Azathioprine/6-mercaptopurine toxicity: The role of the TPMT gene

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SUMMARY

The thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) are widely used in medicine, including inflammatory bowel diseases (IBD). Thiopurine drugs undergo S-methylation catalysed by thiopurine methyltransferase (TPMT) to methylmercaptopurine or oxidation to thiouric acid via xanthine oxidase (XO). Altered TPMT activity predominantly results from single nucleotide polymorphisms (SNPs) in the TPMT gene, which is located on chromosome 6p22.3. In the general population TPMT enzyme activity is normal in 89%, intermediate in approximately 11% and absent in approximately 0.3% of cases. The prevalence of the most frequent single nucleotide polymorphisms (SNPs) in the TPMT gene has been reported to vary worldwide. The mechanisms of AZA/6-MP action are currently unknown. Pharmacogenetics of AZA/6-MP represents an interesting field of research with direct implications in clinical practice. AZA/6-MP metabolism steps, the impact of TPMT gene SNPs on toxicity prediction as well as the 6p loci analysis represents a challenging field of research in IBD and other diseases. When possible, TPMT genotyping prior to the initiation of AZA/6-MP should be considered to decrease the risk of severe adverse event as well as to identify patients who might benefit from higher doses. Clinicians should be aware that TPMT SNPs do not explain all leucopenic events and that TPMT measurements cannot replace the need for continued monitoring of leucocyte counts in AZA/6-MP treated patients.

INTRODUCTION

The thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) are widely used in medicine, including inflammatory bowel diseases (IBD).

Thiopurine drugs undergo S-methylation catalysed by thiopurine methyltransferase (TPMT) to methylmercaptopurine or oxidation to thiouric acid via xanthine oxidase (XO). During the last ten years a lot of clinical and fundamental research has been focused on the role of TPMT gene and TPMT enzyme in predicting AZA/6-MP efficacy and side effects. Indeed, pharmacogenetics in IBD seems currently to be of immediate scientific as well as of capital clinical importance also in view of new therapies.

This review summarizes the outcomes of these studies, highlights the points of current interest in research and stresses fundamental clinical principles related to AZA/6-MP therapy.

It has been generally accepted that TPMT polymorphisms represent one of the best models for the translation of genomic information to guide IBD patient therapeutics.

Keywords: TPMT gene, single nucleotide polymorphism, prevalence, AZA/6-MP, inflammatory bowel disease, toxicity.

Abbreviations:

AZA/6-MP=azathioprine / 6-mercaptopurine
CD=Crohn's disease
IBD=Inflammatory Bowel Disease
6-MP=6-mercaptopurine
SNP(s)=Single nucleotide polymorphism(s)
TPMT=thiopurine methyl transferase (gene)
TPMT alleles: TPMT*1=G238C,
TPMT*3A=A719G & G460A,
TPMT*3B=G460A,
TPMT*3C=A719G
UC=Ulcerative Colitis
with and it has been also suggested that TPMT status is an effective method for tailored thiopurine drug therapy.\textsuperscript{6,20}

Clinically sound pharmacogenetic studies over the last two decades have shown that polymorphisms at the TPMT gene locus play a significant role in the occurrence of various side effects of thiopurine drugs including life-threatening myelosuppression, a serious dose-related toxicity. In addition to toxicity, TPMT polymorphisms have also been related to AZA/6-MP therapeutic efficacy.\textsuperscript{6,20}

**AZA/6-MP METABOLIZATION, KINETICS, ACTIONS AND INTERACTIONS**

Factors that affect AZA/6-MP pharmacokinetics may be involved in the following steps: 1) absorption, 2) distribution, 3) metabolism (TPMT gene) and 4) excretion. Factors affecting AZA/6-MP pharmacodynamics include receptors and transporters. To date no clear genetic factors affecting receptors or transporters have been described. Interactions of AZA/6-MP have been described with allopurinol, warfarin\textsuperscript{21} and Infliximab.\textsuperscript{22}

Azathioprine was introduced approximately forty years ago [Prepn: Hitchings, Elion, US patent 3,056,785 (1962)]. Azathioprine [6-(1-Methyl-4-nitroimidazol-5-methylthio)purine has a relative molecular mass of 277.3, it is insoluble in water and very slightly soluble in ethanol and chloroform. AZA/6-MP may be dissolved in water with addition of one molar equivalent of alkali. AZA/6-MP is stable in solution at neutral or acidic PH but hydrolysis to mercaptopurine occurs in excess sodium hydroxide (0.1N).\textsuperscript{23-26}

AZA/6-MP is well absorbed following oral administration. Maximum serum radioactivity occurs at one to two hours after oral ingestion and decays with a half-life of five hours. No AZA/6-MP is detectable in urine after 8 hours. Azathioprine/6-MP is moderately bound to serum proteins and is partially dialyzable.\textsuperscript{27-29}

Each tablet contains 92.5 to 107.5% of the stated amount. After ingestion AZA/6-MP is rapidly broken down in vivo into 6-MP and a methylthioimidazole moiety. The 6-MP readily crosses cell membranes and is converted intracellularly into a number of purine thio-analogues, which include the main active nucleotide, thioinosinic acid. It is of interest that the rate of conversion varies from one person to another.\textsuperscript{30-34}

The mechanisms of AZA/6-MP action are presently unknown; many mechanisms have been suggested in order to explain also the common clinical place that AZA/6-MP therapeutic effects may be evident only after several weeks or months of treatment. These mechanisms include the release of 6-MP which acts as a purine antimetabolite, the possible blockade of –SH groups by alkylation, the inhibition of many pathways in nucleic acid biosynthesis, hence preventing proliferation of cells involved in determination and amplification of the immune response, the damage to deoxyribonucleic acid (DNA) through incorporation of purine thio-analogues [Figure].

**INTRODUCING THE TPMT GENE AND ITS POLYMORPHISMS**

The human TPMT gene is located on chromosome 6p22.3 and is approximately 34 kb in length, consisting of 10 exons. TPMT activity is inherited as an autosomal dominant trait. The hereditary nature of the TPMT deficiency in humans was initially identified in a study of TPMT activity in red blood cells (RBC). This and subsequent studies determined the distribution of TPMT activity in RBC to be trimodal; 90% of persons have high activity, 10% have intermediate activity and 0.3% have low or no detectable enzyme activity.\textsuperscript{37}

Altered TPMT activity predominantly results from single nucleotide polymorphisms (SNPs). To date, numerous TPMT alleles have been identified.\textsuperscript{38-49} Four prevalent mutant alleles (TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C) account for 75% to 80% of TPMT mutations and are associated with various degrees of reduction in TPMT activity.

The mutant allele TPMT*2 is defined by a single nucleotide transversion (G238C) in the open reading frame (ORF), leading to an amino acid substitution at codon 80 (Ala->Pro) and resulting in a 100-fold reduction in catalytic activity compared to the wild type. The second and more prevalent mutant allele, TPMT*3A, contains two nucleotide transition mutations (G460 and A719G) in the ORF, leading to amino acid substitutions at codon 154(Ala->Thr) and codon 240(Tyr->Cys). The allele TPMT*3B has only the G460A mutation and leads to a nine-fold reduction in catalytic activity. TPMT*3C has only the A719G mutation and leads to a 1.4 reduction in catalytic activity. Thus, G460A mutation carries a higher than A719G risk of toxicity.\textsuperscript{50-54}

**LABORATORY METHODS OF SCREENING FOR TPMT POLYMORPHISMS**

Many methods of screening for TPMT polymorphisms have been developed or are under evaluation.\textsuperscript{55-60} These methods will not be analysed in this review.
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ENZYMES IN AZATHIOPRINE METABOLIZATION

Briefly, these methods include analysis for one or more SNP and visualization in gels using polymerase chain reaction / restriction fragment length polymorphism methods (PCR-RFLP) or other methods investigating simultaneously more than one SNP (i.e multiplex PCR).

Finally sequencing of the TPMT gene still remains an accurate, although expensive method. Laboratory expertise and standardized technique is mandatory for clear-cut results.

PREVALENCE OF TPMT GENE POLYMORPHISMS IN ETHNIC POPULATIONS

The prevalence of the most frequent single nucleotide polymorphisms (SNPs) in the TPMT gene has been reported to vary worldwide\(^a\)\(^b\)\(^c\) [Table 1], however approximately 90-95% of the healthy population has no TPMT variant allele while the rest have one or more of the most frequent variants. Knowing the prevalence of TPMT SNPs in ethnic populations seems to be of help in more accurately assessing and predicting the risk of AZA/6-MP toxicity.\(^d\)^\(^e\)\(^f\)\(^g\)

PREVALENCE OF TPMT GENE POLYMORPHISMS IN PATIENT GROUPS

TMPT turns out nowadays to be an ‘old story’ but as AZA/6-MP are more and more extensively used in many diseases including IBD the need for predicting toxicity is constantly rising.

It seems that the lack of large scale comparative studies on TPMT polymorphisms between patient groups and background population is mainly due to the fact that there is still conflicting data on whether TPMT genotype or TPMT activity are safe in predicting common adverse events to AZA/6-MP. In fact, there are studies in favour or against the clinical importance of TPMT genotyping in AZA/6-MP treated groups of patients.

In the study from Leuven\(^h\) it has been demonstrated that TPMT allele frequency may differ between IBD patients and healthy control populations. This study is not the only one to support that TPMT polymorphisms may differ between a patient and a control group from the same ethnic origin. A significant difference for the TPMT*3C allele has already been demonstrated in lupus erythematosus patients,\(^i\) in children with acute leukemia\(^j\) and
patients with neurological diseases, all of them under AZA/6-MP maintenance therapy [Table 2].

TPMT has been thoroughly investigated as its substrates AZA/6-MP can cause, among other side effects, bone marrow suppression and according to a review of nine retrospective studies there was a 3.2% overall frequency of leucopenia in IBD patients treated with AZA/6-MP. However, none of these studies provided large comparative data on the prevalence of TPMT polymorphisms of IBD patients compared to background population. Furthermore, a restricted number of studies has implicated that IBD patients have the same prevalence and the same pattern of TPMT mutated alleles compared to background populations [Table 2]. Three studies suggested that the frequency of variant alleles does not differ between IBD patients and healthy controls from the same background Caucasian population. It is of importance to mention that TPMT variant alleles may vary among studies in Caucasians and this may reflect ethnic differences.

We believe that this issue still remains debatable and

### Table 1. Prevalence of frequent TPMT gene alleles in ethnic groups.

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>No of alleles</th>
<th>TPMT*1</th>
<th>TPMT*2</th>
<th>TPMT*3A</th>
<th>TPMT*3C</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Caucasians</td>
<td>564</td>
<td>0.963</td>
<td>0.0017</td>
<td>0.032</td>
<td>0.0017</td>
<td>Hon et al.61</td>
</tr>
<tr>
<td>Americans African</td>
<td>496</td>
<td>0.954</td>
<td>0.004</td>
<td>0.008</td>
<td>0.024</td>
<td>Hon et al.61</td>
</tr>
<tr>
<td>Asians Southwest (British)</td>
<td>198</td>
<td>0.990</td>
<td>0</td>
<td>0.010</td>
<td>0</td>
<td>Collie-Duguid et al.62</td>
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<tr>
<td>Asians Southeast</td>
<td>600</td>
<td>0.993</td>
<td>0.0017</td>
<td>0</td>
<td>0.050</td>
<td>Chang et al.63</td>
</tr>
<tr>
<td>Asians (British)</td>
<td>170</td>
<td>N/A*</td>
<td>N/A*</td>
<td>0.011</td>
<td>0.047</td>
<td>Marinaki et al.64</td>
</tr>
<tr>
<td>Argentinian</td>
<td>294</td>
<td>0.960</td>
<td>0.0068</td>
<td>0.031</td>
<td>0</td>
<td>Larovere et al.65</td>
</tr>
<tr>
<td>Belgians</td>
<td>742</td>
<td>0.894</td>
<td>0.008</td>
<td>0.08</td>
<td>0.0013</td>
<td>Katsanos et al.66</td>
</tr>
<tr>
<td>British Caucasians</td>
<td>398</td>
<td>0.947</td>
<td>N/A</td>
<td>0.045</td>
<td>0.003</td>
<td>Ameayew et al.67</td>
</tr>
<tr>
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<td>398</td>
<td>N/A</td>
<td>0.004</td>
<td>0.045</td>
<td>0.003</td>
<td>McLLeod et al.68</td>
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<tr>
<td>British Caucasians</td>
<td>398</td>
<td>0.948</td>
<td>0.005</td>
<td>0.045</td>
<td>0.002</td>
<td>Collie-Duguid et al.62</td>
</tr>
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<td>Brasilian (mixed)</td>
<td>306</td>
<td>N/A</td>
<td>0.0082</td>
<td>0.0163</td>
<td>0.0212</td>
<td>Reis et al.69</td>
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<tr>
<td>Chinese</td>
<td>384</td>
<td>0.977</td>
<td>N/A</td>
<td>0</td>
<td>0.023</td>
<td>Collie-Duguid et al.62</td>
</tr>
<tr>
<td>Chinese (Han)</td>
<td>550</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.026</td>
<td>Zhang et al.70</td>
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<td>Chinese (Han)</td>
<td>624</td>
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<td>N/A</td>
<td>N/A</td>
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<td>Zhang et al.70</td>
</tr>
<tr>
<td>Chinese (Jing)</td>
<td>206</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.019</td>
<td>Zhang et al.70</td>
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<tr>
<td>Chinese (Yao)</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.037</td>
<td>Zhang et al.70</td>
</tr>
<tr>
<td>Chinese (Uyghur)</td>
<td>320</td>
<td>N/A</td>
<td>N/A</td>
<td>0.00625</td>
<td>0.03125</td>
<td>Zhang et al.71</td>
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<tr>
<td>Colombian</td>
<td>280</td>
<td>0.960</td>
<td>0.0038</td>
<td>0.036</td>
<td>0</td>
<td>Isaza et al.72</td>
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<tr>
<td>Egyptian</td>
<td>400</td>
<td>0.984</td>
<td>0</td>
<td>0.003</td>
<td>0.013</td>
<td>Hamdy et al.73</td>
</tr>
<tr>
<td>French Caucasians</td>
<td>382</td>
<td>N/A</td>
<td>0.005</td>
<td>0.057</td>
<td>0.08</td>
<td>Dela Moureyre et al.74</td>
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<tr>
<td>French Caucasians</td>
<td>560</td>
<td>N/A</td>
<td>0.01</td>
<td>0.059</td>
<td>0.07</td>
<td>Ganiere-Monteil et al.75</td>
</tr>
<tr>
<td>Germans</td>
<td>2428</td>
<td>0.898</td>
<td>0.005</td>
<td>0.08</td>
<td>0.006</td>
<td>Schaefer et al.57</td>
</tr>
<tr>
<td>Ghanaians</td>
<td>434</td>
<td>0.924</td>
<td>0</td>
<td>0</td>
<td>0.076</td>
<td>Ameayew et al.67</td>
</tr>
<tr>
<td>Italians</td>
<td>206</td>
<td>N/A</td>
<td>0.005</td>
<td>0.039</td>
<td>0.01</td>
<td>Rossi et al.76</td>
</tr>
<tr>
<td>Indians</td>
<td>400</td>
<td>0.987</td>
<td>0</td>
<td>0.005</td>
<td>0.008</td>
<td>Kham et al.77</td>
</tr>
<tr>
<td>Japanese</td>
<td>1044</td>
<td>0.984</td>
<td>N/A</td>
<td>0</td>
<td>0.015</td>
<td>Kumagai et al.78</td>
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<td>Japanese</td>
<td>142</td>
<td>0.979</td>
<td>N/A</td>
<td>0</td>
<td>0.014</td>
<td>Ando et al.79</td>
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<tr>
<td>Japanese</td>
<td>302</td>
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<td>0</td>
<td>0</td>
<td>0.003</td>
<td>Kubota et al.80</td>
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<tr>
<td>Japanese</td>
<td>192</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>0.008</td>
<td>Hiratsuka et al.81</td>
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<td>Japanese</td>
<td>88</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>0.022</td>
<td>Nishida et al.82</td>
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<td>Kenyans</td>
<td>202</td>
<td>0.946</td>
<td>0</td>
<td>0</td>
<td>0.054</td>
<td>McLLeod et al.68</td>
</tr>
<tr>
<td>Malaysians</td>
<td>400</td>
<td>0.975</td>
<td>0</td>
<td>0</td>
<td>0.023</td>
<td>Kham et al.77</td>
</tr>
<tr>
<td>New Zealand (Caucasians)</td>
<td>200</td>
<td>0.950</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>Gearry et al.83</td>
</tr>
<tr>
<td>Polish</td>
<td>716</td>
<td>N/A</td>
<td>0.004</td>
<td>0.027</td>
<td>0.001</td>
<td>Kurzawski et al.84</td>
</tr>
<tr>
<td>Saami (Norway)</td>
<td>388</td>
<td>0.969</td>
<td>0</td>
<td>0</td>
<td>0.033</td>
<td>Loemmenchen et al.85</td>
</tr>
<tr>
<td>Swedish</td>
<td>1600</td>
<td>0.956</td>
<td>0.00063</td>
<td>0.0375</td>
<td>0.0044</td>
<td>Haglund et al.56</td>
</tr>
<tr>
<td>Taiwanese</td>
<td>498</td>
<td>0.994</td>
<td>0</td>
<td>0</td>
<td>0.014</td>
<td>Chang et al.63</td>
</tr>
<tr>
<td>Thai</td>
<td>400</td>
<td>0.950</td>
<td>N/A</td>
<td>0</td>
<td>0.050</td>
<td>Shrimartpirom et al.86</td>
</tr>
</tbody>
</table>

* N/A = not available or not investigated
Table 2. The TPMT*3C allele prevalence in patient groups treated with azathioprine/6-MP.

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient group</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okada et al.93</td>
<td>Systemic lupus erythematosus (n=68)</td>
<td>174</td>
<td>0.23</td>
</tr>
<tr>
<td>Alves et al.40</td>
<td>Acute lymphoblastic leukemia children (n=43)</td>
<td>43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Yates et al.94</td>
<td>Acute lymphoblastic leukemia children (n=73)</td>
<td>209</td>
<td>&lt;0.05 but only cases with abnormal TPMT enzyme activity genotyped</td>
</tr>
<tr>
<td>Heckman et al.95</td>
<td>Neurologic patients (n=129)</td>
<td>465</td>
<td>NS</td>
</tr>
</tbody>
</table>

PREDICTING AZA/6-MP ADVERSE EVENTS

Two types of adverse reactions can occur in 5-25% of AZA/6-MP treated patients. The first is the allergic type and the second is the non-allergic type.90 The first type usually occurs within the first 3-4 weeks of treatment and is not dose-dependent. It includes pancreatitis, fever, skin rash, general malaise, digestive intolerance and hepatotoxicity. The second type seems to occur later; it is dose-dependent and includes bone marrow toxicity, infections and malignancy. However the potential of long-term AZA/6-MP therapy to substantially increase the risk of malignancy is still debatable.

Patch tests with AZA/6-MP 1% have been suggested to be a safe and reliable tool in the diagnosis of hypersensitivity reactions to this drug.100

Nausea and vomiting may occur within the first few months of AZA/6-MP therapy. The frequency of gastric disturbance can be reduced by administration of the drug in divided doses and/or after meals. However in some patients nausea and vomiting may be severe and may be accompanied by symptoms such as diarrhoea, fever malaise and myalgias. Vomiting with abdominal pain may occur rarely with hypersensitivity pancreatitis.101-104

The most common hematologic abnormality induced by AZA/6-MP appears to be leucopenia (0-10%). If treated with standard doses of thiopurines, TPMT deficient patients accumulate excessive 6-TGN levels in hematopoietic tissues, potentially leading to hematological toxicity, which may be fatal in some cases.105-106 It has been suggested that TPMT status may predict early bone marrow toxicity.107 Interestingly, bone marrow toxicity has been reported to occur up to 11 years after AZA/6-MP therapy initiation.108

ARE TPMT GENE POLYMORPHISMS OF CLINICAL VALUE?

Conflicting data exists as to whether TPMT genotype is useful in predicting common adverse events such as nausea, vomiting and abdominal discomfort as well as rarer recognized complications such as hepatitis and pancreatitis.3 Although the incidence of hepatotoxicity may correlate with 6-MMP levels (the product of TPMT enzymatic reaction), the levels of 6-MMP do not correlate with genotype. Ansari et al. showed that patients with intermediate TPMT activity had a significantly higher rate of AZA/6-MP intolerance when compared to patients with high activity.109 Other studies have failed to show a correlation

Table 3. Studies on TPMT allele prevalence in inflammatory bowel disease patients (CD/UC) and healthy controls

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>IBD patients</th>
<th>No patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corominas et al.97</td>
<td>2000</td>
<td>UC</td>
<td>146</td>
<td>NS</td>
</tr>
<tr>
<td>Reuther et al.98</td>
<td>2003</td>
<td>CD</td>
<td>120</td>
<td>No control group</td>
</tr>
<tr>
<td>Reuther et al.99</td>
<td>2004</td>
<td>CD</td>
<td>52 (mixed)</td>
<td>No control group</td>
</tr>
<tr>
<td>Katsanos et al.66</td>
<td>2006</td>
<td>CD / UC</td>
<td>1031 / 308</td>
<td>&lt;0.0001 in CD for the TPMT*1/3A allele</td>
</tr>
<tr>
<td>Schwab et al.49</td>
<td>2002</td>
<td>CD / UC</td>
<td>77 / 16</td>
<td>NS</td>
</tr>
</tbody>
</table>
between TPMT genotype and the development of these adverse events. Additionally, a report suggested AZA/6-MP intolerance may be imidazole-related and is independent of TPMT.110

Anyway, the great majority of these studies were of retrospective design. We believe that a prospective study may be more informative on the real clinical impact of screening for TPMT polymorphisms in patients on AZA/6-MP therapy.

**CLINICAL VALUE OF TPMT GENE POLYMORPHISMS IN IBD**

AZA/6-MP can cause bone marrow suppression and has been associated with leucopenia, thrombocytopenia, macrocytosis without megaloblastic anemia and rarely pure red cell aplasia.17

TPMT seems of importance in IBD as the largest study108 on AZA toxicity reported a 3.8% prevalence of leucopenia in 739 patients treated with AZA/6-MP; in this cohort two patients died as a result of pancytopenia and sepsis.

In a cost effectiveness analysis study it has been suggested that in 1000 IBD patients treated with AZA/6-MP, 32 will develop myelosuppression and one will die because of this. Of those who develop myelosuppression during AZA/6-MP therapy only 32% is attributed to low TPMT enzyme activity.96

There are many studies supporting the clinical value of screening for TPMT gene SNP in IBD patients before or during AZA/6-MP therapy. According to a study half of the patients with one or two non-functional TPMT mutant alleles will develop AZA/6-MP intolerance leading to withdrawal of therapy.99 Another study showed that patients with Crohn’s disease (CD) and normal TPMT activity who were started on high dose AZA/6-MP and patients with intermediate enzyme activity who were started on reduced doses of AZA/6-MP did not develop acute leucopenia.111

Even though another study confirmed the efficiency of TPMT genotyping in identifying patients at risk for myelosuppression it had its limitation as only 27% of patients carrying the mutated TPMT allele had a parallel enzyme deficiency.9 Another study not favouring TPMT genotyping showed that only one of thirteen patients who experienced leucopenia was heterozygous for TPMT.

The vast majority of patients with drug related toxicity had a wild type TPMT genotype.112 In 56 patients with IBD TPMT genotype did not predict adverse reactions to AZA/6-MP.85 In addition, AZA/6-MP-related gastrointestinal side effects have been suggested to be independent of the TPMT polymorphism in a retrospective analysis of IBD patients taking AZA/6-MP.49

We have to stress here that these studies referred to the three more frequent TPMT SNPs. However, hematotoxicity may occur in the absence of TPMT*2, TPMT*3A, TPMT*3B or TPMT*C variants due to presence of other rare TPMT variants or other factors including viral infections, drugs or environment.113-120

To summarize it seems that TPMT genotyping is able to predict some but not the majority of AZA/6-MP cases which will develop toxicity including bone marrow toxicity.

**CLINICAL VALUE OF TPMT POLYMORPHISMS IN NON-IBD PATIENT GROUPS**

In rheumatoid arthritis patients gastrointestinal intolerance has been related to thiopurine metabolic imbalance resulting in a significant relationship between toxicity and abnormal TPMT activity.121

In a study with neurological patients taking AZA/6-MP it has been suggested that TPMT genotyping is preferable to TPMT activity determination;49 however, only TPMT homozygous deficiency was associated with severe marrow suppression in a study with lupus erythematosus patients.122

Improved methodology for monitoring thiopurine metabolites in patients on thiopurine therapies is mandatory in order to facilitate accurate clinical decisions.123

**AZA/6-MP METABOLITES MONITORING**

There have been many studies in favour of or against the monitoring of AZA/6-MP metabolite levels.124-136 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 AZA/6-MP metabolite assessment requires expertise, standardized methods and is indicated only in cases with unexplained dose-related side effects in patients were AZA/6-MP use is mandatory for disease remission.

**TPMT ENZYME LEVELS IN CLINICAL PRACTICE**

TPMT enzyme is already mature in birth and practically no differences exist between children and adults.75
Some but not all studies have indicated that AZA/6-MP therapy monitoring with erythrocyte 6-thioguanine nucleotides may be clinically useful.

It has been suggested that patients with low or intermediate TPMT activity account for approximately half of early leucopenia (within the first 6 months of AZA/6-MP initiation) whereas patients with normal TPMT activity accounted for approximately 50% of the early leucopenia and nearly all the late (after 6 months of therapy) leucopenia cases. It must be stressed here, that some authors define as ‘early’ all leucopenia cases diagnosed the first 2-3 months of AZA therapy, while ‘intermediate’ cases are those diagnosed during the 4-7 month period of AZA therapy.

A study suggested that TPMT deficiency significantly correlates with haematopoietic toxicity and another study showed that TPMT activity was significantly lower in patients who discontinued AZA/6-MP due to leucopenia than in those who discontinued due to other side effects.

In contrast, another study supported that measurement of TPMT activity has no specific role in identifying risk of haematological toxicity. Along these lines, in addition to a high degree of variability in TPMT activity within both the homozygous wild type and heterozygous groups, some individuals with a heterozygous genotype exhibit high activity whereas some homozygous wild type subjects exhibit an intermediate phenotype; attention has to be paid also to transfused individuals.

Such discrepancies are due to the fact that the SNPs discussed so far are not the only factors regulating catalytic activity. Population genetic studies have shown that the genotype, which regulates TPMT activity, accounted for approximately two-thirds of the total variance in the level of RBC enzyme activity. Other factors such as promoter polymorphisms, drug interactions, and environmental factors could play an important role in the final TPMT activity phenotype.

Blood levels of the drug are of little predictive value for therapy since the magnitude and duration of clinical effects and side effects seem to correlate with thiopurine nucleotide levels in tissues rather than with plasma drug levels.

A definite consensus on the clinical value of TPMT enzyme levels determination is wishful. We do believe like others that combining information on TPMT gene SNPs and TPMT enzyme levels could be of importance not only to predict toxicity but also unrevealing AZ/6-MP kinetics and mechanism of action in several tissues.

### FURTHER PERSPECTIVES

**IN IBD RESEARCH: TPMT LOCUS AND OTHER LOCI**

Screening for new TPMT polymorphisms is of immediate interest in IBD not only in view of firmly assessing the risk of toxicity during AZA/6-MP therapy but also in view of unrevealing disease etiopathogenesis.

TPMT gene is located on chromosome 6p. Previous association and linkage analysis has provided some evidence as to the existence of an IBD/CD-susceptibility locus, referred to as IBD3, in this region. It is also of interest that on chromosome 6p are located the human-leucocyte-antigen (HLA) region, the major histocompatibility complex (MHC) region and the tumour necrosis factor (TNF)-a gene, all of them associated with IBD susceptibility. Interestingly, the group of IBD pedigrees that contained one of the three CARD15 variants had two suggestive linkage results occurring in 6p and 10p.

By contrast, other polymorphisms located on chromosome 6p, including also some HLA and MHC polymorphisms, were not associated with an increased risk for IBD.

### CONCLUSIONS

Pharmacogenetics of AZA/6-MP represents an interesting field of research with direct implications in clinical practice and fundamental research (Table 4). AZA/6-MP metabolism steps, the impact of TPMT gene SNPs on toxicity prediction as well as the 6p loci analysis represents a challenging field of research in IBD as well as in other diseases.

When possible, routine TPMT genotyping prior to the initiation of AZA/6-MP should be considered to decrease the risk of a severe adverse event as well as to identify patients who might benefit from higher doses. Although TPMT testing does not eliminate the risk of bone marrow toxicity it has the potential to warn early of a life-threat-

### Table 4. The clinical usefulness of TPMT testing.

- Possibility to start from very early the optimal dose of AZA/6-mercaptopurine
- Possibility to further increase the dose of AZA/6-mercaptopurine (up to 3mg/kg)
- Possibility to prevent early or intermediate leucopenia in high risk patients (with gene homozygous or heterozygous polymorphisms)
- TPMT testing cannot replace the need for regular peripheral blood testing in all patients on AZA/6-mercaptopurine therapy.
ening adverse event due to very low TPMT enzyme activity in homozygous recessive patients.

Thus, clinicians should be aware that TPMT SNPs do not explain all leucopenic events and that TPMT measurement cannot replace the need for continued monitoring of leucocyte counts in AZA/6-MP treated patients.

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