A allele contains mutations at both nucleotides 460 and 719 (18).

An allele-specific PCR was used to analyse the G238C mutation (18,20). DNA was amplified in buffer A (Invitrogen, San Diego, CA) with 0.3 µM primers. Forward primers P2W (5′-GTA TTA TAT TAT CAC GGA GGT TTG-3′) or P2M (5′-GTA TTA TAT TAT CAC GGA GGT TTC-3′) were used with reverse primer P2C (5′-TAA ATA GGA ACC ATC GGA CAC-3′) in wild-type-specific and mutant-specific reactions, respectively. The PCR products (254 bp) were analysed on a 2.5% agarose gel (Fig. 1).

The PCR–restriction fragment length polymorphism (RFLP) for the detection of G460A point mutations used a new set of primers different from that previously described (18,20). A PCR assay using 0.24 µM primer P460Fb (5′-AGG CAG CTA GGG AAA AAG AAA GGT G-3′) identical to nucleotides 756–780 of intron 6 and 0.25 µM primer P460Rb (5′-CAA GCC TTA TAG CCT TAC ACC GCA G-3′) reverse complement of nucleotides 1425–1449 of intron 7 was performed with buffer L (Invitrogen). This was followed by digestion of a 694 bp PCR product with restriction enzyme MwoI (New England Biolabs, Hertfordshire, UK) for 1 h at 60°C. Digested products were separated on a 2.5% agarose gel. MwoI digestion of wild-type DNA yields fragments of 443 and 251 bp. The G460A mutation destroys the restriction site and MwoI digestion yields an uncleaved fragment of 694 bp (Fig. 1). Automated sequencing of the PCR fragment confirmed that the expected sequence of TPMT exon 7 was amplified from genomic DNA with these new primers.

The PCR–RFLP used for detection of A719G point mutations used a new forward primer different from that previously described (18,20). For the A719G mutation, the PCR assay was performed using 0.27 µM each of primers P719Fb (5′-GAG ACA GAG TTT CAC CAT CTT GG-3′) identical to nucleotides 401–423 in intron 9 and P719R (5′-CAG GCT TTA GCA TAA TTT TCA ATT CCT C-3′) reverse complement of nucleotides 746–773 in exon 10 and buffer I (Invitrogen). The PCR product was digested with restriction enzyme AccI (New England Biolabs) for 1 h at 37°C, and separated on a 2.5% agarose gel. The A719G mutation introduces an AccI restriction site in the amplified fragment (373 bp), yielding fragments of 283 and 90 bp (Fig. 1). Automated sequencing of the PCR products confirmed specific amplification of the region in exon 10 of the TPMT gene defined by P719Fb and P719R.

Statistical analysis

The difference in allele or genotype frequency between the British and Ghanaian populations and between the Ga, Fanti and Ewe tribes was determined using a χ² test or Fisher’s exact test.

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REFERENCES


