

Positive TPMT Genotype-Phenotype Correlation Underscores Importance of TPMT Genotyping for Personalized Thiopurine Dosing

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ABSTRACT

This study explored TPMT genotype-phenotype correlation in a group of acute lymphoblastic leukemia (ALL) patients to investigate the potential of TPMT genotyping for personalized thiopurine dosing. Genotyping for G238C (TPMT*2), G460A (TPMT*3B) and A719G (TPMT*3C) loci was determined in 89 subjects via PCR, while TPMT activity was measured using HPLC. TPMT*3C was the only mutant allele detected in 4 heterozygous carriers. These patients had significantly lower (23.0 nmol/g Hb/h) TPMT activity compared to wildtype patients (51.0 nmol/g Hb/h) ($p = 0.003$). Positive correlation between TPMT genotype and phenotype projects the possibility of using TPMT genotyping as a guide prior thiopurine drug administration.

INTRODUCTION

The Thiopurine S-methyltransferase (TPMT) gene codes for a cytosolic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulphhydryl compounds, including thiopurines such as 6-mercaptopurine (6-MP) [1]. This gene is subjected to genetic polymorphisms, where various mutant alleles (TPMT*2 – TPMT*18) has been reported [2]. Different populations have been reported to have different allelic frequencies of TPMT mutant alleles [3-6].

It has been reported that the TPMT enzyme exhibits autosomal codominant genetic polymorphism, and individuals carrying mutant alleles will result in TPMT deficiency [7]. These patients have been reported to be at risk of developing severe toxicity if treated with standard doses of thiopurines.

The aim of this study was to investigate the relationship between TPMT genotype and its corresponding phenotype (enzyme activity) in a group of paediatric acute lymphoblastic leukemia (ALL) patients undergoing treatment at a university teaching hospital in Malaysia. The results would determine the potential of utilizing TPMT genotyping for safe thiopurine dosing.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PROCESSING

Ethics approval for this study was given by the Universiti Kebangsaan Malaysia Medical Ethics Committee. Sample collection was carried out from July 2008 until December 2009. Informed consent was obtained from the guardian of every pediatric patient. Patients who have reached adulthood signed the consent form themselves. Blood (3 – 5 ml) from each patient was collected in an EDTA tube and kept at 4°C until processed. Genomic DNA was extracted from each blood sample using QIAamp DNA Blood Kit (QIAGEN Inc., California, USA), according to manufacturer's instructions. Each blood sample was also centrifuged to obtain packed RBCs for TPMT enzyme activity determination.

TPMT GENOTYPING

Genotyping of each DNA sample for the G238C (TPMT*2), G460A (TPMT*3B) and A719G (TPMT*3C) loci was carried out as described previously, using PCR-based techniques with some modifications [3, 8]. The prevalence of

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TPMT*3A, which contains mutations at both nucleotides 460 and 719 was also investigated.

All PCR amplifications were carried out using the Promega GoTaq Master Mixes (Promega, Madison, USA). Both allele-specific PCR and PCR-RFLP products were analysed by electrophoresis using a 1.2% agarose gel.

TPMT PHENOTYPING

Erythrocyte TPMT activity was measured by a HPLC assay with minor modifications as described previously on the Agilent 1200 series (California, United States) liquid chromatography system [9]. An Eclipse XDB C18 column (4.6 × 150 mm) with a 5-µm particle size (Agilent, California, USA) was used. The ChemStation software system controlled all equipment and carried out the data processing.

The hemoglobin levels were determined by quantitative determination of cyanmethemoglobin in the blood. Cyanmethemoglobin standard and reagent were obtained from Eagle Diagnostic (St Louis, USA). Protocol was in accordance with the manufacturer's instructions. Absorbance measurements were recorded at 540 nm.

RESULTS

A total of 89 patients, aged 2 to 25 years old who were undergoing treatment or under follow-up for ALL in the UKM Medical Centre, Kuala Lumpur, Malaysia were included into the study. All patients were in remission. The ratio of male: female was 1:1. Of the 89 patients, 73% were Malays, 17% were Chinese, 9% were Indians and 1% from other ethnic group.

Genotyping for four mutant alleles of the TPMT gene, i.e.: TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C, was carried out. The results revealed that TPMT*3C was the only mutant allele detected in these patients giving an allelic frequency of 0.02. All TPMT*3C alleles detected were heterozygotes. The mutant alleles of TPMT*2, TPMT*3A and TPMT*3B were not detected.

For TPMT phenotyping, all patients who did not have any TPMT mutations had median TPMT activity of 51.0 nmol/g Hb/h (range 18.0 – 575.0 nmol/g Hb/h); while median TPMT activity of TPMT*3C heterozygous patients was 23.0 nmol/g Hb/h (range 17.0 – 36.0 nmol/g Hb/h). The TPMT activity was significantly lower in patients with heterozygous TPMT* 3C compared to those who did not have any TPMT mutations ($p = 0.003$). Two patients of Chinese ethnicity harbouring heterozygous TPMT* 3C had TPMT activity of 17 and 18 nmol/g Hb/h, respectively. However, 2 other patients of Malay origin carrying this mutant allele had slightly higher TPMT activity measurements of 28 and 36 nmol/g Hb/h, respectively. Nevertheless, when all data was analyzed together, ethnicity ($p = 0.659$) and gender ($p = 0.990$) were not found to have an effect on TPMT activity.

DISCUSSION

This study was done to determine the allelic frequency and the phenotype presentation of each corresponding genotype of the TPMT gene in a population of ALL pediatric patients in UKMMC, Malaysia. To the best of our knowledge, there are minimal reports available on the TPMT genetic profile of South East Asians. These include populations of Malay, Chinese and Indian origin in this region.

In our investigation, 95.5% of our study population was homozygous wildtype for TPMT. The only variant detected was TPMT*3C. This finding was in line with the results from Singapore, Thailand and also countries in East Asia where TPMT*3C is also the most prevalent variant found in these populations [4-6]. In contrast, Caucasian populations tend to have TPMT*3A as the most frequent variant allele, where the variant allele carrier frequency was 4.5%, 5.7%, 3.9% and 3.4% for the British, French, Italian and Norwegian populations, respectively [10-12]. In comparison, the heterozygote carrier frequency in our sample population was 4.5%.

Mutations in the TPMT gene have been reported to cause malformation on its enzyme isoforms, which would then affect its efficacy to inactivate thiopurine drugs [13]. Several correlation studies found that TPMT genotype-phenotype was highly correlated in subjects having either high or low TPMT activity, respectively, though subjects with intermediate TPMT activity show a lower concordance in the correlation [12, 14, 15]. This phenomenon is also present in our study, where 2 heterozygous patients had slightly higher TPMT activity measurements, while another 2 were found to have lower TPMT activity. Nevertheless, all together, patients who are heterozygous mutant carriers had significantly lower TPMT activity compared to wildtype patients.

For heterozygous patients, factors other than TPMT genotype might have resulted in them having low TPMT genotype-phenotype correlation; some of the reported factors include S-adenosylmethionine (SAM) concentration and genotypes of genes involved in folate metabolism such as MTHFR (methylenetetrahydrofolate reductase) and TYMS (thymidylate synthase) [13]. Karas-Kuzelicki and Mlinaric-Rascan supported the use of a metabolic network approach rather than only TPMT genotyping to evaluate TPMT activity in patients prior administration of thiopurine drugs [13]. However, the practicality of using a metabolic approach to deduce TPMT activity needs to be examined, especially in study populations such as ours where mutation frequencies are low; all the more so when cost – effective analyses of TPMT genotyping or phenotyping prior thiopurine dosing had produced varied conclusions [16-18].

As a conclusion, even though there are contrasting reports about the importance of TPMT genotyping [19-21], TPMT genotyping is still considered important prior

administration of thiopurine drugs, as it could detect homozygous TPMT mutants and prevent the corresponding patient from thiopurine toxicity. The correlation between TPMT genotype-phenotype projects the possibility of utilizing TPMT genotyping results as a guide before the administration of thiopurine drugs, so that safety and efficacy of these drugs could be improved and enhanced.

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