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Pharmacogenomics of Thiopurine S-Methyltransferase: Clinical Applicability of Genetic Variants

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1. Introduction

Sequence variability among individual human genomes has become a key resource for modern medicine in the search for genetic markers affecting disease susceptibility, disease manifestation and response to treatment. Genetic markers have been used for years as an indispensable tool for the diagnosis and follow-up of a number of diseases. They are also used as prognostic and predictive markers. Their application as pharmacogenetic markers is especially important.

Pharmacogenetics is referred to as the study of the variations in a DNA sequence as related to drug efficacy and toxicity. It began with studying differences among individuals. However, as it developed, it became clear that genetic differences between populations should also be taken into account. Following great progress in understanding the molecular basis of health and disease, pharmacogenetics has evolved into pharmacogenomics, a much newer discipline which can be described as the whole-genome application of pharmacogenetics. More precisely, pharmacogenomics is the study of variations of DNA and RNA characteristics as related to drug response.

Genetic variability can affect various aspects of drug therapy: disposition of the drug (pharmacokinetics), efficacy of the drug (pharmacodynamics) and adverse drug reactions (ADRs). Genetic factors are estimated to account for 15-30% of inter-individual differences in drug metabolism and response.

The ultimate goal of pharmacogenetic testing is to aid physicians in the prescription of the appropriate medication at the correct dose prior to the initiation of the therapy. This would lead to minimizing adverse events and toxicity and maximizing efficacy by excluding those who are unlikely to benefit (non-responders) or who may be harmed (adverse responders).

Here, we provide an overview of the genetic variants of thiopurine S-methyltransferase (*TPMT*) gene that influence inter-individual dosing of thiopurine drugs, to highlight a tangible benefit of translating genomic knowledge into clinical practice. Particular single nucleotide polymorphisms (SNPs) in *TPMT* gene have proven to be applicable for optimising the dosage in pursuit of maximum efficacy and minimum adverse effects. Thus,

they set an important paradigm of the implementation of pharmacogenomics in mainstream clinical practice.

2. Pharmacogenetics

The role of genetics in response to drugs was first predicted by Sir Archibald Garrod in the early 1930s (Garrod 1931). Pharmacogenetics, as it is known today, originated as a new scientific discipline in the late 1950s by the merging of two older ones: pharmacology and genetics. Pharmacogenetics examines the role of inherited individual differences in response to drugs. It is a branch of science that explains variability in response to drugs and genetic basis as the cause of this variability. Initially, the focus was on individual human differences, but over time the area of interest of this science extended to genetic differences between populations. Many professionals from this field deal exclusively with humans, but this science has applied its principles to all living organisms that are able to respond to a drug or other chemicals.

Research in the field of pharmacogenetics is being developed into two main directions: first, the identification of specific genes and their products that are associated with various diseases and that could represent targets for new therapeutics; and second, the identification of genes and allelic variants of genes that might influence the response to already existing drugs (Wolf *et al.,* 2000).

3. Human genetic variations

In 2003, after more than a decade, the Human Genome Project was completed. It was clear that the information obtained from the Project had the potential to forever transform healthcare and that genome-based medicine, frequently called personalized medicine, is the future of healthcare. Ever since, the main goal was achieving faster and cheaper sequencing of the whole human genome. The key advantage is the possibility to identify very rare or new, "private" genetic variants. Among a patient's personal genome data, the most important data is about inter-individual genetic differences.

More than ninety-nine percent of the DNA sequence is identical among individuals. The remaining DNA is responsible for genetic diversity (Kidd *et al.,* 2004). Polymorphisms are common genetic variations in the human genome. They represent sequence variations that occur with a frequency >1% in the general population. The most studied polymorphisms are SNPs (single nucleotide polymorphisms). They are distributed over the whole genome. The number of SNPs is estimated to range from 0.3 to 1 SNP per 100 base pairs (bp). Besides SNPs, there are other important classes of polymorphisms, such as VNTRs (variable number of tandem repeats, polymorphic sequence containing 20-50 copies of 6-100 bp repeats), STRs (short tandem repeats, a subclass of VNTR in which repeat unit consists of only 2-7 nucleotides) and CNPs (copy number polymorphisms, variation in the number of copies (CNV) of a DNA sequence in the > 1 kb size range, which are common and widely distributed in the human genome).

The totality of these genetic variations found in an individual, a variome, should carry an answer about inborn diseases, compliance with drug therapies and other processes - all specific to that individual. However, in order to be fully understood and finally translated into the everyday clinical practice, variome data needs to be adequately interpreted. The most important approach of interpretation is to correlate genetic variation with clinical data.

4. Thiopurine S-methyltransferase (TPMT)

One of the best examples of the application of pharmacogenetics in clinical practice is the discovery that different individual responses to purine antagonists as therapeutics are caused by individual variations in thiopurine S-methyltransferase (TPMT) enzyme activity (Weinshilboum *et al*., 1980). Patients who have reduced TPMT enzyme activity can develop toxic effects after the application of standard doses of these drugs (Weinshilboum *et al*., 1980). On the other hand, there are patients in whom the activity of this enzyme is extremely high and they do not respond to standard doses of drugs (Weinshilboum *et al*., 1980). The characterization of mutations within the *TPMT* gene enabled the explanation of these inter-individual differences in enzyme activity. Consequently, the goal of pharmacogenetics, the individualization of therapy, becomes a step closer. The characterization of mutations in *TPMT* gene is also a model system that illustrates how knowledge in the field of pharmacogenetics is successfully used in clinical practice.

5. Thiopurine drugs

Antimetabolites or structural analogs are compounds analogous to natural cell compounds such as folic acid, purines and pyrimidines. The mechanism of their action is based on the fact that they replace natural metabolites in the biochemical processes of cells. Antimetabolites have the greatest impact on the biochemical pathways that are involved in the metabolism of nucleotides and nucleic acids. Purine antagonists as thiopurine drugs have been widely used in medical practice for over 50 years. The structural analogues of purines are 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine (AZA) (Coulthrad *et al.,* 2005).

Thiopurine drugs are indicated for the treatment of various diseases. 6-MP and 6-TG are mainly used in the treatment of hematologic malignancies, such as acute leukemia and lymphoma in children and adults. In childhood acute lymphoblastic leukemia (ALL), 6-TG is primarily used in the induction phase, and 6-MP in the consolidation phase of ALL therapy. The immunosuppressive drug AZA is the drug of choice in the treatment of inflammatory bowel diseases, rheumatoid arthritis, autoimmune hemolytic anemia, systemic lupus erythematosus, as well as in transplantation medicine.

Thiopurine drugs were synthesized in 1951 (Elion 1986). It was shown that newly synthesized drugs inhibit the use of natural purines and act upon the reduction of some tumors in rats (Elion 1967). Soon the activity of these drugs was confirmed in childhood ALL. At that time, the prognosis of this disease was extremely poor. The discovery that 6-MP can lead to the complete remission of childhood ALL, resulted in the approval of the use of these drugs in medical practice by the U.S. Food and Drug Administration in 1953 (Burchenal *et al.,* 1953). AZA was introduced in therapy later, in 1963, after its successful use in kidney transplantation (Murray *et al.,* 1963).

The basic principle of how thiopurine drugs act is the inhibition of many pathways in nucleic acid biosynthesis. Consequently they prevent proliferation of cells involved in determination and amplification of the immune response, causing suppression of the immune system. Thiopurine drugs are also used in cancer treatment (Katzung 2004). An important biochemical feature of cancer cells is excessive synthesis of nucleic acids. Thiopurine drugs are able to stop this synthesis, and thus prevent the division of neoplastic cells (Katzung 2004). Anticancer and

immunosuppressive activity is accomplished through the incorporation of thioguanine nucleotides, metabolic products of thiopurine drugs, into DNA.

Thiopurine drugs are inactive in their original form. They are precursors of the active drug, so-called pro-drugs, and they have to be metabolized first in order to exhibit cytotoxic, therapeutic effect (Lennard 1992). The first step is the non-enzymatic degradation of AZA to 6-MP and imidazole group. 6-MP and 6-TG go through metabolic changes prior to the expression of their cytotoxic effects. After metabolic conversion, 6-MP and 6-TG are incorporated into the DNA and RNA molecules as thioguanine nucleotides (6-TGN) (Bertino 1991). 6-MP can also inhibit *de novo* synthesis of purine nucleotides (Dervieux *et al.,* 2001; Coulthard *et al.,* 2002). 6-TGN are incorporated into DNA as "false" bases, causing DNA damage by single strand breaking, inter-strand cross-linking and DNA-protein cross-linking (Maybaum *et al.,* 1981; Maybaum *et al.,* 1983; Christie *et al.,* 1984; Tay *et al.,* 1969; Pan *et al.,* 1990; Bodell 1991). Also, the inhibition of normal DNA replication may occur, through the partial inhibition of DNA polymerase and DNA ligase (Ling *et al.,* 1992) as well as through the significant inhibition of RNase H (Krynetskaia *et al.,* 1999).

6. Metabolism of thiopurine drugs

As already mentioned, 6-MP and 6-TG are metabolically converted to 6-TGN before expressing their cytotoxic effect (Fig 1). Metabolic conversion begins with the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT, EC: 2.4.2.8). After a series of metabolic steps, 6-TGN are formed and incorporated into DNA and RNA molecules.

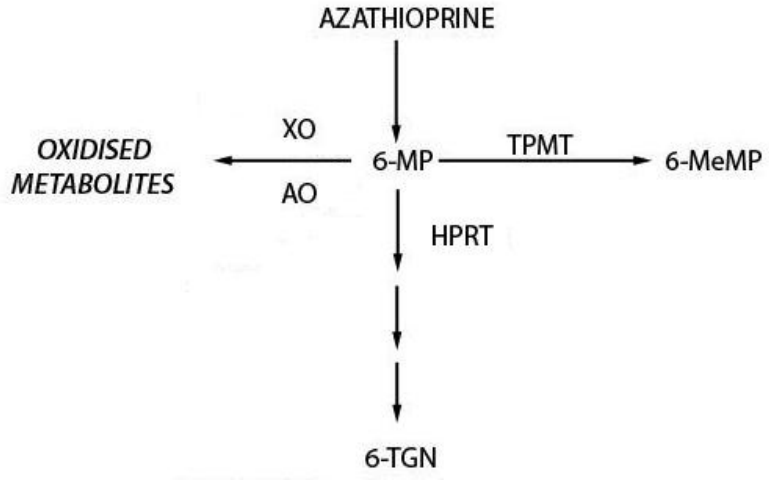

Fig. 1. Thiopurine drug metabolism. The figure shows a simplified representation of thiopurine drug biotransformation, with azathioprine being converted *in vivo* to 6-mercaptopurine (6-MP), followed by the metabolic activation of 6-MP mediated by hypoxanthine-guanine phosphoribosyltransferase (HPRT), to form 6-thioguanine nucleotides (6-TGN). 6-MP is inactivated by xanthine oxidase (XO), aldehyde oxidase (AO) or thiopurine S-methyltransferase (TPMT). TPMT enzyme uses S-adenosyl-L-methionine as a methyl group donor. One of the reaction products is methyl-6-mercaptopurine (6-MeMP).

Thiopurine drugs are inactivated in the organism by oxidation (mediated by aldehyde oxidase (AO, EC 1.1.3.13) and xanthine oxidase (XO, EC 1.1. 3.22)) and methylation (mediated by thiopurine S-methyltransferase (TPMT, EC 2.1.1.67)), reactions which are needed to prevent high drug concentrations and adverse drug-related events. XO and AO produce metabolites that have little or no cytotoxic effect. XO activity in hematopoietic tissue is very low, almost insignificant. This is the reason why the main pathway of thiopurine drug inactivation goes through the TPMT enzyme (Remy 1963).

The influence of TPMT enzyme activity on cytotoxicity induced by thiopurine drugs was first documented in 1987 (Van Loon *et al.,* 1987).

7. TPMT allozymes

Thiopurine S-methyltransferase is a cytosolic monomeric enzyme that catalyzes S-methylation of heterocyclic aromatic sulfhydryl compounds, and consequently, partial inactivation of immunosuppressive thiopurine medications. The molecular weight of TPMT protein is 28.18 kDa and it consists of 245 amino acids. The natural substrate for TPMT is unknown, although this enzyme is expressed in nearly all human tissues (Weinshilboum *et al.,* 1978). Structural and biochemical analyses of TPMT protein revealed the existence of certain protein variants with altered activity. In some individuals, TPMT enzyme activity is significantly decreased or increased compared to the normal TPMT activity level.

One of the first studies of TPMT activity in red blood cells determined the distribution of TPMT activity to be trimodal. Namely, it was found that approximately 90% of individuals express high TPMT activity. These individuals are referred to as high methylators. Intermediate methylators represent approximately 10% of the population. Low or undetectable TPMT activity is reported in 0.3% individuals (Weinshilboum *et al.,* 1980). This study initially identified the hereditary nature of the TPMT deficiency in humans. Trimodal frequency distribution of TPMT activity corresponds to monogenic co-dominant inheritance. Additionally, ultra-high methylators have been observed (Spire-Vayron de la Moureyre *et al.,* 1999; Roberts *et al.,* 2008). It has been shown that these different TPMT allozymes are defined by certain *TPMT* gene polymorphisms.

8. Genetic variants in *TPMT* gene

Human *TPMT* gene (NG_012137, NM_000367, GeneBank: 7172 or MIM: 187680) was discovered using a "classical" molecular biology strategy. Firstly, TPMT enzyme from kidneys was purified and a partial amino acid sequence was obtained (Van Loon *et al.,* 1982). This information served for the successful cloning of human *TPMT* cDNA (Honchel *et al.,* 1993).

Initially, on chromosome 18q.21.1 the pseudogene for *TPMT* has been discovered, containing a similar sequence to the *TPMT* gene (Lee *et al.,* 1995). The human gene for *TPMT* was cloned and mapped on the short arm of chromosome 6, at the position 6p22.3 (Szumlanski *et al.,* 1996). *TPMT* gene comprises a region of 34 kb and 10 exons, 8 of which encode *TPMT* protein. Krynetski et al. thoroughly characterized the *TPMT* gene and its adjacent sequences (Krynetski *et al.,* 1997).

TPMT gene exhibits significant genetic heterogeneity. It has been shown that certain polymorphisms in *TPMT* gene define different TPMT allozymes with different enzyme activity. At present, the *TPMT* allele nomenclature comprises at least 27 *TPMT* alleles (Feng

et al., 2010), with wild type allele designated as *TPMT**1. There are several *TPMT* variant alleles comprising one or more SNPs (Table 1).

TPMT variant allele	Genetic variant	Molecular alteration	Position in the *TPMT* gene	Reference
*TPMT*1*	Wt			
*TPMT*1A*	-178C>T		Exon 1	Spire-Vayron de la Moureyre *et al.*, 1998b
*TPMT*1S*	c.474T>C	p.Ile158Ile	Exon 7	Alves *et al.*, 1999
*TPMT*2*	c.238G>C	p.Ala80Pro	Exon 5	Krynetski *et al.*, 1995
*TPMT*3A*	c.460G>A and c.719A>G	p.Ala154Thr and p.Tyr240Cys	Exon 7, Exon 10	Tai *et al.*, 1997
*TPMT*3B*	c.460G>A	p.Ala154Thr	Exon 7	Szulmanski *et al.*, 1996; Tai *et al.*, 1996
*TPMT*3C*	c.719A>G	p.Tyr240Cys	Exon 10	Szulmanski *et al.*, 1996; Tai *et al.*, 1996
*TPMT*3D*	c.460G>A, c.719A>G and c.292G>T	p.Ala154Thr, p.Tyr240Cys and p.Glu98STOP	Exon 7, exon 10 and exon 5	Otterness *et al.*, 1997
*TPMT*4*	IVS9–1G>A	exon 10 is shortened as a result of use of the cryptic splice site created by G>A substitution	intron 9/exon 10	Otterness *et al.*, 1998
*TPMT*5*	c.146T>C	p.Leu49Ser	Exon 4	Otterness *et al.*, 1997
*TPMT*6*	c.539A>T	p.Tyr180Phe	Exon 8	Otterness *et al.*, 1997
*TPMT*7*	c.681T>G	p.His227Glu	Exon 10	Spire-Vayron de la Moureyre *et al.*, 1998b
*TPMT*8*	c.644G>A	p.Arg215His	Exon 10	Hon *et al.*, 1999
*TPMT*9*	c.356A>C	p.Lys119Thr	Exon 5	Schaeffeler *et al.*, 2004
*TPMT*10*	c.430G>C	p.Gly144Arg	Exon 7	Colombel *et al.*, 2000
*TPMT*11*	c.395G>A	p.Cys132Tyr	Exon 6	Schaeffeler *et al.*, 2003
*TPMT*12*	c.374C>T	p.Ser125Leu	Exon 6	Hamdan-Khalil *et al.*, 2003
*TPMT*13*	c.83A>T	p.Glu28Val	Exon 3	Hamdan-Khalil *et al.*, 2003
*TPMT*14*	c.1A>G	p.Met1Val	Exon 3	Lindqvist *et al.*, 2004
*TPMT*15*	IVS7–1G>A	p.Arg140_Cys165del (deletion of the entire exon 8 in the final protein, resulting in a frame shift and a premature stop codon in exon 9)	intron 7/ exon 8	Lindqvist *et al.*, 2004

TPMT variant allele	Genetic variant	Molecular alteration	Position in the *TPMT* gene	Reference
*TPMT*16*	c.488G>A	p.Arg163His	Exon 7	Schaeffeler *et al.*, 2004
*TPMT*17*	c.124C>G	p.Gln42Glu	Exon 3	Schaeffeler *et al.*, 2004
*TPMT*18*	c.211C>A	p.Gly71Arg	Exon 4	Schaeffeler *et al.*, 2004
*TPMT*19*	c.365A>C	p.Lys122Thr	Exon 5	Hamdan-Khalil *et al.*, 2005
*TPMT*20*	c.712A>G	p.Lys238Glu	Exon 10	Schaeffeler *et al.*, 2006
*TPMT*21*	c.205C>G	p.Leu69Val	Exon 4	Schaeffeler *et al.*, 2006
*TPMT*22*	c.488G>C	p.Arg163Pro	Exon 7	Schaeffeler *et al.*, 2006
*TPMT*23*	c.500C>G	p.Ala167Gly	Exon 8	Lindqvist *et al.*, 2007
*TPMT*24*	c.537G>T	p.Gln179His	Exon 8	Garat *et al.*, 2008
*TPMT*25*	c.634T>C	p.Cys212Arg	Exon 10	Garat *et al.*, 2008
*TPMT*26*	c.622T>C	p.Phe208Leu	Exon 9	Kham *et al.*, 2009
*TPMT*27*	c.319T>G	p.Tyr107Asp	Exon 5	Feng *et al.*, 2010
*TPMT*28*	c.611T>C	p.Ile204Thr	Exon 9	Appell *et al.*, 2010

HGVS nomenclature has been applied. IVS-intron

Table 1. Summary of the currently known SNPs in the *TPMT* gene.

The common nonfunctional alleles include *TPMT*2* (containing a single c.238 G>C polymorphism), *TPMT*3A* (containing both c.460 G>A and c.719 A>G polymorphisms), *TPMT*3B* (containing a single c.460 G>A polymorphism), *TPMT*3C* (containing a single c.719 A>G polymorphism) and *TPMT*4* (containing a single nucleotide G>A substitution at the 3'end of intron 9*)* (Krynetski *et al.,* 1995; Tai *et al.,* 1996; Loennechen *et al.,* 1998; Otterness *et al.,* 1998). Most of these SNPs are located in the coding region of the *TPMT* gene and lead to non-synonymous amino acid substitutions (p.Ala80Pro, p.Ala154Thr and p.Tyr240Cys for c.238 G>C, c.460 G>A and c.719 A>G polymorphisms, respectively), which cause a decrease in activity of TPMT enzyme in comparison to the wild type. On the contrary, *TPMT*4* allele contains a frameshift within exon 10, leading to low-enzyme activity.

The majority of genetic variants detected in the *TPMT* gene represent sequence variations that alter the encoded amino acid (Weinshilboum *et al.,* 2006). Besides these, there are genetic variants that influence transcription and mRNA splicing, resulting in variable *TPMT* gene expression.

Recently, a great deal of evidence has confirmed the existence of such modifiers of TPMT activity within non-coding regions of the *TPMT* gene. In particular, it has been demonstrated that the presence of variable number of tandem repeats, VNTRs, ranging from three to nine, in the *TPMT* gene promoter, directly alters TPMT activity, most likely due to the alteration of promoter *cis*-regulatory elements (Zukic *et al.,* 2010; Pavlovic 2009; Pavlovic *et al.,* 2010; Georgitsi *et al.,* 2011).

TPMT promoter VNTRs include GC-rich blocks that are putative binding sites of various transcriptional factors (Krynetski *et al.,* 1997, Fessing *et al.,* 1998). The VNTR region architecture is defined by three types of repeats (A, B and C) that vary amongst each other by length and nucleotide sequence. Repeats are always arranged in the same order: A is followed by B and then C, with no intervening sequences. The number of A and B repeats varies, while the C repeat is always present in only one copy (Spire-Vayron de la Moureyre *et al.,* 1999). An inverse correlation between the total number of repeats and the enzymatic activity was observed (Spire-Vayron de la Moureyre *et al.,* 1998a), while findings from Zukic and co-workers suggest that on top of the total number of VNTRs, the type/architecture of the repeat has crucial impact on *TPMT* gene transcriptional regulation as well (Zukic *et al.,* 2010).

Recently, trinucleotide repeat variants in the *TPMT* promoter region have been described which may explain the 1-2% of Caucasians who demonstrate ultra-metabolizer phenotype (Roberts *et al.,* 2008).

9. Functional characterization of TPMT allozymes

The functional characterization and expression analysis in human cells and yeast system (Tai *et al.,* 1997; Otterness *et al.,* 1997; Hamdan-Khalil *et al.,* 2003; Lindqvist *et al.,* 2004; Schaeffeler *et al.,* 2006; Ujiie *et al.,* 2008), revealed that alleles *TPMT**2, *TPMT*3A, TPMT*5, TPMT*12, TPMT*14,* and *TPMT*22* encode for TPMT enzymes that have a very reduced activity in comparison to wild type allele designated as *TPMT*1.* In addition, it has been shown that *TPMT*18* allele encodes for an enzyme that has a slightly reduced activity compared to wild-type allele. Alleles *TPMT*9, TPMT*19* and *TPMT*24* express TPMT proteins whose activity is not statistically different from the activity of wild-type enzyme (Garat *et al.,* 2008; Hamdan-Khalil *et al.,* 2005). Polymorphisms in the alleles *TPMT*4* and *TPMT*15* cause alternative processing of *TPMT* mRNA and consequently, the expression level of the TPMT enzyme is reduced. They belong to the so-called quantitative polymorphisms (Otterness *et al.,* 1998; Lindqvist *et al.,* 2004). The molecular mechanism that leads to the reduction of TPMT activity was studied in the most common *TPMT* genetic variants. Expression studies of *TPMT**2 and *TPMT*3A* alleles showed that both alleles are about 100 times less expressed than wild type, *TPMT*1* allele (Tai *et al.,* 1997). Also, the expression of *TPMT**2 and *TPMT*3A* allelic variants was not in correlation with the activities of TPMT*2 and TPMT*3A proteins. The mechanism of accelerated degradation of TPMT*2 and TPMT*3A proteins is responsible for the reduced level of TPMT proteins and thereby for the reduced catalytic ability of enzymes (Tai *et al.,* 1997). More detailed studies have confirmed that in the accelerated degradation of TPMT*3A protein, through a ubiquitin-mediated system, the molecular chaperones from the family of heat shock proteins are involved (Wang *et al.,* 2003).

10. Population-specific distribution of *TPMT* variant alleles

Pharmacogenetics is generally focused on inter-individual differences in drug metabolism and on variations in response to drugs. The frequency of pharmacogenetic markers studied so far, is different between certain racial and ethnic groups. Historically, the practical application of pharmacogenetic achievements, i.e., the individualization of therapy, has been based on studies conducted on Caucasians. With time, other ethnic groups have been included in clinical trials, and it became clear that responses to drug therapy may depend on

ethnic background (Relling *et al.,* 2011a). Thus, if the metabolism of drugs varies among different ethnic groups, then the pharmacogenetic data of one population cannot be extrapolated to another one without prior assessment. Knowledge of the pharmacogenetic differences between populations can be of great importance for the pharmaceutical industry.

The distribution of clinically relevant *TPMT* alleles is population specific (Spire-Vayron de la Moureyre *et al.,* 1998a; Hon *et al.,* 1999; Collie-Duguid *et al.,* 1999; Schaffeler *et al.,* 2004). The *TPMT*3A* allele is the most common variant allele in Caucasians (frequency approximately 3.5%) (Relling *et al.,* 2011a), while *TPMT*3C* is predominant in subjects with Asian or African ancestry (frequencies of 0.3–5.3% and 2.4–10.9 % respectively) (Kubota *et al.,* 2001; Hongeng *et al.,* 2000; Hon *et al.,* 1999; McLeod *et al.,* 1999). Additionally, *TPMT*8* has been reported to be common in the African population (Hon *et al.,* 1999; Oliveira *et al.,*2007; Alves *et al.,* 2004).

The common *TPMT* variant alleles in Caucasian include *TPMT*2, TPMT*3A, TPMT*3B* and *TPMT*3C* (Krynetski *et al.,* 1995; Tai *et al.,* 1996; Loennechen *et al.,* 1998). These variant alleles are detected in over 80-95% of Caucasians characterized to have low or intermediate TPMT activity (Yates *et al.,* 1997).

11. Methodology for TPMT phenotype and genotype testing

The TPMT phenotype and genotype can be defined in several ways. Phenotypic analysis of TPMT enzyme activity could be performed by radiochemical activity assays (McLeod *et al.,* 1995; Weinshilboum *et al.,* 1978), or an assay based on high performance liquid chromatography (HPLC) method (Kroplin *et al.,* 1998). Genotyping is performed using PCR-based methods (Yates *et al.,* 1997; Coulthard *et al.,* 1998), denaturing high performance liquid chromatography (DHPLC) (Hall *et al.,* 2001; Schaeffeler *et al.,* 2001), Real Time-PCR (Lindqvist *et al.,* 2003), a combination of microchip and sequencing (arrayed primer extension - APEX) (Yi *et al.,* 2002), molecular haplotype analysis (McDonald *et al.,* 2002) and pirosequencing (Haglund *et al.,* 2004).

Methods based on PCR are used to detect the most common *TPMT* variant alleles that lead to reduced TPMT activity. These analyses are helpful in identifying individuals with a high risk of developing potentially fatal hematologic toxicity caused by thiopurine drugs. Measurement of TPMT enzyme activity was, until recently, very expensive and relatively inaccessible to patients. A concordance of more than 95% exists between actual TPMT enzyme activity and the prediction of its activity based on detection of *TPMT* variant alleles (McLeod *et al.,* 2000; Schwab *et al.,* 2001). Therefore, molecular genetic analysis represents a quick and efficient method to identify patients at risk for toxicity and adverse effects-free guidance of the therapy.

Commercially available genetic tests change over time. Many of them include only the **2, *3A, *3B* and **3C* alleles. There is no doubt that sequencing of the *TPMT* gene remains the most accurate, although expensive method.

12. Clinical applicability of *TPMT* genetic variants: Individualization of thiopurine therapy

All patients with decreased TPMT activity are at risk of hematologic toxicity owing to the accumulation of high levels of 6-thioguanine nucleotides (Weinshilboum 2003). Thiopurine-

induced myelosuppression can result in increased morbidity, hospitalization and/or treatment discontinuation (Leung *et al.,* 2009; Ugajin *et al.,* 2009). Myelosuppression increases an individual's risk of developing an infection and sepsis (Campbell *et al.,* 2001; Posthuma *et al.,* 1995; Connell *et al.,* 1993; Schütz *et al.,* 1993). The incidence of mild leukopenia is approximately 5-25% (Gurwitz *et al.,* 2009). Rare, but severe leukopenia can develop suddenly and unpredictably in approximately 3% of patients (Carter *et al.,* 2004). A 27-year analysis showed that AZA contributed to the incidences of myelosuppression in 5% of patients (Connell *et al.,* 1993). Over an 18-year period, 2% of patients with IBD experienced 6-MP-induced leukopenia that resulted in hospitalization (Present *et al.,* 1989). The incidence of myelosuppression occurred more frequently during the first eight weeks after treatment initiation, and was more likely to occur with a higher drug dose (Present *et al.,* 1989; Lewis *et al.,* 2009).

Consequently, thiopurine drug dose reduction is necessary to avoid toxicity (Weinshilboum *et al.,* 1980). Therefore, it is of great importance to determine TPMT status before initiating thiopurine therapy (Relling *et al.,* 2011a; Gurwitz *et al.,* 2009; Schmiegelow, K. *et al.,* 2009; Relling *et al.,* 2010). TPMT genotyping is commonly used for determination of TPMT status.

Patients who are homozygotes or compound heterozygotes for nonfunctional genetic variants, treated with standard drug doses, develop severe, eventually fatal, myelosuppression and require AZA, 6-MP or TG reduced doses by at least 10-fold (Schwab *et al.,* 2001; Evans *et al.,* 2001; Schwab *et al.,* 2002; Slanar *et al.,* 2008; Relling *et al.,* 2011a).

Patients with intermediate TPMT activity, heterozygous carriers of nonfunctional genetic variants in the *TPMT* gene, also require dose reduction (Weinshilboum *et al.,* 1980; Dokmanovic *et al.,* 2006). The initial dose of AZA or 6-MP should be reduced by 30-70%. The AZA dose can be titrated as tolerated. The 6-MP dose should be adjusted based on the severity of myelosuppression and disease-specific guidelines. The initial dose of 6-TG should be reduced by 30-50%, and adjusted based on the severity of myelosuppression and disease-specific guidelines (Weinshilboum 2001; Krynetski *et al.,* 2003; Weinshilboum 2003; Dokmanović *et al.,* 2008; Relling *et al.,* 2011b).

In addition, 1 - 2% of patients are ultra-high methylators, who experience thiopurine treatment resistance and hepatotoxicity as a result of treatment with elevated 6-MP concentrations (Spire-Vayron de la Moureyre *et al.,* 1999; Roberts *et al.,* 2008). These patients often do not respond to therapy, although doses of drugs up to 50% higher than the standard doses are given. (Schaeffeler *et al,* 2004; Spire-Vayron de la Moureyre *et al.,* 1998a; Dokmanovic *et al.,* 2006).

13. Guidelines for thiopurine dosing based on *TPMT* genotype

The Clinical Pharmacogenetics Implementation Consortium (CPIC), as a part of the National Institutes of Health's Pharmacogenomics Research Network, developed the first guideline for the dosing of thiopurines based on *TPMT* genotype (updates at http://www.pharmgkb.org) (Relling *et al.,* 2011b).

Dose adjustments based on *TPMT* genotype have reduced thiopurine induced adverse effects without compromising the desired antitumor and immunosuppressive therapeutic effects in several clinical settings (Relling *et al.,* 2011a).

Although the information on *TPMT* genotype is recommended rather than required as part of thiopurine drug treatment, some groups (Relling *et al.,* 2011a; Gurwitz *et al.,* 2009; Relling *et al.,* 2010) advocate testing for *TPMT* status prior to initiating thiopurine therapy, so that starting dosages can be adjusted accordingly. This is very important, since, if one starts with low doses in all patients in order to avoid severe toxicity, in the minority with a *TPMT* defect, one risks disease progression during the period of upward dosage titration (Sandborn 2001). Therefore, the use of this genetic test in routine clinical practice is recommended, while clinicians should continue to evaluate markers of disease progression and/or myelosuppression to adjust thiopurine doses upward or downward from the genotype-directed starting doses (Relling *et al.,* 2011a).

Besides CPIC guideline, there are recommendations and guidelines offered by other groups (Nguyen *et al.,* 2011). Namely, the Royal Dutch Association for the Advancement of Pharmacy Pharmacogenomic Working Group recommends for patients who are intermediate metabolizers that the dose of AZA or 6-MP should be reduced by 50% and titrated based on hematologic monitoring and efficacy. For patients who are poor metabolizers, the dose of AZA or 6-MP should be reduced by 90% and titrated based on hematologic monitoring and efficacy. Moreover, patients who are intermediate or poor metabolizers should not be treated with 6-TG as there are "insufficient data to allow calculation of dose adjustment" (Swen *et al.,* 2011).

The US Food and Drug Administration (FDA) and prescribing information for AZA and 6-MP recommend either TPMT genotyping or phenotyping prior to initiating therapy to help identify patients who are at an increased risk of developing toxicity (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm). The prescribing information for 6-TG indicates that patients with TPMT deficiency "may be unusually sensitive to the myelosuppressive effects of 6-TG. Substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression". In addition, the American College of Gastroenterology treatment guidelines prefer TPMT phenotyping over genotyping in patients who are being treated with thiopurines for ulcerative colitis (Korbluth *et al.,* 2010).

On the other hand, there are research groups that do not advocate for TPMT genotyping before treatment with thiopurines (Booth *et al.,* 2011). The Agency for Healthcare Research and Quality (AHRQ) concluded that "there is currently insufficient evidence regarding the effectiveness of determining TPMT status prior to thiopurine treatment in terms of improvement in clinical outcomes and incident myelotoxicity in comparison with routine monitoring of full blood counts and adverse events" (http://www.ahrq.gov/clinic/tp/tpmttp.htm#Report).

Also, the British Society of Gastroenterology does not require either TPMT genotyping or phenotyping as a prerequisite to initiating thiopurine therapy because the use of AZA has been shown to be safe in patients with Crohn's disease or ulcerative colitis (Carter *et al.,* 2004).

14. From TPMT pharmacogenetics to TPMT pharmacogenomics

It is worth noting that there is no clear boundary between the low and intermediate, or intermediate and high methylators. Even individuals within the same "methylation" group show different enzymatic activity, and these differences are genetically determined (Vuchetich *et al.,* 1995). Also, there are many patients with wild type *TPMT* who develop

toxicity. All this leads to the conclusion that the association of a particular genetic variation with adverse drug effects could be softened or enhanced by the other genetic variations present in the same individual that influence other processes (drug absorption, transportation, metabolism, *TPMT* gene transcription and consequently the abundance of TPMT protein). Moreover, the effects and clinical relevance of genetic variants in another gene, encoding an enzyme involved in mercaptopurine metabolism (inosine-triphospate-pyrophosphatase, *ITPA*), on mercaptopurine pharmacogenetics has been demonstrated (Stocco *et al.,* 2010).

Finally, a myriad of other genetic factors, which influence interactions between thiopurines and other drugs, could play an important role in the final TPMT activity phenotype.

Therefore, more comprehensive study of the modifying role of different genetic factors in TPMT pharmacogenetics, will in time lead to our understanding of controversial results published in our era. The new era of pharmacogenomics will bring more consistent and reliable guidelines for thiopurine dosing based on patient's genotype.

In recent years, the term pharmacogenomics is more and more present alongside the term pharmacogenetics. Due to rapid technological development and the great success of the human genome sequencing project, the variations and interactions of multiple genes, rather than variations in individual genes, have been recognized as the cause of diverse responses to drugs (O'Brien *et al.,* 1999; Kennedy *et al.,* 2003). Genotyping methods, the application of microarrays and GWAS analyses (Genome Wide Association Studies) that are used in pharmacogenomics, provide an insight into a number of individual genes at one time, their possible interactions and changes in their expression. The final goal of pharmacogenomics is the individualization of therapy in accordance with a patient's genotype and gene expression profile. Thus, by using the appropriate therapeutics and adequate doses without side effects, the cheapest, fastest and the most efficient treatment for patients would be achieved. Under these conditions, patients would not be faced with complications, time and money for additional drugs and hospital days would not be wasted for treatment of complications, while the primary disease progresses. Unfortunately, widely available pharmacogenomic tests for particular diseases still do not exist. The individualization of therapy in medical practice, if at all implemented, is conducted based on pharmacogenetic achievements, by testing polymorphisms in a single gene.

15. Conclusions

Although pharmacogenetics is one of the most promising fields of biomedicine, only a few pharmacogenetic markers have been introduced in routine clinical practice. Among them are genetic variants in the *TPMT* gene which can be used for determination of the cause of unusual therapeutic response in patients treated with thiopurine drugs.

TPMT genotyping is recommended prior to initiating thiopurine therapy by several groups and consortia, so that starting dosages can be adjusted accordingly. Doses customized on the basis of *TPMT* status reduce the likelihood of acute myelosuppression without compromising disease control.

Nowadays, *TPMT* genetic testing comprise the analysis of DNA sequence at each of the important single-nucleotide polymorphism in the *TPMT* gene associated with altered level of enzyme activity.

However, the understanding of a single genetic variation is far more complex when it is put in the context of other genetic variations. In that sense, the future of personalized medicine is heading towards variomics - the study of the overall genetic variations found in an individual.

Although the knowledge of pharmacogenomics is incomplete and still in expansion, evidence presented in this chapter show that up to date knowledge can already be used for more successful, personalized patient treatment. There is no doubt that pharmacogenomics together with gene therapy will change the future of medicine and will steadily pave the path to personalized medicine.

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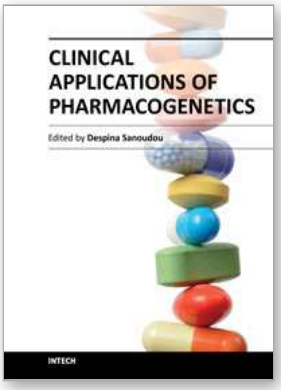

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The rapidly evolving field of Pharmacogenetics aims at identifying the genetic factors implicated in the inter-individual variation of drug response. These factors could enable patient sub-classification based on their treatment needs thus expediting drug development and promoting personalized, safer and more effective treatments. This book presents Pharmacogenetic examples from a broad spectrum of different drugs, for different diseases, which are representative of different stages of evaluation or application. It has been designed so as to serve both the unfamiliar reader through explanations of basic Pharmacogenetic concepts, the clinician with presentation of the latest developments and international guidelines, and the research scientist with examples of Pharmacogenetic applications, discussions on the limitations and an outlook on the new scientific trends in this field.

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