JPR INFO .com
Journal of Pharma Research

Journal of Pharma Research
Available online through
www.jprinfo.com

Review Article
ISSN: 2319-5622

Impact of Thiopurine S-Methyl Transferase gene (TPMT) Polymorphism on Thiopurine drug Metabolism: A way to Individualized therapy

Singh Balbir*, Kumar Tirath, Godiyal Aditya
**Department of Pharmaceutical sciences Kumaun University, Campus Bhimtal, Nainital.*

Received on: 23-04-2013; Revised and Accepted on: 16-05-2013

ABSTRACT

Individualization of drug regimens is today's need because drug metabolism vary significantly among individuals i.e. patients given similar dosages can have widely divergent results as seen in case of thiopurine drugs due to genetic polymorphism of TPMT gene(s) responsible for the metabolism of these drugs. The genetic polymorphism of thiopurine methyltransferase (TPMT) gene is one of the most mature examples of pharmacogenetics, which could be helpful in clinical diagnostic for individualization of thiopurine drug therapy (i.e. Azathioprine, Mercaptopurine, and Thioguanine). Determining the molecular mechanisms and biochemical consequences of TPMT deficiency illustrate the potential of pharmacogenomics to optimize cancer therapy by avoiding toxic side effects in genetically distinct subgroups of patients. It concludes that TPMT pretesting is necessary.

Key Words: *TPMT Gene, Single nucleotide polymorphisms (SNPs), Inter-patient variability, Thiopurine drugs and Pharmacogenetics.*

INTRODUCTION

Interest in thiopurine S-methyltransferase (TPMT) emerged initially because of the important role this enzyme plays in metabolic transformation of the widely used anticancer and anti-inflammatory drugs mercaptopurine (MP), thioguanine (TG), and Azathiopurine (AZA) [1]. Introduction of thiopurine, along with other antileukemic agents, increased the survival rates of children with acute lymphoblastic leukemia (ALL), from less than 5% in the early 1940s to 80% today. It is estimated that approximately 60,000 patients per year commence thiopurine drug treatment in the UK. Although thiopurine drugs are widely used, gastrointestinal intolerance, pancreatitis, hypersensitivity and myelosuppression are observed in up to 30% of patients. These ADR often result in the withdrawal of treatment [2-5]. In some instances the unexpected reaction to therapy can be fatal, for example in cases of severe myelosuppression. If this occurs, all cells produced in the bone marrow become suppressed, causes reduction in leucocytes, leaves the body susceptible to infection, while reduced platelets cause clotting abnormalities and red cell anaemia.

Current concepts of drug therapy is treating large patient populations as groups; without considering the potential of individual genetically based differences in drug response [6] i.e. most medications exhibit wide inter-patient variability in their efficacy and toxicity. Clinical pharmacogenomics is the study which tells us how genetic basis affects variability in drug response [7-9]. Also, the advent of modern highly efficient and specific genomic technologies enables the search for relevant genes and their variants in the genome, and these new technologies have essentially spawned a new discipline in pharmacogenomics using genome-wide approaches [10]. Moreover, pharmacogenomics analysis can identify disease susceptibility genes representing potential new drug targets. Numerous genes, in particular those encoding drug metabolizing enzymes, drug transporters and drug targets, have been identified to play a role in drug response and toxicity [10]. All of this will lead to novel approaches in drug discovery, individualized dosing of medications, and new insights into disease susceptibility and prevention [11].

Variability in Drug Metabolism:

Metabolism is an important process to end the effects of any drug, but sometime can be essential for the activity of a drug i.e. in case of prodrugs. The large number of active combination chemotherapy regimens for most cancers has led to the need for better information to guide the 'standard' treatment for each patient. In fact, it is estimated that properly prescribed medication causes 2 million Americans to get sick and result in 100000 deaths each year because of adverse drug reactions [12-15]. Clearly the current regimen i.e. 'one dose fits all' for chemotherapy treatment is not ideal for patients and is not cost effective for the health service.

TPMT is a very important cytoplasmic transmethylase enzyme that preferentially catalyses S- mehtythylation of aromatic and heterocyclic sulfhydryl compounds like thiopurine drugs activating or detoxifying the anti-inflammatory or immunosuppressant AZA and the anticancer agents, MP and TG [16] via S- adenosyl-L-Methionine as the S-methyl donor and s- adenosyl-L-homocysteine as by product. (**Fig. 1**) [17-18].

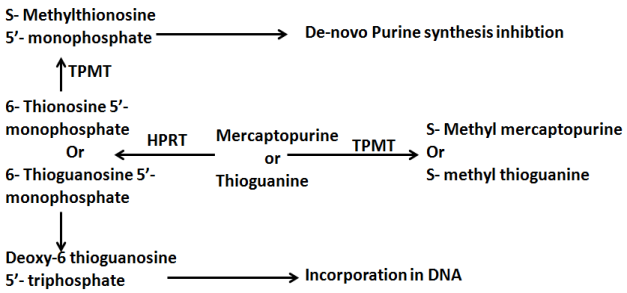

Fig. 1: Simplified schematic representation of thiopurine metabolism within cells, a step in metabolic activation of mercaptopurine and thioguanines

Charles Remy was the first to describe the specific thiopurine S-methyltransferase activity in several species [19-20] and the partially purified protein was subsequently assayed and characterized in human tissue [21-22]. The first inactive allele of TPMT in humans was identified in 1995 [23]. After that various studies demonstrated ethnic difference in mean TPMT activity and observed evidence of polymorphic distribution of TPMT activity in each of the large racial groups studied to date [24].

TPMT Gene Location, Structure and Polymorphisms:

TPMT gene is located on chromosome 6, is about 34 kilobases in length and has 8 exon [25] later this was modified that TPMT chromosomal gene is 27 kB long [26-27] maps to chromosome 6p22.3 and has 10 exons [25-26]. The TPMT encodes 245-amino acid protein. Analysis of the 5-promoter region revealed a 71% GC content, more detailed analysis identified stimulating protein 1 (Sp1) as an

***Corresponding author:**
Balbir Singh
Department of Pharmaceutical sciences, Kumaun University, Campus Bhimtal, Nainital, India.
Telephone: +91 9654135631, +91 9418316731.
**E-Mail: balbirpharmacologist@gmail.com*

important *trans*-activator essential for constitutive activity in cell culture [28] and the presence of a variable number tandem repeat (VNTR) region which may affect levels of expression [29-30].

The wild type allele is designated as TPMT*1 and to date, at least 26 variant alleles of the TPMT gene have been identified [31-35]. Four alleles (TPMT*2, *3A, *3B and *3C) account for 80-95% of the inherited TPMT deficiency and have been biochemically characterized [32-36]. The diagram below (**Fig. 2**) [37] is representing the TPMT gene, showing the exons as boxes. The first 'wild type' is the most common version. In other versions, the TPMT gene is associated with low enzyme activity TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C and TPMT*8. In TPMT *3B there is one single nucleotide change in exon 7 from G to A at position 460 causing substitution of threonine for alanine at 154 position. TPMT*3A has two single nucleotide polymorphisms (SNPs), or changes in single DNA nucleotide bases or transitions (in exon 7 from 'G' to 'A' at position 460 and in exon 10 from 'A' to 'G' at position 719), leading to substitution of threonine for alanine at position 154 and cysteine for tyrosine at position 240, and TPMT*3C has one single nucleotide polymorphism, or changes in single DNA nucleotide base or transition (from 'A' to 'G' at position 719 leading to substitution of cystine for tyrosine at position 240 in the enzyme. This in turn, affects the enzyme's function.

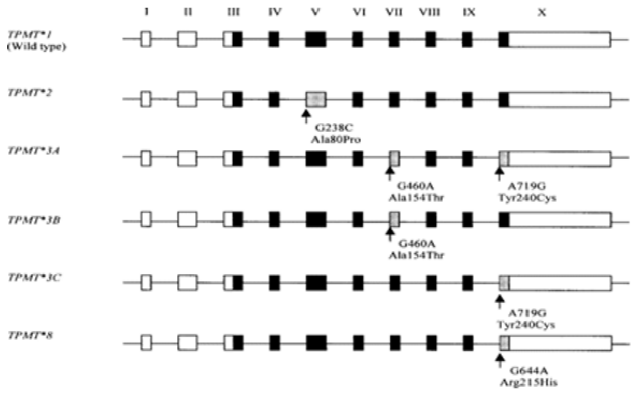

Fig. 2: Alleles varients at human TPMT locus. Boxes depict the exons in human TPMT gene. White boxes are untranslated exonic regions and black boxes represents the exons in ORF, gray boxes represents exons that contains mutations that result in changes to amino acids

There was an enhanced rate of proteolysis of mutant TPMT proteins encoded by TPMT*2 and TPMT*3A alleles, with degradation half lives of 15 min for both mutant proteins compared with 18 hr for the wild type protein [18]. A number of rare mutant TPMT alleles (TPMT*3D, *4, *5, *6, *7, *8, *10, *11, *12, *13, *14, *15, *16, and *19) have been identified [38-41]. TPMT*4 has a GA transition at the intron 9-exon 10 junction, which disrupts the final nucleotide of the intron at the 3' acceptor splice site sequence [40]. TPMT*5 was identified as a T146C transition in heterozygous individual and has intermediate TPMT activity. In TPMT*6 mutation results in a Leu, Ser amino acid substitution at codon 49, [40] the A539T transversion in exon 8 results in a TyrPhe substitution at codon180 in TPMT*7, results in intermediate activity [42]. In TPMT*8 there is G to A substitution at position 644 resulting in exchange of R215H at exon 10, this also resulted in intermediate enzyme activity. A few new missense mutations, TPMT*10 (G430C, codon 144 GlyArg), TPMT*12 (C374T, codon 125 SerLeu) and TPMT*13 (A83T, codon 28 GluVal), were identified in the 10 exons of the TPMT gene when DNA samples from four leucopenic patients were screened by PCR analysis [43]. TPMT 11 is a missense in mutation (G395A) in exon 6, resulting in an amino acid exchange C132Y with reduced enzyme activity [44] TPMT*14 and TPMTP*15 were recently reported [43]. TPMT*14 contained an AG transition in the start codon (exon 3, MetVal), whereas TPMT*15 had a GA transition in the acceptor splice site in intron 7/exon 8 (IVS7 -1GA) [45]. Both TPMT*14 and TPMTP*15 resulted in the loss of enzyme activity. Recently, two novel missense mutations, TPMT*16 (G488A, Arg163His) and TPMT*19 (A365C, codon 122 LysThr) were identified in a Caucasian and a Moroccan patient, respectively [46]. The Lys122Thr exchange did not significantly affect the intrinsic clearance value (Vmax/Km) of the variant enzyme, whereas the Arg163His substitution significantly decreased the intrinsic clearance value by 3-fold. The frequencies and contributions of these alleles to reduced TPMT activity in different ethnic groups have not been defined yet. TPMT*23, substitution occur between C to G at position 500 result substitution of A167G at exon 8, these all results in decreased activity of that enzyme [47].

Polymorphisms in the 5'-flanking promoter region of TPMT gene have also been identified due to a variable number of tandem repeats (VNTR) with three kinds of motifs (A, B, and C) differing by the length of the unit core and nucleotide sequence [48-51]. Each repeat consists of 17 or 18 bp unit and contains a potential binding site for the transcription factor Sp1. The polymorphic tandem repeat within the 5'-flanking promoter region of the TPMT gene appears to participate in the regulation of level of erythrocyte. TPMT activity [30] allele VNTR*6 was found to be consistently associated with decreased levels of TPMT activity in humans [50]. However, those effects are probably small in a quantitative sense [46]. A few recent studies demonstrated that the variable number tandem repeats (VNTR*3 to VNTR*9) had no significant impact on enzyme activity in British Asians and Caucasians [52-65]. It has been shown that there might be a negative correlation between the variable number of tandem repeats within the 5'-flanking region of the TPMT gene and the level of TPMT activity. TPMT VNTR length varied from three to nine repeats (*V3 to *V9), but the most commonly occurring were *V4 and *V5. The lowest levels of TMPT activity were found with genotypes that included an allele with more than 5 repeat elements. The *V4/*V5 were associated with higher activity levels than *V4/*V4 and *V5/*V5 [30]. In another study, the *V6 was found to be consistently associated with decreased levels of TPMT activity [49]. However, the effect of VNTR length may not be as drastic as TPMT deficiency caused by genetic mutations. The mechanistic effect of VNTR on TPMT activity remains to be elucidated.

The most prevalent TPMT mutant allele in the Caucasian and Latin American population is TPMT*3A [37, 55] whereas TPMT*3C is predominant in Chinese, Egyptians, and African-Americans [39, 55]. In Caucasians, the TPMT activity in erythrocytes shows a trimodal distribution: 89–94% of individuals have high activity, 6– 11% have intermediate activity due to heterozygosity of TPMT variants, and 0.3% have low or undetectable enzyme Activity. 1 of every 300 subjects was homozygous for the trait of low level of RBC TPMT activity as in figure 3 [56] unlike in most Caucasians, TPMT*3C is a common mutant allele in Kazaks, whereas TPMT*3A is a rare mutant allele. Further studies are needed to explore the clinical impact of these TPMT mutants to thiopurine therapy in Kazak patients [57].

Techniques used for TPMT Genotyping Include:

A. Restriction Fragment Length Polymorphism (RFLP) - in which DNA molecule is cleaved at a specific nucleotide sequences, using restriction endonucleases and the patterns derived is analyzed [36].

B. Amplification Refractory Mutation System (ARMS) - it takes advantage the fact that the 3'-OH end of a primer bound to template DNA is required for the polymerase enzyme to synthesize a new copy of DNA. Any mismatch leads to PCR failure. Consequently, any point mutation can be detected by synthesizing two primers specific to the wild type or mutant DNA. Several mutations can be screened for at once, using multiplex reactions containing more than one set of primers [36].

C. Single Strand Conformational Polymorphism (SSCP) - this is a method to screen the exons of the TPMT gene for mutations using electrophoresis. This method, although relatively simple and low cost, is time consuming and is not suited for routine use in a clinical setting.

D. Denaturing High-Performance Liquid Chromatography (DHPLC) - identifies mutations by detecting sequence variation in re-annealed DNA strands (hetero-duplexes). This method efficiently detects single nucleotide and insertion/deletion variation in crude PCR products directly without DNA sequencing.

E. DNA Microchip technology - in this fluorescently labeled amplified DNA is hybridized with oligo-nucleotide DNA probes immobilized in gel pads on a biochip. This specially designed costly biochip can recognize six point mutations in the TPMT gene and seven corresponding alleles associated with TPMT deficiency.

F. Mass spectrometry - highly multiplexed genotyping of TPMT variants has been performed using Matrix-Assisted Laser Desorption/Ionization - Time of Flight analysis (MALDI -TOF). A matrix is used to protect the molecules during ionisation, transforming the energy from the laser into excitation energy. The time-of-flight analyser separates the ions according to their mass (m) to charge (z) (m/z) ratio by measuring the time it takes for them to travel through a field free region [44].

G. SNaP shot method- a non time consuming, half automated method which enables testing of TPMT SNPs in a multiplex reaction. The method is based on a dideoxy single - base (ddNTP) extension of primers complementary to the sequences of three most common TPMT polymorphisms. The addition of

one of four ddNTPs lebeled with different fluorescent dyes at the position of the SNP is followed by electrophoresis and analysis of data [58].

Clinical Implications of TPMT Polymorphism and Thiopurine Drugs:

Anti-metabolites are one of the most common chemotherapeutic agents used in cancer therapy. They are structural analogues of important intermediates in the DNA and RNA bio-synthetics. Anti-metabolites act by inhibiting synthesis of nucleic acids and their building units or are incorporated into the DNA or RNA, resulting in impaired synthesis. Three Anti-metabolites among thiopurine drugs are in common usage, and have been used for over years. 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) are part of many cytotoxic drug regimens for the treatment of acute leukemia, and azathiopurine(AZA) is a widely used immunosuppressant given to patients with autoimmune conditions, inflammatory bowel disease (Crohn's) or following kidney TPMT or heart transplantation [33]. Thiopurine drugs are effective in inducing remission in 50% to 60% of patients with inflammatory bowel disease and permit steroid reduction or withdrawal in up to 65% of patients [59].

TPMT catalyses the predominant inactivation pathway of thiopurines; hence, patients who inherit TPMT deficiency accumulate excessive concentrations of the active thioguanine nucleotides (TGN), with standard doses of these medications. Thiopurine methyltransferase (TPMT) polymorphisms are the major determinants of inter-individual differences in the severe haematological toxicity of 6-mercaptopurine. Since there is considerable inter-individual variability in TPMT activity, genotyping of the TPMT gene or phenotypic TPMT enzyme activity measurements are routinely performed prior to thiopurine dosing [60, 29]. Weinshilboum and colleagues were the first to report large inter-individual variations in TPMT activity. Using a radiochemical assay to assess red blood cell (RBC) activity they demonstrated a trimodal distribution with approximately; 90% of individuals inherit high activity, 10% intermediate activity due to heterozygosity, and 0.3% have low or no detectable enzyme activity because they inherit two non-functional TPMT alleles [5, 36]. The differences in TPMT activity result predominantly from single nucleotide polymorphisms (SNPs). TPMT deficiency has also been associated with a higher risk of irradiation-induced brain tumors in patients treated with thiopurines concomitant with radiation therapy [61].

Several studies [62-63] have highlighted the importance of TPMT in thiopurine drug metabolism, because reduced or absent TPMT activity may place patient at higher risk for drug-related toxicity. The various adverse effects include myelosuppression, hepatic toxicity, pancreatic and flu like symptoms. Severe myelosuppression, one of the most serious dose dependent reactions, is believed to be caused by 6-thioguanine nucleotides, the active metabolites [28].

Thiopurine Toxicity proofs in relation to TPMT Polymorphism:

There are various examples which provide or explain thiopurine drug toxicity is significantly related with the TPMT polymorphism:

a) A significant negative correlation between erythrocyte cytotoxic TGNs and TPMT activity among children with acute lymphoblastic leukemia (ALL) treated with 6MP [64-66] Two of 3 adult patients with very high TGN concentrations and 6MP-induced leukopenia had no detectable TPMT activity, presumed to be an inherited deficiency of the enzyme [66]. Children with low concentrations of TGNs had higher TPMT activity and a higher subsequent relapse rate [58]. Lennard *et al* concluded that individuals with inherited low TPMT activity may be at risk for increased TGNs and acute myelosuppression when treated with standard doses of thiopurine drugs. In addition, genetically determined TPMT activity may regulate the cytotoxic effect of 6MP and thus influence outcome of therapy for childhood ALL.

b) 5 patients treated with standard doses of azathioprine which developed acute myelosuppression. Compared to control patients who did not develop myelosuppression, the 5 patients had very low TPMT activities and abnormally high levels of cytotoxic thioguanine nucleotides, consistent with inherited low TPMT activity [67].

c) An 8-year-old girl with acute lymphocytic leukemia who developed severe hematopoietic toxicity with conventional oral doses of 6-mercaptopurine due to severe TPMT deficiency. The level of 6-thioguanine nucleotide in the patient's erythrocytes was seven times the population median value [68].

d) Two unrelated children with ALL taking 6MP who developed profound myelosuppression on 25% of the standard protocol dose. Both were found to have undetectable intracellular TPMT activity and both produced higher cytotoxic drug metabolites at decreased 6MP dosage compared to other patients taking 100% of the dose, concluded that both children had the recessive trait lacking TPMT activity and noted the importance of recognizing such individuals in order to avoid fatal bone marrow failure through inadvertent over dosage [69].

e) Azathiopurine-induced myelosuppression in a heart transplant recipient with TPMT deficiency has been reported [70].

f) A 14-year-old girl who developed severe pancytopenia seven weeks after starting azathioprine for HLA-B27-associated juvenile spondylarthritis. She was found to have toxic levels of 6-thioguanine nucleotides and was TPMT-deficient. Withdrawal of azathioprine allowed recovery 8 weeks later [50].

g) 6 (9%) of 67 patients with autoimmune disease taking AZA were heterozygous for a mutant TPMT allele. Five of the 6 patients discontinued treatment within 1 month because of low leukocyte levels [71]. The authors concluded that TPMT heterozygotes taking AZA are at increased risk for adverse side effects.

Of 23 patients who developed excessive toxicity while taking 6MP or AZA [7] found that 6 were homozygous for TPMT deficiency, 9 were heterozygous, and 8 had normal TPMT activity (homozygous wild type). The 65% frequency of homozygous or heterozygous individuals among these patients was significantly greater than the expected 10% in the general population. Hematologic toxicity occurred in more than 90% of the patients, while hepatotoxicity occurred in 6 (26%) patients. Dosage adjustment in these patients resulted in tolerance of treatment without toxicity. 3 (60%) of 5 patients who developed hematopoietic toxicity from azathioprine had 1 or more TPMT variant alleles [72].

h) Patients developing hematopoietic toxicity with thiopurine therapy were found to have overrepresentation of TPMT variant alleles [7]. In addition; their studies showed that hematopoietic toxicity can occur in TPMT heterozygotes, not just homozygous-deficient patients [7].

i) A 38-year-old Polish man with compound heterozygosity for two variants in the TPMT gene (TPMT*3A and TPMT*3C) underwent kidney transplant due to chronic glomerulo-nephritis, he was treated with AZA as immunosuppressive agents. Two months later he developed severe myelosuppression and AZA was withdrawn. Upon reintroduction of AZA two weeks later, the patient again developed myelosuppression. After final withdrawal of AZA, the kidney was still functioning 10 years after transplant [73].

Table No. 1: Most prevalent TPMT mutant allele in different ethnic populations

Population	TPMT allele	Reference
Chinese	TPMT*3C	[74]
Japanese	TPMT*3C	[75-76]
Taiwanese	TPMT*3C	[75]
South- west Asians	TPMT*3A	[74]
South- East Asians	TPMT*3C	[77]
Kenayans	TPMT*3C	[78]
Ghanaians	TPMT*3C	[79]
American Caucasian	TPMT*2,*3A,*3C	[39]
British Caucasian	TPMT*2,*3A,*3C	[79]
Jordanians	TPMT*3A,*3C	[80]
Iranian	TPMT*2,*3A,*3B	[81]
Israeli	TPMT*3A	[82]
Russians	TPMT*3A	[83]
Turkish	TPMT*3A	[84]
Serbian	TPMT*3A	[85]
Kazaks	TPMT*3C	[57, 86]
Indians	TPMT*3A, *3C	[87]
Polish	TPMT*2,*3A,*3C	[73]
German	TPMT*1,*2,*3A,*3C	[83]
African- Americans	TPMT*3C	[37]

TPMT Monitoring and TPMT Test before Thiopurine Therapy:

There have been many studies in favour of or against the monitoring of thiopurine drug metabolite levels [88-99]. In favouring studies, hepatotoxicity correlated with very high 6-MMP levels. It is not the aim of this review to further discuss the methods of monitoring these metabolites. We would like to stress here that thiopurine drug (AZA/6-MP) metabolite assessment requires expertise, standardized methods and is indicated only in cases with unexplained dose-related side effects in patients where AZA/6-MP use is mandatory for disease remission.

From above proofs and other thiopurine toxicity studies it become important to test TPMT activity or screening for variant TPMT

allele before initiating thiopurine therapy. The drug monograph for azathiopurine approved by the USFDA also recommends pretesting.The evidence base for this recommendation is unclear, particularly the crucial evidence that pretherapy TPMT testing decreases myelotoxicity-specific mortality [100-101]. However enzymatic analysis should identify most patients at risk, with the exception of those with a recent blood transfusion [102] whether genotyping is sufficiently sensitive for routine use in clinical practices is also unclear, because most laboratories identify only the most common variant alleles and miss rare variants.

Utility of TPMT Review:

Usually homozygous TPMT deficient patients screened prior to starting thiopurine drugs are not treated with thiopurine drugs. If treatment has already started and the patient presents with myelotoxicity, this must be managed supportively and thiopurine drug treatment withdrawn. Heterozygous / low TPMT activity patients are often started on a reduced dose, which then may be adjusted depending on the clinical efficacy and toxicity of the treatment. Thiopurine metabolites 6-thioguanine nucleotides (6TGN) and 6-methytlmercaptopurine nucleotides (6MMPN) are increasingly monitored in TPMT heterozygote's to allow titration of the dose or in patients on thiopurine drugs presenting with toxicity symptoms. Withdrawal of the thiopurine drug allows recovery of the myelotoxicity in many patients. In short TPMT study helps:

- ✓ To identify patients who will benefit from a substantial decrease in dosage [38].
- ✓ Pre-therapy identification of at-risk patients allows for drug adjustment, maximizing therapeutic efficacy [68].
- ✓ TPMT status is an early indicator of bone marrow toxicity and dose-related myelosuppression in at-risk patients [65].
- ✓ Approximately 40% of Inflammatory Bowel Disease patients receiving 6-MP/Azathioprine (AZA) therapy either fail to respond or are intolerant [103].
- ✓ In AZA-treated Rheumatoid Arthritis patients, intermediate TPMT activity is indicative of severe side effects, necessitating cessation of therapy [42].
- ✓ In reducing cost of cancer chemotherapy.

Future Perspectives:

Despite significant progress in the study of this important TPMT enzyme, several questions still remain to be answered. There are certain points which may be useful in improving thiopurine-based therapeutic protocols in future:

- ✓ Characterization of new TPMT polymorphisms and their effect on the level of enzyme activity could be a subject of future studies.
- ✓ Elucidation of the spatial structure of TPMT could be helpful in understanding the root mechanism of its action, as well as provide a rational strategy to develop specific inhibitors of TPMT which could be useful in improving thiopurine-based therapeutic protocols, if they can reduce inter-patient variability in thiopurine absorption and disposition.
- ✓ Investigate the phenotypic consequences of TPMT deficiency, the murine TPMT gene was isolated and characterized by Krynetski and Evans, opening the way to a mouse model in which the TPMT gene has been disrupted (i.e. TPMT-KO). Finally, gene expression and regulation patterns in TPMT-deficient verses TPMT-proficient cells and mice using DNA arrays may reveal the functional roles this protein plays in homeostasis.

DISCUSSION

Currently available drug regimens like thiopurine drugs for management of Cancer have certain drawbacks. Therefore there is a need for safer and more effective anticancer drugs therapy which is not still available or is costly. Therefore there is a need to use the already available drugs safely and effectively. But the question arises how? The only answer is to study the molecular pharmacokinetics or pharmacogenomics of thiopurine drugs; this could provide us why these drugs are producing such life threatening side effects (Myelotoxicity, hepatoxicity, pancreatitis) and how to overcome them, what this study is providing. TPMT pharmacogenetics has been studied extensively because of its clinical significance. Many patients got benefited from the knowledge of TPMT genotype-phenotype correlation.

As these drugs are metabolised by Thiopurine methyl trasferase enzyme, whose activity is controlled by TPMT genes. TPMT deficiency is caused by mutations of the TPMT gene. Both TPMT activity measurement and genotyping methods can be used to diagnose TPMT deficiency [104]. Heterozygous individuals have intermediate TPMT enzymatic activity; homozygous individuals have very low or absent enzymatic activity. Myelotoxicity as a side effect of thiopurine drugs may also occur in the absence of TPMT deficiency. Furthermore, other reported side effects of thiopurine drugs, e.g., hepatoxicity, pancreatitis are not necessarily associated with deficient TPMT activity. Transmission is autosomal co-dominant. Therefore it becomes necessary to test the TPMT profile of every patient before starting the thiopurine therapy. Thus studying the genetic polymorphism of TPMT gene could provide a baseline data for individualization of thiopurine drugs therapy.

CONCLUSION

The number of clinically important applications of molecular genetics of TPMT is constantly increasing, from the initial screening of TPMT polymorphism in ALL patients to prevent toxicity, to the increasingly common use of TPMT phenotyping/genotyping in other patient populations where thiopurines are prescribed, including solid organ transplant recipients, patients with Crohn's disease, systemic lupus erythematosus, nonbullous inflammatory dermatoses, rheumatoid arthritis, and other autoimmune diseases. Based on the patient's TPMT genotype, more rational planning of thiopurine therapy has now become possible.

TPMT polymorphism offers a broad perspective of how pharmacogenomics can help to achieve therapeutic goals while avoiding toxic effects in genetically distinct groups of patients. Elucidation of the molecular mechanisms and biochemical consequences of TPMT deficiency demonstrates how pharmacogenetic traits can be identified, characterized but it requires integration of diverse approaches from different fields of biomedical science, also there is a need of pre-TPMT testing and standard guidelines for prescribing thiopurine drugs.

ABBREVIATIONS:

6-MMP = 6-Methytlmercaptopurine
6-MMPN = 6-Methytl Mercaptopurine Nucleotide
6-MP = 6-Mercaptopurine
6-TG = 6-Thioguanil
6-TGNs = 6-Thioguanine nucleotides
ALL= Acute lymphoblastic Leukemia
ARMS = Amplification Refractory Mutation System
AZA= Azathiopurine
ddNTP = Dideoxy single- Base
DHPLC = Denaturating High Performance Liquid Chromatography
HPRT= Hypoxanthine Phosphoribosyl Transferase
kB = Kilo Bases
m/z = Mass/Charge ratio
MALDI-TOF = Matrix-assisted Laser Desorption/Ionization- Time of Flight analysis
PCR = Polymerase Chain Reaction
RFLP = Restriction Fragment Length Polymorphism
SAM =S-Adenosyl Methionine
SNPs = Single Nucleotide Polymorphisms
SSCP = Single Strand Conformational Polymorphism
TPMT = Thiopurine S-Methyl Transferase
TPMT-KO = Thiopurine S-Methyl Transferase knock Out

REFERENCES:

1. Elion GB. The purine path to chemotherapy, In Vitro Cell Dev. Biol., **1989**; 125: pp 321-30.
2. Anstey AV, Wakelin S, Reynolds NJ. British Association of Dermatologists Therapy, Guidelines and Audit Subcommittee, Guidelines for prescribing azathioprine in dermatology, Br. J. Dermatol., **2004**; 151: pp 1123-32.
3. Clunie GP, L ennard L. Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine, Rheumatology, **2004**; 43: pp 13-18.
4. Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism, Clin. Pharm., Ther., **1989**; 46: pp 149-54.
5. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity, Am. J. Hum. Genet., **1980**; 32: pp 651-62.
6. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics, Science, **1999**; 286: pp 487-91.
7. Evans WE, Hon YY, Bomgaars L, Coutre S, Holdsworth M, Janco R, et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to

mercaptopurine or azathioprine, J. Clin. Oncol., **2001**; 19: pp 2293-01.
8. Evans WE, McLeod HL. Pharmacogenomics - drug disposition, drug targets, and side effects, N. Engl. J. Med., **2003**; 348: pp 538-549.
9. McLeod HL, Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy, Annu. Rev. Pharmacol. Toxicol., **2001**; 41: pp 101-21.
10. Evans WE, Johnson JA. Pharmacogenomics: the inherited basis for interindividual differences in drug response, Annu. Rev. Genom. Human. Genet., **2001**; 2: pp 9-39.
11. Milos PM, Seymour AB. Emerging strategies and applications of pharmacogenomics, Hum. Genomics, **2004**; 1: pp 444-55.
12. Bader JS. The relative power of SNPs and haplotype as genetic markers for association tests, Pharmacogenomics, **2001**; 2: pp 11-24.
13. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies, JAMA, **1998**; 279: pp 1200-05.
14. Pirmohamed M, Park BK. Genetic susceptibility to adverse drug reactions, Trends Pharmacol. Sci., **2001**; 22: pp 298-305.
15. Wolf CR, Smith G, Smith RL. Science, medicine, and the future, Pharmacogenetics, BMJ, **2000**; 320: pp 987-990.
16. McLeod HL, Pritcha SC, Githang J, Indalo A, Ameyaw MM, Powrie RH, et al. Ethnic differences in thiopurine methyltransferase pharmacogenetics: evidence for allele specificity in Caucasian and Kenyan individuals, Pharmacogenetics, **1999**; 9: pp 773-76.
17. Deininger M, Szumlanski CL, Otterness DM, Van Loon J, Ferber W, Weinshilboum RM. Purine substrates for human thiopurine methyltransferase, Biochem. Pharmacol., **1994**; 48: pp 2135-38.
18. Krynetski EY, Krynetskaia NF, Yanishevski Y, Evans WE. Methylation of mercaptopurine, thioguanine, and their nucleotide metabolites by heterologously expressed human thiopurine S-methyltransferase, Mol. Pharmacol., 1995a; 47: pp 1141-47.
19. Remy CN, Metabolism of thiopyrimidines and thiopurine. S-Methylation with S-adenosylmethionine transmethylase and catabolism in mammalian tissues, J. Biol. Chem., **1963**; 238: pp 1078-1084.
20. Remy CN. Ribonucleotides and ribonucleosides as methyl acceptors for S-adenosylmethionine: (amino- and thio-) purine methyl-transferases. Incorporation of 6-amino-2-methylaminopurine into ribonucleic acids, Biochim. Biophys. Acta., **1967**; 138: pp 258-275.
21. Weinshilboum RM, Raymond FA and Pazmino PA. Human erythrocyte thiopurine methyltransferase: Radiochemical microassay and biochemical properties, Clin. Chim. Acta., **1978**; 85: pp 323-333.
22. Woodson LC and Weinshilboum RM. Human kidney thiopurine methyltransferase. Purification and biochemical properties, Biochem. Pharmacol., **1983**; 32: pp 819-826.
23. Krynetski EY, Schuetz JD, Galpin AJ, Pui CH, Relling MV, Evans WE. A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase, Proc. Natl. Acad. Sci. U S A, **1995b**; 92: pp 949-953.
24. McLeod HL, Lin JS, Scott EP, Pui CH, Evans WE. Thiopurine methyltransferase activity in American white subjects and black subjects, Clin. Pharmacol. Ther., 1994; 55: pp 15-20.
25. Szumlanski C, Otterness D, Her C, Lee D, Brandriff B, Kelsell D, et al., Thiopurine methyltransferase pharmacogenetics: human gene cloning and characterization of a common polymorphism, DNA Cell Biol., **1996**; 15: pp 17-30.
26. Krynetski EY, Fessing MY, Yates CR, Sun D, Schuetz JD and Evans WE. Promotor and intronic sequences of the human thiopurine S-methyltransferase (TPMT) gene isolated from a human PAC1 genomic library, Pharm. Res., **1997**; 14: pp 1672-78.
27. Seki T, Tanaka T and Nakamura YI. Genomic Structure and multiple single- nucleotide polymorphisms (SNPs) of thiopurine S-methyltransferase gene, Human Genet., **2000**; 45: pp 299-302.
28. Fessing MY, Krynetski EY, Zambetti GP, Evans WE. Functional characterization of the human thiopurine S-methyltransferase (TPMT) gene promoter, Eur. J. Biochem., **1998**; 256: pp 510-17.
29. Spire-Vayron de la Moureyre C, Debuysere H, Fazio F, et al. Characterization of a variable number tandem repeat region in the thiopurine S-methyltransferase gene promoter. Pharmacogenetics, **1999**; 9: pp 189-98.
30. Yan L, Zhang S, Eiff B, Szumlanski CL, Powers M, O'Brien JF. Thiopurine methyltransferase polymorphic tandem repeat: genotypephenotype correlation analysis, Clin. Pharmacol. Ther., **2000**; 68: pp 210-219.
31. Kham SK, Soh CK, Aw DC, and Yeoh AE. TPMT*26 (208F– >L), a novel mutation detected in a Chinese, Br. J. Clin. Pharmacol., **2009**; 68: pp 120-23.
32. Rutherford K, Daggett V. Four human thiopurine smethyltransferase alleles severely affect protein structure and dynamics, J. Mol. Biol., **2008**; 379: pp 803-814.
33. Sahasranaman S, Howard D, Roy S. Clinical pharmacology and pharmacogenetics of thiopurines, Eur. J. Clin. Pharmacol., **2008**; 64: pp 753-767.
34. Samochatora EV, Chupova NV, Mokarova O, Nasedkina TV, Fedorova OE, Glotov AS, et al. TPMT genetic variation in population of the Russian federation, Pediator. Blood centre, **2008**.
35. Zhou S. Clinical pharmacogenomics of thiopurine S-methyltransferase, Curr. Clin. Pharmacol., **2006**; 1: pp 119-128.
36. Yates CR, Krynetski EY, Loennechen T. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance, Ann. Intern. Med., **1997**; 126: pp 608-14.
37. Hon YY, Fessing MY, Pui CH, Relling MV, Krenetski EV and Evans WE. Polymorphism of the thiopurine S- methyltransferase gene in African and Americans, Human molecular genetics, **1999**; pp 371-376.
38. Colombel JF, Ferrari N, Debuysere H. Genotypic analysis of thiopurine *S*-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy, Gastroenterology, **2000**; 118: pp 1025-30.
39. Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine S-methyltransferase gene in African-Americans, Hum. Mol. Genet., **1999**; 8: pp 371-76.
40. Otterness D, Szumlanski C, Lennard L, Klemetsdal B, Aarbakke J, Park-Hah JO, et al. Phenotyping and genotyping study of thiopurine S-methyltransferase in healthy Chinese children: A comparison of Han and Yao ethnic groups, Clin. Pharmacol. Ther., **1997**; 62: pp 60-73.
41. Otterness DM, Szumlanski CL,Wood TC, Weinshilboum RM. Human thiopurine methyltransferase pharmacogenetics. Kindred with a terminal exon splice junction mutation that results in loss of activity, J. Clin. Invest, **1998**; 101: pp 1036-44.
42. Stolk JN, Boerbooms AMT, de Abreu RA. Reduced thiopurine methyltransferase activity and development of side effects of azathioprine treatment in patients with rheumatoid arthritis, Arthritis Rheum., **1998**; 41: pp 1858-66.
43. Hamdan-Khalil R, Allorge D, Lo-Guidice JM. In vitro characterization of four novel non-functional variants of the thiopurine S-methyltransferase, Biochem. Biophys. Res. Commun., **2003**; 309: pp 1005-10.
44. Schaeffeler E, Stanulla M, Greil J. A novel TPMT missense mutation associated with TPMT deficiency in a 5-year-old boy with ALL, Leukemia, **2003**; 17: pp 1422-4.
45. Lindqvist M, Haglund S, Almer S. Identification of two novel sequence variants affecting thiopurine methyltransferase enzyme activity, Pharmacogenetics, **2004**; 14: pp 261-5.
46. Hamdan-Khalil R, Gala JL, Allorge D. Identification and functional analysis of two rare allelic variants of the thiopurine Smethyltransferase gene, TPMT*16 and TPMT*19, Biochem. Pharmacol., **2005**; 69: pp 525-29.
47. Busti Aj, Lehew DS, Daves BJ, McKeever GC. What are common genetic polymorphisms to thiopurine methyltransferase (TPMT) that could impact drug metabolism, PW. Pharmacogenet. News, **2010**; 2: pp 143-46.
48. Alves S, Amorim A, Ferreira F, Prata MJ. Influence of the variable number of tandem repeats located in the promoter region of the thiopurine methyltransferase gene on enzymatic activity, Clin. Pharmacol. Ther., **2001**; 70: pp 165-74.
49. Alves S, Ferreira F, Prata MJ, Amorim A. Characterization of three new VNTR alleles in the promoter region of the TPMT gene, Hum. Mutat., **2000**; 15: pp 121.
50. Liepold G, Schutz E, Haas JP, Oellerich M. Azathioprine-induced severe pancytopenia due to a homozygous two-point mutation of the thiopurine methyltransferase gene in a patient with juvenile HLA-B27-associated spondylarthritis, Arthritis Rheum., **1997**; 40: pp 1896-98.
51. Spire-Vayron de la Moureyre C, Debuysere H, Fazio F, et al. Characterization of a variable number tandem repeat region in the thiopurine S-methyltransferase gene promoter, Pharmacogenetics, **1999**; 9: pp 189-98.
52. Arenas M, Duley JA, Ansari A. Genetic determinants of the pre- and post- azathioprine therapy thiopurine methyltransferase activity phenotype, Nucleosides Nucleotides Nucleic Acids, **2004**; 23: pp 1403-05.

53. Marinaki AM, Arenas M, Khan ZH. Genetic determinants of the thiopurine methyltransferase intermediate activity phenotype in British Asians and Caucasians, Pharmacogenetics, **2003**; 13: pp 97-05.
54. McLeod HL, Relling MW, Liu Q, Pui CH, Evan WE. Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphocytic leukemia, Blood, **1995**; 85: pp 1897-902.
55. Collie-Duguid ES, Pritchard SC, Powrie RH, Sludden Collier JD, Li T, McLeod HL. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations, Pharmacogenetics, **1999**; 9: 37-42.
56. Weinshilboum R. Thiopurine pharmacogenetics: Clinical and molecular studies of thiopurine methyltransferase. Drug Metabolism Disposition, The American Society for Pharmacology and Experimental Therapeutics, **2001**; 29: 601-605.
57. Hong Wei, Shufeng Zhou, Chengrong Li, Jianping Zhang, Jueheng Wu and Min Huang. Phenotyping and Genotyping Studies of Thiopurine *S*-Methyltransferase in Kazaks, Pharmaceutical Research, **2005**; 22: pp 1762-66.
58. Kapoor G, Maitra A, Somlata, Brahmachari V. Application of SNaPshot for analysis of thiopurine methyltransferase gene polymorphism, Indian J. Med. Res., **2009**; 129: pp 500-5.
59. Prefontaine E, Sutherland LR, Macdonald JK, Cepoiu M. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease, Cochrane Database Syst. Rev., **2009**; CD000067.
60. Fargher EA, Tricker K, Newman W, Elliott R, Roberts SR, Shaffer HL, et al. Current use of pharmacogenetic testing: a national survey of thiopurine methyltransferase testing prior to azathiopurine prescription, J. Clin. Pharm. Ther., **2007**; 32: 187-95.
61. Relling MV, Rubnitz JE, Rivera GK, Boyett JM, Hancock ML, Felix CA, et al. High incidence of secondary brain tumours after radiotherapy and antimetabolites, Lancet., **1999c**; 354: pp 34-39.
62. Gisbert JP, Gomollón F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: a review, Am. J. Gastroenterol., **2008**; 1031: pp 783-800.
63. Meggitt SJ, Reynolds NJ. Azathioprine for atopic dermatitis, Clin. Exp. Dermatol., **2001**; 26: pp 369-75.
64. Lennard L, Lilleyman J S, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia, Lancet., 1990; 336: 225-29.
65. Lennard L, Lilleyman JS. Individualising therapy with 6-mercaptopurine and 6-thioguanine related to the thiopurine methyl transferase genetic polymorphism, Ther. Drug Monit., **1996**; 18: pp 328-34.
66. Lennard L. TPMT in the treatment of Crohn's disease with azathioprine, Gut, **2002**; 51: pp 143-6.
67. Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM. Pharmacogenetics of acute Azathiopurine toxicity: Relationship to thiopurine methyltransferase genetic polymorphism, Clin. Pharmacol. Ther., **1989**; 46: pp 149-54.
68. Evans WE, Horner M, Chu YQ, Kalwinsky D and Roberts WM. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia, J. Pediat., **1991**; 119: pp 985-89.
69. Lennard L, Gibson BES, Nicole T, Lilleyman JS. Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia, Arch. Dis. Child, **1993**; 69: pp 577-79.
70. Schutz E, Gummert J, Mohr F, Oellerich M. Azathioprine-induced myelosuppression in thiopurine methyltransferase deficient heart transplant recipient, (Letter) Lancet., **1993**; 341: pp 436.
71. Black AJ, McLeod HL, Capell HA, Powrie RH, Matowe LK, Pritchard SC,et al. DM. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine, Ann. Intern. Med., **1998**; 129: pp 716-18.
72. Schwab M, Schaffeler E, Marx C, Fischer C, Lang T, Behrens C, Gregor M, Eichelbaum M, Zanger UM, Kaskas BA. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism, Pharmacogenetics, **2002**; 12: pp 429-436.
73. Kurzawski M, Dziewanowski K, Ciechanowski K, Drozdzik M. Severe azathioprine-induced myelotoxicity in a kidney transplant patient with thiopurine S-methyltransferase-deficient genotype (TPMT*3A/*3C), (Letter) Transplant Int., **2005**; 18: pp 623-25.
74. Collie-Duguid ES, Pritchard SC, Powrie RH, Sludden Collier JD, Li T, McLeod HL. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations, Pharmacogenetics, **1999**; 9: pp 37-42.
75. Ando M, Ando Y, Hasegawa Y, Shekido Y, Shimokata K, et al. Genetic polymorphisms of thiopurine S-methyltransferase and 6- mercaptopurine toxicity in Japanese with acute lymphoblastic leukaemia, Pharmacogenetics, **2001**; 11: pp 269-73.
76. Kumagai K, Hiyama K, Ishioka S. Allelotype frequency of the thiopurine methyltransferase (TPMT) gene in Japanese, Pharmacogenetics, **2001**; 11: pp 275-78.
77. Chang JG, Lee LS, Chen CM. Molecular analysis of thiopurine S-methyltransferase alleles in South- east Asian populations, Pharmacogenetics, **2002**; 12: pp 191-5.
78. McLeod HL, Relling MW, Liu Q, Pui CH, Evan WE. Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphocytic leukemia, Blood, **1995**; 85: pp 1897-902.
79. Ameyaw MM, Collie-Duguid ES, Powrie RH, Ofori-Adjei D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations, Hum. Mol. Genet., **1999**; 8: pp 367-70.
80. Hakooz N, Arafat T, Payne D, Ollier W, Pushpakom S, Andrews J, et al. Genetic analysis of thipurine methyltransferase polymorphism in Jordian population, Eur. J. Clin. Pharmacol., **2010**; 66: pp 999-03.
81. Bahari A, Hashemi H, Bari Z, Karim A, Roodi A, Kaykhaei MA, Narovine B. Frequency of thiopurine S- metyltransferase (TPMT) allele in shoutheast Iranian population, Nucleoside Nucleotide Nucleic acid., **2010**; 29: pp 237-44.
82. Efrati E, Adler L, Krivoy N, Sprecher E. Distribution of TPMT risk alleles for thiopurine toxicity in Israeli population, Eur. J. Clin. Pharmacol., **2009**; 65: pp 257-62.
83. Schaeffeler E, Fischer C, Brockmeier D, et al. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants, Pharmacogenetics, **2004**; 14: pp 407-417.
84. Albayrak M, Konyssova V, Kaya Z, Gursel Turkiz, Guntekin Sezen, Percin EF, *et al*. Thiopurine methyltransferase polymorphism and mercaptopurine tolerance in Turkish children with acute leukemia, Cancer Chemotherapy Pharmacol., **2011**; 68: pp 1155-59.
85. Dokmanovic L, Urosevic Jelena, Janic D, Jovanovic N, Petruecv B, Tosic N, *et al*. Analysis of Thiopurine methyltransferase polymorphism in the population of Seia and Montenegro and mercaptopurine therapy tolerance in childhood acute lymphoblastic leukemia, The Drug monit., **2006**; 28: pp 800-6.
86. Wei H, Zhou S, Li C, Zhang J, Wu J and Huang M. Phenotyping and Genotyping Studies of Thiopurine S-Methyltransferase in Kazaks, Pharmaceutical Research, **2005**; pp 1762-66.
87. Kham SK, Tan PL, Tay AH, et al. Thiopurine methyltransferase polymorphisms in a multiracial asian population and children with acute lymphoblastic leukaemia, J. Pediatr. Hematol. Oncol., **2002**; 24: pp 353-59.
88. Al Hadithy AFY, De Boer NKH, Derijks LJJ, Escher JC, Mulder CJJ and Brouwers JRBJ. Thiopurines in inflammatory bowel disease: pharmacogenetics, therapeutic drug monitoring and clinical recommendations, Dig. Liver Dis., **2005**; 37: pp 282-97.
89. Belaiche J, Desager JP, Horsemans Y and L ouis E. Therapeutic drug monitoring of azathioprine and 6-mercaptopurien metabolites in Crohn disease, Scand. J. Gastroenterol., **2001**; 37: pp 371-72.
90. Bloomfeld RS, Onken JE. Mercaptopurine metabolite results in clinical gastroenterology practice, Aliment. Pharmacol. Ther., **2003**; 17: 69-73.
91. Clunie GP, L ennard L. Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine, Rheumatology, **2004**; 43: pp 13-18.
92. Decaux G, Horsmans Y, Houssiau F, and Desager JP. High 6-thioguanine nucleotide levels and low thiopurine methyltransferase activity in patients with lupus erythematosus treated with AZA/6-MPthioprine, Am. J. Therapeutics., **2001**; 8: pp 147-50.
93. DeLeve LD, Wang X, Kuhlenkamp JF, and Kaplowitz N. Toxicity of Azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic venooclusive disease, Hepatology, **1996**; 23: 589-99.

94. Hindorf U, Lyrenas E, Nilsson A, et al. monitoring of longterm thiopurine therapy among adults with inflammatory bowel disease, Scand. J. Gastroenterol., **2004**; 39: pp 1105-12.
95. Hindorf U, Peterson C, and Almer S. Assessment of thiopurine methyltransferase and metabolite formation during thiopurine therapy-results from a large Swedish patient population, Ther. Drug. Monit., **2004**; 26: pp 673-78.
96. Kelleher D, Farrell R, Mc Manus R. Pharmacogenetics of inflammatory bowel disease, Novarrtis Found Symp., **2004**; 263: pp 41-53.
97. Kontorinis N, Agarwal K, Gondolesi G, Fiel MI, O'Rourke M, and Schiano TD. Diagnosis of 6 mercaptopurine hepatotoxicity post liver transplantation utilizing metabolite assays, Am. J. Transplant, **2004**; 4: pp 1539-42.
98. Rumbo C, Emerick KM, Emre S, et al. Azathioprine metabolite measurements in the treatment of autoimmune hepatitis in pediatric patients: a preliminary report, J. Ped. Gastroenterol. Nutr., **2002**; 35: pp 391-8.
99. Wusk B, Kullak-Ublick GA, Rammert C, et al. Therapeutic drug monitoring of thiopurine drugs in patients with inflammatory bowel disease or autoimmune hepatitis, Eur. J. Gastroenterol. Hepatol., **2004**; 16: pp 1407-13.
100. Anstey A, Lennard L, Mayou SC, and Kirby JD. Pancytopenia related to azathioprine – an enzyme deficiency caused by a common genetic polymorphism, J. Royal Soc. Med., **1992**; 85: pp 752-56.
101. Lichtenstein GR, Abreu MT, Cohen R and Tremaine W. American Gastroenterological Association, American Gastroenterological Association Institute medical position statement on corticosteroids, immunomodulators and infliximab in inflammatory bowel disease, Gastroenterology, **2006**; 130: pp 935-39.
102. Payne K, Newman W, Fargher E, Tricker K, Bruce IN and Ollier WE. TPMT testing in rheumatology: any better than routine monitoring, Editorial Rheumatology (Oxford), **2007**; 46: pp 727-29.
103. Dubinsky MC, Yang H and Hassard PV. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease, Gastroenterology, **2002**; 122: pp 904-15.
104. Coulthard SA, Rabello C, Robson J, *et al*. A comparison of molecular and enzyme-based assays for the detection of thiopurine methyltransferase mutations, Br. J. Haematol., **2000**; 110: pp599-604.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil