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REVIEW

Genetic polymorphism of thiopurine methyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia

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Thiopurine methyltransferase (TPMT) catalyses the S-methylation of thiopurines, including 6-mercaptopurine and 6-thioguanine. TPMT activity exhibits genetic polymorphism, with about 1/300 inheriting TPMT deficiency as an autosomal recessive trait. If treated with standard doses of thiopurines, TPMT-deficient patients accumulate excessive thioguanine nucleotides in hematopoietic tissues, leading to severe hematological toxicity that can be fatal. However, TPMT-deficient patients can be successfully treated with a 10- to 15-fold lower dosage of these medications. The molecular basis for altered TPMT activity has been defined, with rapid and inexpensive assays available for the three signature mutations which account for the majority of mutant alleles. TPMT genotype correlates well with *in vivo* enzyme activity within erythrocytes and leukemic blast cells and is clearly associated with risk of toxicity. The impact of 6-mercaptopurine dose intensity is also being clarified as an important determinate of event-free survival in childhood leukemia. In addition, there are emerging data that TPMT genotype may influence the risk of secondary malignancies, including brain tumors and acute myelogenous leukemia. Ongoing studies aim to clarify the influence of TPMT on thiopurine efficacy, acute toxicity, and risk for delayed toxicity. Together, these advances hold the promise of improving the safety and efficacy of thiopurine therapy. *Leukemia* (2000) **14,** 567–572.

Keywords: thiopurine methytransferase; childhood acute lymphoblastic leukemia; pharmacogenetics; 6-mercaptopurine

Introduction

6-Mercaptopurine (6-MP) is one of the most widely used medications for childhood acute lymphoblastic leukemia (ALL).[1,2] In standard protocols, 6-MP is administered as a daily oral dose for a majority of the 2–3 years of maintenance therapy. 6-MP and the other thiopurines, azathioprine and thioguanine, are all inactive prodrugs, requiring metabolism to thioguanine nucleotides (TGN) in order to exert cytotoxicity.[3,4] The principal cytotoxic mechanism of these agents is generally considered to be mediated via incorporation of TGN into DNA and RNA (Figure 1). For 6-MP, TGNs are formed by a multi-step pathway which is initiated by hypoxanthine phosphoribosyl transferase.[4] Alternatively, these agents can undergo S-methylation catalyzed by thiopurine methyltransferase (TPMT) to methylmercaptopurine (meMP) or oxidation to thiouric acid via xanthine oxidase. Metabolism via either TPMT or xanthine oxidase reduces formation of the active TGNs. TPMT can also S-methylate 6-thioinosine 5 monophosphate (TIMP), yielding the S-methylated derivative (methylTIMP). MethylTIMP is a potent inhibitor of *de novo* purine synthesis and represents an alternative mechanism for cytotoxicity.[3,4]

Clinicians have known for some time that a small subset of patients is intolerant to thiopurine therapy. Individual differences in TGN accumulation after 6-MP therapy have been shown to be a significant determinate of hematopoietic toxicity and anti-leukemic effects.[5–7] The cellular accumulation of TGN is inversely related to TPMT activity, presumably because high TPMT activity shunts more drug down the methylation pathway, leaving less for activation to TGNs.[3,4] Furthermore, several studies have documented that TPMT-deficient patients accumulated very high TGN concentrations in erythrocytes (RBC).[6,7] This will result in severe hematopoietic toxicity in TPMT-deficient patients, unless the thiopurine dosage is reduced 10- to 15-fold.[6,7] The use of azathioprine in a patient with complete TPMT deficiency resulted in death from neutropenic sepsis.[8]

Population studies have found the distribution of TPMT activity in erythrocytes (RBC) to be trimodal; approximately 90% of persons have high activity, 10% have intermediate activity, and 0.3% have low or no detectable enzyme activity.[4,9] Family and molecular genetic studies have documented that the 10% with intermediate activity are heterozygous at the TPMT gene locus and the TPMT-deficient subjects are homozygous for low activity alleles. Ethnic variation in TPMT has been observed, with a median 20% lower activity in African–American subjects compared with Caucasians from the same location.[9]

Molecular basis for altered TPMT activity

The molecular basis for altered TPMT activity has now been defined for the majority of patients.[10,11] To date, eight TPMT alleles have been identified, including three alleles (*TPMT*2*, *TPMT*3A* and *TPMT*3C*) which account for 80–95% of intermediate or low enzyme activity cases (Figure 2).[10–15] The mutant allele *TPMT*2* is defined by a single nucleotide transversion (G238C) in the open reading frame, leading to an amino acid substitution at codon 80 (Ala Pro).[16] When assessed in a yeast heterologous expression system, this mutation lead to a 100-fold reduction in TPMT activity relative to wild-type cDNA, despite a comparable level of messenger RNA expression.[13] The second and more prevalent mutant allele, *TPMT*3A*, contains two nucleotide transition mutations (G460A and A719G) in the open reading frame, leading to amino acid substitutions at codon 154 (Ala Thr) and codon 240 (Tyr Cys).[12] When heterologously expressed in yeast or COS-1 cells, *TPMT*3A* had 200-fold lower TPMT activity and immunodetectable protein compared to wild-type cDNA.[13] Heterologous expression in yeast established an enhanced rate of proteolysis of mutant TPMT proteins enco-

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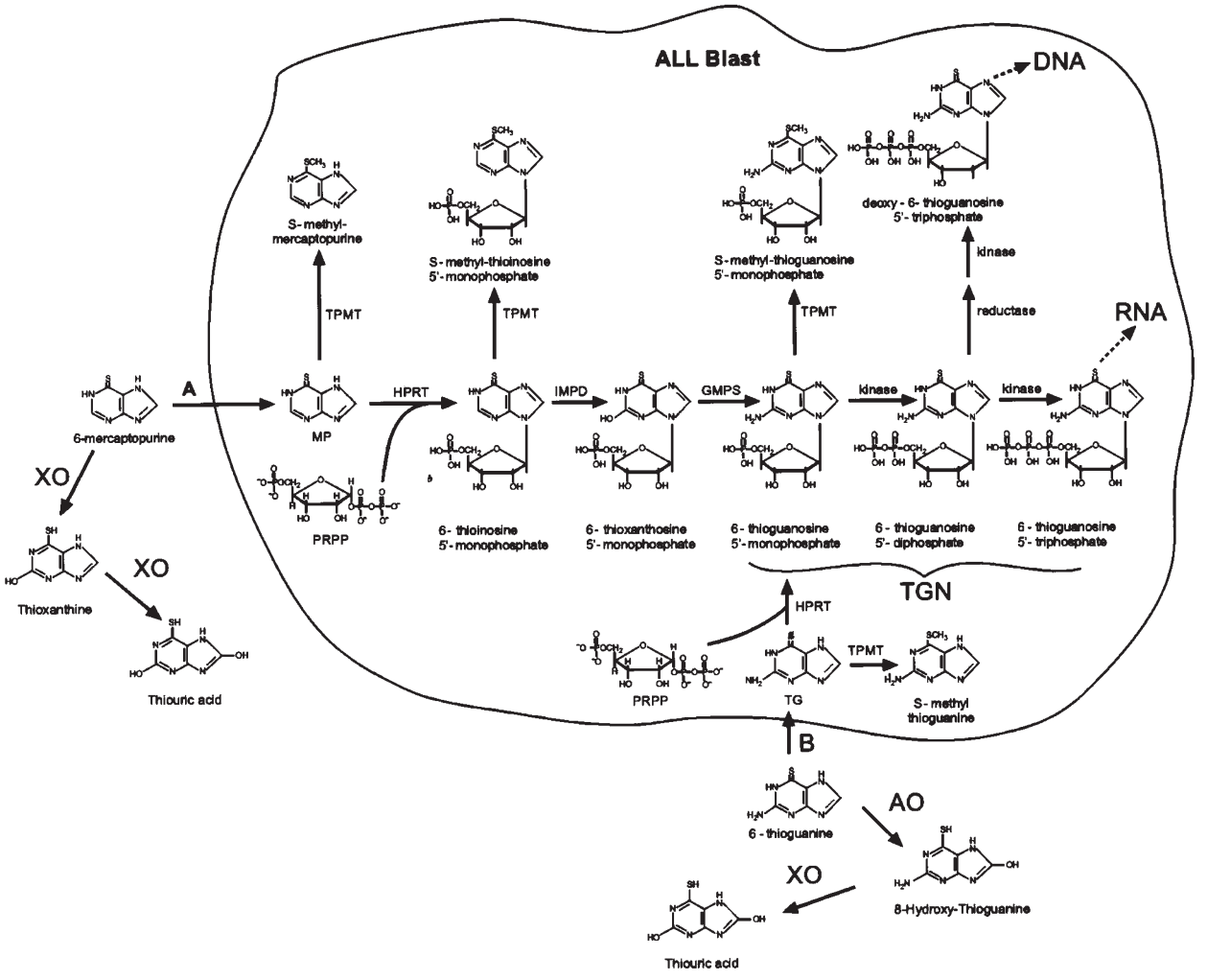

Figure 1 Metabolism of 6-mercaptopurine (A), and 6-thioguanine (B) in human leukemia cells. Modified from Refs 33 and 34.

ded by *TPMT*2* and *TPMT*3A* alleles, with degradation half lives of approximately 15 min for both mutant proteins compared with 18 h for the wild-type protein.[13] Subsequent studies also established that *TPMT*3B* and *TPMT*3C* proteins have an enhanced rate of proteolysis when expressed in mammalian cells,[17] consistent with the lower protein levels in individuals who inherit these alleles.[18] The mutant alleles *TPMT*4–8* have also been identified during clinical genotype-phenotype analysis (Figure 2). *TPMT*4* has a G A transition at the intron 9–exon 10 junction which disrupts the final nucleotide of the intron at the 3 acceptor splice site sequence.[11,15] *TPMT*5* was identified as a T146C transition in a heterozygous individual of undefined ethnicity who had intermediate TPMT activity.[11] This mutation results in a Leu Ser amino acid substitution at codon 49. *TPMT*6* was identified in a Korean subject with intermediate activity.[11] This A539T transversion in exon 8 results in a Tyr Phe substitution at codon 180. *TPMT*7* was identified in a single European subject with intermediate TPMT activity.[14] This allele contains a T681G transversion in exon 10 which results in a His Glu amino acid substitution at codon 227. Lastly, *TPMT*8* contains a single nucleotide transition (G644A) leading to an amino acid change at codon 215 (Arg His).[19] This allele has been identified in one African–American individual with intermediate activity.

Based on the population genotype–phenotype studies performed to date, assays for the molecular diagnosis of TPMT deficiency have focused on alleles *TPMT*2, TPMT*3A* and *TPMT*3C*.[10] By using allele-specific PCR or PCR-RFLP to detect the three signature mutations in these alleles, a rapid and relatively inexpensive assay can be performed which will identify 80–95% of all mutant alleles.[10,11] Population studies in Caucasian, East and West African, African–American, Chinese, Japanese and Southwest Asian populations have demonstrated the utility of this approach[10,19–23] (Figure 3). However, the frequency and pattern of mutant TPMT alleles is different among various ethnic populations. For example, Southwest Asians (Indian, Pakistani) have a lower frequency of mutant TPMT alleles and all mutant alleles identified to date are *TPMT*3A* (Table 1).[21] This is in contrast with the East and West African population in which the frequency of mutant alleles is similar to Caucasians, but all mutant alleles in the African populations are *TPMT*3C* (Table 1).[20,22] Among African–Americans, *TPMT*3C* is the most prevalent, but *TPMT*2* and *TPMT*3A* are also found, reflecting the integration of Caucasians and African–Americans genes in the US populations.[19]

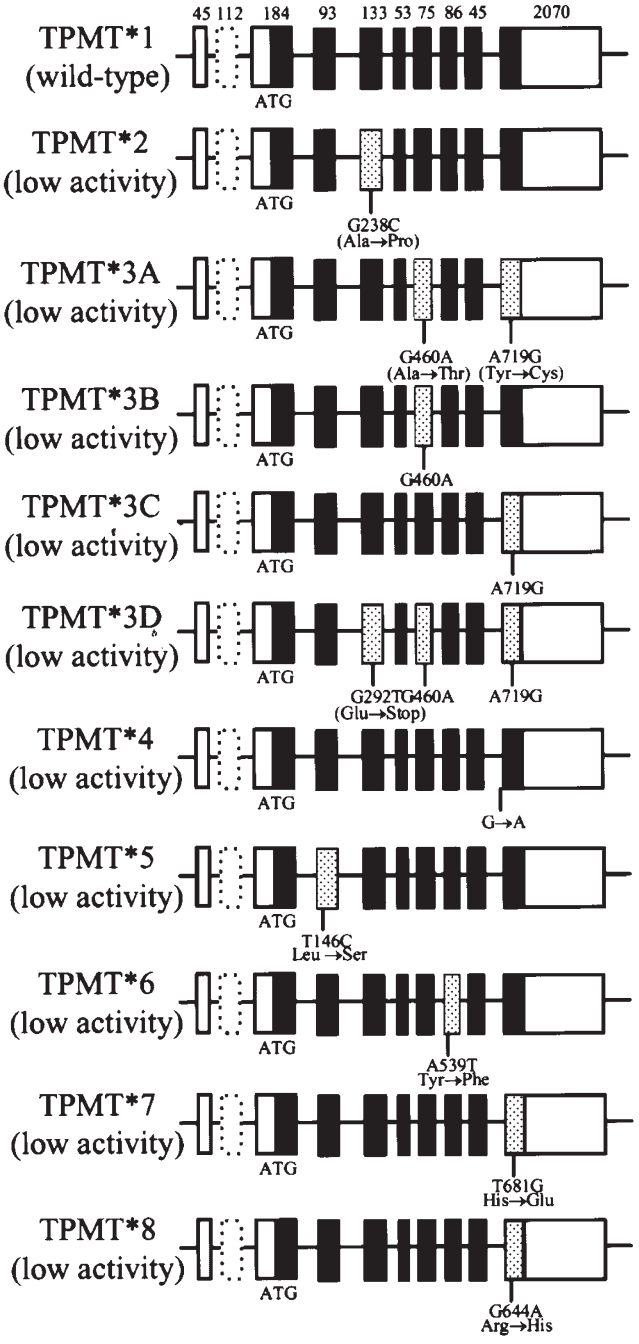

Figure 2 Allele variants at the human thiopurine methyltransferase locus. Gray boxes represent exons that contain mutations that result in changes of amino acids. White boxes are untranslated regions and black boxes represent exons in the open reading frame. The dashed box represents exon 2, which was detected in one of 16 human liver cDNAs.

Genotype–phenotype relationship

The relationship between TPMT genotype and phenotype has been most clearly defined for *TPMT*2*, *TPMT*3A* and *TPMT*3C* in patients with leukemia and normal volunteers.[10,24] *TPMT*2* is the least common of the three alleles, representing 0.2–0.5% of all alleles in Caucasian populations.[10,21,22,24–26] In Caucasian subjects, *TPMT*3A* is the most common of the three alleles, with a frequency of 3.2–5.7%, while *TPMT*3C* has an allele frequency 0.2–0.8%.[10,22,24–26] The presence of *TPMT*2*, *TPMT*3A* or *TPMT*3C* is predictive for phenotype, in that patients heterozygous for these alleles all have intermediate activity and subjects homozygous for these alleles are TPMT deficient.[10] In addition, compound heterozygotes (*TPMT*2/*3A*, *TPMT*3A/*3C*) are also TPMT deficient,[10] as would be expected. While most studies to date have used erythrocytes as a surrogate tissue for measuring TPMT activity, a recent study demonstrated that TPMT genotype also influences TPMT activity in blast cells from leukemia patients,[24] as would be predicted from previous studies of TPMT activity in these two tissues.[27] The median TPMT activity among 50 children and adults with ALL, who were homozygous for a wild-type genotype, was 0.25 nU/mg protein compared with 0.1 nU/mg protein in the five patients heterozygous for *TPMT*3A*.[24] A high degree of variability in TPMT activity was observed within both the homozygous wild-type and heterozygous patient groups, suggesting that these TPMT nucleotide polymorphisms are not the only factors regulating catalytic activity (eg promoter polymorphisms, drug interactions, diagnosis, environment, etc).

Relationship between genotype and toxicity

The enthusiasm for TPMT pharmacogenetics has been stimulated by the confirmation that TPMT genotype does identify patients who are at risk of toxicity from 6-MP or azathioprine. In a study of 67 patients treated with azathioprine for rheumatic disease, six patients (9%) were heterozygous for mutant TPMT alleles.[26] Five of the six patients discontinued therapy because of low leukocyte counts within 1 month of starting treatment. The sixth patient had documented non-compliance with azathioprine therapy. Patients with wild-type TPMT received therapy for a median 39 weeks compared with a median of 2 weeks in patients heterozygous for mutant TPMT alleles. This study clearly demonstrated that prospective knowledge of TPMT genotype will aid the clinical management of patients receiving thiopurine therapy. In addition, it highlights the importance of not relying on molecular biology alone. One of the patients with a heterozygous genotype had no toxicity, because she was non-compliant with her azathioprine therapy. Therefore the combination of molecular testing and patient counselling is needed to make management more effective.

The impact of a heterozygous TPMT genotype in ALL has recently been examined. A study of 147 British children with ALL identified a heterozygous variant TPMT allele frequency of 10.9%, with one patient (0.7%) homozygous for low TPMT activity.[2] There was no clear difference in the percentage of weeks where no therapy could be administered for the homozygous wild-type and heterozygous patients. However, the patient with a homozygous mutant TPMT genotype received no therapy for 53% of the maintenance period, due to severe toxicity from 6-MP.[2] With the recent data demonstrating the clinical importance of 6-MP dose intensity on survival, clinical situations such as that found in this homozygous mutant individual could place them at greater risk for a poor outcome from ALL therapy. A recent study in 180 children with ALL treated at St Jude Children's Research Hospital also identified an important role for TPMT genotype on tolerance to 6-MP therapy.[28] The two TPMT-deficient patients tolerated full dose 6-MP for only 7% of weeks, whereas heterozygous and homozygous wild-type patients tolerated full doses for 65% and 84% weeks of therapy over the 2.5 years of treatment, respect-

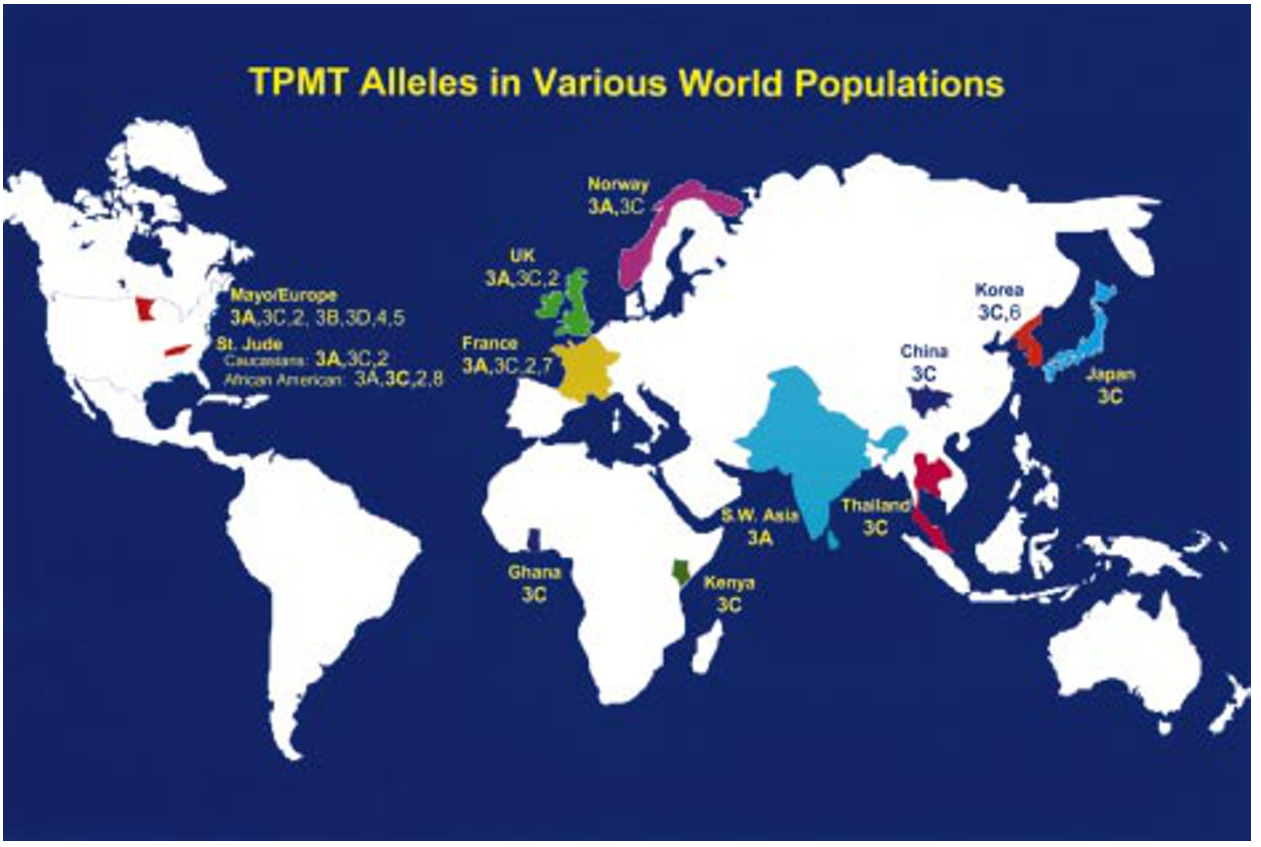

Figure 3 Ethnicity and mutant TPMT alleles. The observed mutant TPMT alleles are indicated for each country, with the most common mutant allele in bold.

Table 1 Ethnic variation in TPMT alleles

	n	*wt/wt %*	*wt/mut* %*	*mut/mut* %*	*TPMT*2 %*	*TPMT*3A %*	*TPMT*3C %*
British Caucasians (Ameyaw *et al*, 1999)	199	89.9	9.6	0.5	0.5	4.5	0.3
French Caucasians (de la Moureyere *et al*, 1998)	191	85.9	13.6	0.5	0.5	5.7	0.8
American Caucasian (Yates *et al*, 1997)	cal.	92.5	7.4	0.14	0.2	3.2	0.2
African–American (Hon *et al*, 1999)	cal.	90.7	9.2	0.2	0.4	0.8	2.4
Kenyan (McLeod *et al*, 1999)	101	89.1	10.9	0	0	0	5.4
Ghanaian (Ameyaw *et al*, 1999)	217	85.3	14.4	0.5	0	0	7.6
Chinese (Collie-Duguid *et al*, 1999)	192	95.3	4.7	0	0	0	2.3
Japanese (Kumagai *et al*, in press)	553	97.3	2.4	0.4	0	0	1.5
Southwest Asian (Collie-Duguid *et al*, 1999)	99	98	2	0	0	1	0

n, number of subjects, calculated; *, including *TPMT*2*, *TPMT*3A* and *TPMT*3C*.

ively. The percentage of weeks in which 6-MP dosage had to be decreased to prevent toxicity was 2%, 16% and 76% in wild-type, heterozygous, and homozygous mutant individuals.[28] Heterozygous patients were significantly more likely to miss weeks of 6-MP than homozygous wild-type patients, although they were not more likely to be hospitalised for fever or infection.[28] The British and St Jude studies had a similar 6-MP dosage and frequency of TPMT heterozygotes, but the heterozygous TPMT phenotype was associated with increased toxicity only in the St Jude study. One possible explanation for this discordance is the higher intensity of pulse chemotherapy during the consolidation phase in the St Jude study.[28] Consolidation therapy may influence bone marrow reserve and unmask the influence of a heterozygous TPMT genotype on 6-MP myelosuppression. Overall, these studies demonstrated that the influence of TPMT genotype is most dramatic for homozygous mutant patients, but is also of clinical relevance for heterozygous individuals.

Effect of 6-mercaptopurine dose intensity on outcome

The prognostic importance of 6-MP has recently been intensively evaluated in 182 children receiving the St Jude Children's Research Hospital Total Therapy XII study protocol for ALL.[1] A number of demographic, biochemical, molecular and pharmacological variables were evaluated as predictors of event-free survival, including RBC TGN, TPMT activity,

methotrexate polyglutamates, area under the curve for methotrexate, teniposide, or cytarabine, or the dose intensity for 6-MP (mg/m^2/week). The most powerful predictor of event-free survival was the dose intensity for 6-MP in both univariate and multivariate models.[1] The occurrence of neutropenia was also associated with a worst outcome. In the multivariate analysis, only higher dose intensity of 6-MP was a significant predictor of event-free survival, with lower TPMT activity tending to associate with better outcome. 6-MP dose intensity was also associated with event-free survival among patients with a homozygous wild-type TPMT phenotype. Lower 6-MP dose intensity was primarily due to missed weeks of therapy and not to reductions in daily dose. This study concluded that increased dose intensity of 6-MP is an important determinant of event-free survival in childhood ALL, particularly among children with a homozygous wild-type TPMT phenotype.[1] The study also highlighted that increasing intensity of therapy, such that neutropenia precludes other chemotherapy administration, would be counter productive.

TPMT and secondary malignancy

Several recent studies have identified an interaction between 6-MP pharmacology and the incidence of secondary malignancies, including brain tumors after radiotherapy. During St Jude Children's Research Hospital Total Therapy study XII, a higher incidence of brain tumors was observed, particularly among those who received prophylactic cranial radiotherapy (6/52; 12.8%) compared with patients in the same study who did not receive radiotherapy (0/101) and with other protocols that included cranial radiotherapy.[29] This protocol differed from previous protocols, in that more intensive systemic antimetabolite therapy was given before and during radiotherapy. Of the six children with secondary brain tumors, four had erythrocyte TGN concentrations higher than the 70th percentile of the entire cohort and three had a genetic defect in TPMT.[29] The 8 year cumulative incidence of brain tumor among children with defective TPMT was 42.9% *vs* 8.3% in wild-type TPMT patients (Figure 4).[29] Although specific biochemical or molecular mechanisms behind this apparent interaction between radiation and 6-MP are not clear, this study suggests that concurrent therapy should be avoided. Several studies have also evaluated the influence of 6-MP pharmacology on secondary leukemia. In a study of eight patients who developed secondary AML and 23 matched patients from the same protocol, no significant difference between RBC TPMT activity or 44 h methotrexate concentration was observed, although TPMT activity tended to be lower in the AML group ($P = 0.16$).[30] There was also the

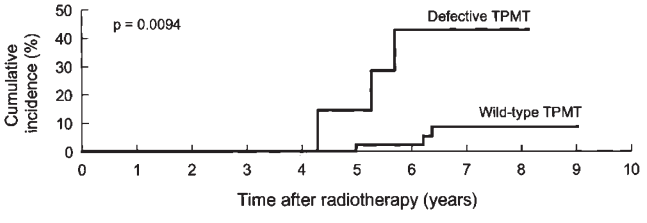

Figure 4 Estimated cumulative incidence of radiation associated secondary malignant brain tumour for seven children in St Jude Children's Research Hospital total XII study for ALL who received preventative cranial radiotherapy and had genetic defects in thiopurine methyltransferase compared with 45 with wild-type status (reproduced from Ref. 29, with permission).

suggestion that lower TPMT activity tended to be associated with shorter onset of secondary AML. A second study from the Nordic Society for Paediatric Hematology and Oncology Group (ALL-1992) evaluated TPMT activity and RBC TGN and MeMP concentrations in 439 children with ALL, including five who developed secondary myelodysplasia or AML.[31] RBC TPMT activity was significantly lower in patients who developed myelodysplasia or AML ($P = 0.03$). The 55 patients with TPMT activity 14 U/ml RBC had a 5 year risk of myelodysplasia/AML of 9% *vs* 1% for the remaining patients ($P = 0.002$).[31] All five patients with secondary malignancies had TGN and/or MeMP levels greater than the 90th percentile or had TPMT activity 14 U/ml RBC.[31] These data demonstrate an increased leukemogenic risk with 6-MP in patients with low TPMT activity and suggest that high TGN and/or the methylmetabolites may lead to DNA damage. Together, these studies raise important issues about the use of this class of medications and give further impetus to developing clinically useful tests for TPMT.

The way forward

There is now a growing body of evidence that prospective information on functional TPMT status would be of high utility, especially in the treatment of childhood ALL, as these patients often require acute transfusions at diagnosis. The common use of RBC as a surrogate tissue of the measurement of TPMT activity is not appropriate in patients who have recently received donor erythrocytes, since this will yield spuriously high activity results.[10] However, using current technologies, genotype analysis can successfully predict TPMT status in 80–95% of patients and with newer genomic approaches, such as DNA chip technology to screen for all known TPMT inactivating mutations, this should approach 100% predictive ability and allow genotype-guided use of thiopurine medications to soon become a reality.[32]

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References

1 Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. *Blood* 1999; **93**: 2817–2823.
2 McLeod HL, Coulthard S, Thomas AE, Pritchard SC, King DJ, Richards SM, Eden OB, Hall AG, Gibson BES. Analysis of thiopurine methyltransferase variant alleles in childhood acute lymphoblastic leukaemia. *Br J Haematol* 1999; **105**: 696–700.
3 Krynetski EY, Evans WE. Pharmacogenetics as a molecular basis for individualized drug therapy: the thiopurine S-methyltransferase paradigm. *Pharmaceut Res* 1999; **16**: 342–349.
4 Krynetski EY, Tai HL, Yates CR, Fessing MY, Loennechen T, Schuetz JD, Relling MV Evans WE. Genetic polymorphism of thiopurine S-methyltransferase: clinical importance and molecular mechanisms. *Pharmacogenetics* 1996; **6**: 279–290.
5 Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukemia. *Lancet* 1990; **336**: 225–229.

6 Evans WE, Horner M, Chu YO, Kalwinsky D, Roberts WM. Altered mercaptopurine metabolism, toxic effects and dosage requirement in a thiopurine methyltranferase-deficient child with acute lymphoblastic leukemia. *J Pediatr* 1991; **119**: 985–989.
7 McLeod HL, Miller DR, Evans WE. Azathioprine induced myelosuppression in thiopurine methyltransferase deficient heart-transplant recipient. *Lancet* 1993; **341**: 1151.
8 Schutz E, Gummert J, Mohr F, Oellerich M. Azathioprine induced myelosuppression in thiopurine methyltransferase deficient heart transplant recipient. *Lancet* 1993; **341**: 436.
9 McLeod HL, Lin JS, Scott EP, Pui CH, Evans WE. Thiopurine methyltransferase activity in American white subjects and black subjects. *Clin Pharm Ther* 1994; **55**: 15–20.
10 Yates CR, Krynetski EY, Loennechen T, Fessing MY, Tai HL, Pui CH, Relling MV, Evans WE. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann Intern Med* 1997; **126**: 608–616.
11 Otterness D, Szumlanski C, Lennard L, Klemetsdal B, Aarbakke J, ParkHah JO, Iven H, Schmiegelow K, Branum E, Obrain J, Weinshilboum R. Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. *Clin Pharmacol Ther* 1997; **62**: 60–73.
12 Tai HL, Krynetski EY, Yates CR, Loennechen T, Fessing MY, Krynetskaia NF, Evans WE. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am J Hum Genet* 1993; **58**: 694–702.
13 Tai HL, Krynetski EY, Schuetz EG, Yanishevski Y, Evans WE. Enhanced proteolysis of thiopurine S-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2): mechanisms for the genetic polymorphism of TPMT activity. *Proc Natl Acad Sci USA* 1997; **94**: 6444–6449.
14 delaMoureyre CSV, Debuysere H, Sabbagh N, Marez D, Vinner E, Chevalier ED, LoGuidice JM, Broly F. Detection of known and new mutations in the thiopurine S-methyltransferase gene by single-strand conformation polymorphism analysis. *Hum Mutat* 1998; **12**: 177–185.
15 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**: 1036–1044.
16 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 USA* 1995; **92**: 949–953.
17 Tai HL, Fessing MY, Bonten EJ, Yanishevsky Y, d'Azzo A, Krynetski EY, Evans WE. Enhanced proteasomal degradation of mutant human thiopurine S-methyltransferase (TPMT) in mammalian cells: mechanism for TPMT protein deficiency inherited by *TPMT* 2, TPMT*3A, TPMT*3B or TPMT*3C. Pharmacogenetics* 1999; **9**: 641–650.
18 Loennechen T, Yates CR, Fessing MY, Relling MV, Krynestski EY, Evans WE. Isolation of a human thiopurine S-methyltransferase (TPMT) complementary DNA with a single nucleotide transition A719G (TPMT*3C) and its association with loss of TPMT protein and catalytic activity in humans. *Clin Pharmacol Ther* 1998; **64**: 46–51.
19 Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine S-methyltransferase gene in African–Americans. *Hum Mol Gen* 1999; **8**: 371–376.
20 Ameyaw MM, Collie-Duguid ESR, Powrie RH, Ofori-Adjei D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations. *Hum Mol Gen* 1999; **8**: 367–370.
21 Collie-Duguid ESR, Prichard SC, Powrie RH, Sludden J, Collier DA, Li T, McLeod HL. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. *Pharmacogenetics* 1999; **9**: 37–42.
22 McLeod HL, Pritchard SC, Githang'a J, Indalo A, Ameyaw MM, Powrie RH, Booth L, Collie-Duguid ESR. Ethnic differences in thiopurine methyltransferase pharmacogenetics: evidence for allele specificity in Caucasian and Kenyan subjects. *Pharmacogenetics* 1999; **9**: 773–776.
23 Kumagai K, Hiyama K, Ishioka S, Sato H, Yamanishi Y, McLeod HL, Konishi F, Maeda H, Yamakido M. Allelotype frequency of the thiopurine methyltransferase gene in Japanese. *Pharmacogenetics* (in press).
24 Coulthard SA, Howell C, Robson J, Hall AG. The relationship between thiopurine methyltransferase activity and genotype in blasts from patients with acute leukemia. *Blood* 1998; **92**: 2856–2862.
25 delaMoureyre CSV, Debuysere H, Mastain B, Vinner E, Marez D, LoGuidice JM, Chevalier D, Brique S, Motte K, Colombel JF, Turck D, Noel C, Flipo EM, Pol A, Lhermitte M, Lafitte JJ, Libersa C, Broly F. Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene (TPMT) in a European population. *Br Pharmacol* 1998; **125**: 879–887.
26 Black AJ, McLeod HL, Capell HA, Powrie RH, Matowe LK, Pritchard SC, Collie-duguid ESR, Reid DM. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann Intern Med* 1998; **129**: 716–718.
27 McLeod HL, Relling MV, Liu Q, Pui CH, Evans WE. Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphoblastic-leukemia. *Blood* 1995; **85**: 1897–1902.
28 Relling MV, Hancock HL, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, Pui CH Evans WE. Intolerance to mercaptopurine therapy related to heterozygosity at the thiopurine methyltransferase gene locus. *J Natl Cancer Inst* 1999; **91**: 2001–2008.
29 Relling MV, Rubnitz JE, Rivera GK, Boyett JM, Hancock ML, Felix CA, Kun LE, Walter AW, Evans WE, Pui CH. High incidence of secondary brain tumours after radiotherapy and antimetabolites. *Lancet* 1999; **354**: 34–39.
30 Relling MV, Yanishevski Y, Nemec J, Evans WE, Boyett JM, Behn FG, Pui CH. Etoposide and antimetabolite pharmacology in patients who develop secondary acute myeloid leukemia. *Leukemia* 1998; **12**: 346–352.
31 Thomsen JB, Schroder H, Kristinsson J, Madsen B, Szumlanski C, Weinshilboum R, Andersen JB, Schmiegelow. Possible carcinogenic effect of 6-mercaptopurine on bone marrow stem cells. *Cancer* 1999; **86**: 1080–1086.
32 Krynetski EY, Evans WE. Pharmacogenetics of cancer therapy: getting personal. *Am Hum Genet* 1998; **63**: 11–16.
33 Kitchen BJ, Moser A, Lowe E, Balis FM, Widemann B, Anderson L, Strong J, Blaney SM, Berg SL, O'Brien M, Adamson PC. Thioguanine administered as a continuous intravenous infusion to pediatric patients is metabolized to the novel metabolite 8-hydroxythioguanine. *J Pharmacol Exp Ther* 1999; **2**: 870–874.
34 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* 1995; **6**: 1141–1147.