GENETIC POLYMORPHISMS AND ENZYME ACTIVITY OF THIOPURINE S-METHYLTRANSFERASE GENE (TPMT): DEVELOPMENT OF MOLECULAR DIAGNOSTICS AND A BIOASSAY TO REDUCE TOXICITY AND ENHANCE THERAPEUTIC EFFICACY IN ACUTE LYMPHOBLASTIC LEUKAEMIA

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ABSTRACT

Thiopurine drugs such as 6-mercaptopurine (6-MP) used in the treatment of leukemia are methylated by the Thiopurine S-methyltransferace (TPMT) enzyme which exhibits autosomal codominant genetic polymorphism. It has been reported that individuals carrying mutant TPMT alleles will result in TPMT deficiency. Decreased level of TPMT activity is always associated with severe hematopoietic toxicity after administration of standard doses of thiopurine drugs. As its activity is polymorphic and leading to inter-individual variations in drug metabolism, TPMT genotyping is particularly important to determine population at risk of TPMT deficiency.

To date, TPMT*2, 3A, 3B and 3C are the more common alleles that have been reported. TPMT*2 contains a G → C substitution at nucleotide 238 on exon V, while TPMT*3B carries a G460A mutation on exon VII. TPMT*3C contains a A → G substitution at nucleotide 719 on exon X. TPMT*3A contains both G460A and A719G nucleotide transition mutations.

Different populations have been reported to have different allelic frequencies of TPMT mutant alleles. A total of 10.1% of British subjects were detected to have variant alleles consisting of TPMT*2, TPMT*3A and TPMT*3C, while TPMT*3C is more common among African Ghanaian populations. German-causasians have a distribution of 4.4% TPMT*3A, 0.4% of TPMT*3C and 0.2% of TPMT*2 in their population. In Asian populations of Koreans, Japanese, Chinese, Thai and also in multiethnic Singaporeans, TPMT*3C was the most common mutant allele detected.

In this study, the TPMT genetic profile of acute lymphocytic leukemia (ALL) patients who are in remission follow up from July 2008 until September 2009 at a pediatric clinic of a Malaysian university teaching hospital was investigated and their corresponding TPMT activity determined. Informed consent was obtained from 80 patients and blood (3 – 5 ml) was then collected. Blood components were separated for genomic DNA and packed red blood cell.

Genotyping of each patient's DNA sample for the G238C (TPMT*2), G460A (TPMT*3B) and A719G (TPMT*3C) loci was carried out as follows: For TPMT*2, an allele specific-PCR was carried out to detect the G238C mutation. Forward primers P2W (5'-GTA TGA TTT TAT GCA GGT TTG-3') or P2M (5'-GTA TGA TTT TAT GCA GGT TTG-3') were used with reverse primer P2C (5'-TAA ATA GGA ACC ATC GGA CAC-3') to respectively amplify wild-type-specific or mutant-specific TPMT*2 alleles. For TPMT*3B and TPMT*3C, PCR-restriction fragment length polymorphism (PCR-RFLP) was used to detect these mutant alleles. For TPMT*3B, DNA was amplified using primers P3F (5'-AGG CTC CTA AAA CCA TGA GGG-3') and P3R (5'-GTA TAC TAA AAA ATT AAG ACA GC-3') and the resulting PCR product was digested with MwoI at 60ºC for 2 hours. Genotyping for TPMT*3C was carried out by generating a PCR product using primers P719Fb (5'-GAG ACA GAG TTT CAC CAT CTT GG-3') and P719R (5'-CAG GCT TTA GCA TAA TTT TCA ATT CCT C-3') and digesting the product with AccI at 37ºC for 2 hours. 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.

For TPMT phenotyping, erythrocyte TPMT activity was measured by a HPLC assay. The principle of the test is based on the catalysation of S-methylation of 6-MP by TPMT, resulting in the production of 6-MMP. HPLC analysis was performed on Agilent 1200 series (California, United States) liquid chromatography system equipped with a diode array detector and auto injection valve. An Eclipse XDB C18 column (4.6 X 150 mm) with a 5-μm particle size (Agilent, California, United States) was used. ChemStation software system controlled all equipment and carried out the data processing. Elution was performed isocratically with a mixture of 7.0 % acetonitrile in 0.01 M sodium phosphate buffer adjusted to pH 2.7 with phosphoric acid (85%). The flow rate was 0.5 ml/min and the injection volume was 50 μl. Chromatograms were acquired at 290 nm. Retention time for 6-MMP was detected at minute of 12.8.

In this study, patients recruited were age between 2 – 25 years old with the male to female ratio of 1:1. Of 80 patients recruited, 72.5% of the patients were Malay, 20% were Chinese, and 7.5% Indians. We found that the TPMT*3C was the only mutant allele present in our study population. Four out of the 80 patients (2 Malays and 2 Chinese) were found to be heterogeneous carriers of this allele, giving an overall allelic frequency of 5%. This finding is in line with the results from Singapore, Thailand and also countries in East Asia (China, Japan, Korea), where TPMT*3C is the most prevalent variant found. The mutant alleles of TPMT*2, TPMT*3A and TPMT*3B were not detected in our study population.

TPMT activity was then determined in 80 previously genotyped patients. TPMT activity was expressed as the amount of TPMT enzyme needed for the formation of 1 nmol of 6-MMP in 1 gram of hemoglobin in one hour of time. For patients who did not have any TPMT mutations (n=76), TPMT activity was in the range of 21 – 109 nmol/g Hb/h, while TPMT activity of 2 patients habouring heterozygous TPMT* 3C allele were in the low range of 17 and 18 nmol/g Hb/h, respectively. However, the other 2 patients carrying this mutant allele had normal TPMT activity of 28 and 36 nmol/g Hb/h, respectively. This is in line with reports that heterozygous TPMT mutant alleles still retain TPMT activity, though the level may be lower than that of wild type populations. There are reports that question the importance of TPMT genotyping, as heterozygous individuals do not show a significantly different phenotypic profile from those who

are wild type, as shown also in the results of our study. Nevertheless, as homozygous mutants will have zero TPMT activity, we suggest that TPMT genotyping is still important before the administration of thiopurine drugs, as it could detect homozygous TPMT mutants and predict the corresponding patient who is at risk of developing thiopurine toxicity.

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