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

HUNG-LIANG TAI, EUGENE Y. KRYNETSKI, ERIN G. SCHUETZ, YURI YANISHEVSKI, AND WILLIAM E. EVANS†

St. Jude Children's Research Hospital, 332 North Lauderdale Street, Memphis, TN 38101

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ABSTRACT TPMT is a cytosolic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulphydryl compounds, including medications such as mercaptopurine and thioguani ne. TPMT activity exhibits autosomal codominant genetic polymorphism, and patients inheriting TPMT deficiency are at high risk of potentially fatal hematopoietic toxicity. The most prevalent mutant alleles associated with TPMT deficiency in humans have been cloned and characterized (TPMT*2 and TPMT*3A), but the mechanisms for loss of catalytic activity have not been elucidated. In the present study, we established that erythrocyte TPMT activity was significantly related to the amount of TPMT protein on Western blots of erythrocytes from patients with TPMT activities of 0.4-23 units/ml pRBC (r_s = 0.99; P < 0.001). Similarly, heterologous expression of wild-type (TPMT*1) and mutant (TPMT*2 and TPMT*3A) human cDNAs in yeast and COS-1 cells demonstrated comparable levels of TPMT mRNA but significantly lower TPMT protein with the mutant cDNAs. Rates of protein synthesis were comparable for wild-type and mutant proteins expressed in yeast and with in vitro translation in rabbit reticulocyte lysates. In contrast, pulse-chase experiments revealed significantly shorter degradation half-lives for TPMT*2 and TPMT*3A (–0.25 hr) compared with wild-type TPMT*1 (18 hr). The degradation of mutant proteins was impaired by ATP depletion and in yeast with mutant proteasomes (pre-1 strain) but unaffected by the lysosomal inhibitor chloroquine. These studies establish enhanced degradation of TPMT proteins encoded by TPMT*2 and TPMT*3A as mechanisms for lower TPMT protein and catalytic activity inherited by the predominant mutant alleles at the human TPMT locus.

The inherited polymorphism of TPMT activity in humans has now been elucidated at the molecular level with the identification of inactivating mutations in the human TPMT gene (9, 10). The initial molecular defect to be discovered was a single G238 → C transversion mutation leading to an amino acid substitution (Ala → Pro) at codon 80 (9). Heterologous expression of this mutant allele (TPMT*2) in yeast demonstrated TPMT mRNA levels comparable to wild type (TPMT*1), but approximately a 100-fold reduction in S-methylation activity. The second and more prevalent mutant allele (TPMT*3A) contains two nucleotide transition mutations (G460 → A and A719 → G) in the open reading frame, leading to amino acid substitutions Ala-154 → Thr and Tyr-240 → Cys (10, 11). Heterologous expression of the TPMT*3A cDNA in yeast produced TPMT mRNA levels comparable to wild type, but TPMT protein levels were about 400-fold lower, with no detectable catalytic activity. However, the post-transcriptional mechanism for loss of TPMT function has not been elucidated for either TPMT*2 or TPMT*3A.

If the mechanism for TPMT deficiency in humans results in lower TPMT protein levels, instead of a modification in the active site(s) for metabolism of thiopurine medications, then these patients will have reduced metabolism of all TPMT substrates, including endogenous or environmental substrate(s) that have not yet been identified. The current studies were, therefore, undertaken to elucidate the mechanism(s) for loss of TPMT activity in cells expressing proteins encoded by human TPMT*2 and TPMT*3 alleles.

METHODS

Human Subjects. After informed consent was obtained from the patient, parent, or guardian, blood was obtained for the measurement of TPMT activity in erythrocytes (RBCs) and for preparation of RBC lysates for Western blots. These studies were performed in unrelated individuals selected on the basis of their TPMT phenotypes: three patients known to have TPMT deficiency (4, 9, 10), four with a heterozygous TPMT phenotype (5–10 units/ml pRBC), and four with homozygous wild-type TPMT activity (>10 units/ml pRBC), by using published criteria for phenotype assignment (1). TPMT genotypes of TPMT-deficient patients were determined by cDNA cloning and sequencing, as described (9, 10).

Expression and Western Blot of TPMT Proteins. Human TPMT cDNAs were cloned and expressed in yeast as described (9, 10). Subsequently, the coding region of TPMT cDNA was amplified by PCR using previously constructed plasmids containing TPMT*1, TPMT*2, or TPMT*3A cDNA as templates, with addition of restriction sites at each end and change of thymidine to cytidine at position –1, as described (12). The primers used were the sense primer 5'-cggatctAAGACCATG-

Abbreviations: TPMT, thiopurine S-methyltransferase; RBC, red blood cell; SAM, S-adenosylmethionine; pRBC, packed RBC.

†To whom reprint requests should be addressed. e-mail: william.evans@stjude.org.

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GATGGTACAGAACTTCACGTTG-3' and the antisense primers 5'-cggatctTATTTTTTCTGTAAGTATACATACTTTTC-3' or 5'-cggatctTATTTTTTCTGTAAGTATACATACTTTTC-3', depending on the template used. PCR products were digested with EcoRI and BamHI and then ligated with similarly digested pGEM-TZ(+) vector (Promega). The resultant clones were subcloned into pSP64 Poly(A) vector (Promega) for in vitro translation through SalI/XhoI and BamHI restriction sites. The in vitro translation reaction was performed with TNT-Coupled Reticulocyte Lysate Systems (Promega) with the addition of 1.5 µg of plasmid DNA. TPMT cDNA inserts in pSP64 Poly(A) vector were then subcloned into pcDNA3.1(+) vector (Invitrogen) through HindIII and BamHI sites for TPMT expression in COS-1 (monkey kidney) cells transfected by the calcium phosphate method (13). Nucleotide structures of all cDNA inserts in these plasmids were confirmed by sequencing. Enzymatically active 6×His-tagged TPMT was expressed in *Escherichia coli* strain M15[pREP4] driven by a cDNA fragment containing the TPMT ORF (9) inserted into pQE-30 vector (Qiagen) and subsequently purified according to manufacturer's instructions. Glutathione S-transferase–TPMT fusion protein was generated and purified as described (10); this fusion protein was digested with thrombin to release C-terminal TPMT and subsequently purified according to manufacturer's instructions. Enzymatically active 6×His-tagged TPMT was expressed in *Escherichia coli* strain M15[pREP4] driven by a cDNA fragment containing the TPMT ORF (9) inserted into pQE-30 vector (Qiagen) and subsequently purified according to manufacturer's instructions. Glutathione S-transferase–TPMT fusion protein was generated and purified as described (10); this fusion protein was digested with thrombin to release C-terminal TPMT and used for Western blot analysis. Expressed human TPMT proteins and TPMT in human RBC lysates and liver cytosol were analyzed by SDS–PAGE (16 cm × 16 cm; 12.5% gel) and immunodetected with TPMT-specific antibody as described (10).

**Pulse–Chase Analysis.** Pulse–chase experiments were performed as described (14–16), modified as follows. Saccharomyces cerevisiae cells of the strain 2805 transformed with plasmids containing wild-type or mutant human TPMT cDNAs (10) were grown at 30°C to an *A*~600~ of 1.5 in 2% galactose, 0.67% yeast nitrogen base (Difco) without amino acids, adenine (20 µg/ml), and a mixture of amino acids lacking methionine or cysteine. Four mutant TPMT cDNAs were studied: TPMT*+2* with the G238C transition (9), TPMT*+3A* with both the G460A and A719G transitions (10), TPMT*+3B* with only the G460A mutation, and TPMT*+3C* with only the A719G mutation. These cDNAs were cloned and expressed in yeast as described (9, 10). For pulse-labeling proteins, [35S]methionine (Tran35S-label, ICN) at 20 µCi/ml was added to cell cultures for 15 min. Cells were then washed and resuspended in a glucose-containing medium with amino acids including unlabeled methionine (2 mg/ml) and cysteine (0.5 mg/ml) and chased in the same medium for the indicated durations. To determine the effect of chloroquine on protein degradation, 0.2 mM chloroquine was included in the medium throughout the pulse–chase experiment. To determine the effect of ATP depletion, 2-deoxyglucose (20 mM) and 2,4-dinitrophenol (0.2 mM) were added to the medium after pulse labeling. For each experiment, 1 ml of cell culture was harvested at each time point by centrifugation, and the cell pellet was resuspended in 200 µl of 2% SDS/10 mM dithiothreitol/10 mM Tris-HCl, pH 7.5, and heated in a boiling-water bath for 3 min. After centrifugation, the supernatant was diluted in buffer A (1% Triton X-100/0.15 M NaCl/1 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride/50 mM Tris-HCl, pH 7.5) and centrifuged in a Millipore Ultrafree-15 filter unit with Biomax-10 membrane, followed by dilution in buffer B (buffer A plus 10% glycerol) (17). The concentration in the filtrate was measured by pulse labeling in the galactose-containing medium for 5–20 min. After electrophoresis, gel slices corresponding to the area of the Western blot were cut out and analyzed by SDS–PAGE and by PhosphorImager (Molecular Dynamics). An aliquot of preincubation mixture was then assayed for TPMT activity with 2 mM 6-mercaptopurine and 1 mM SAM as substrates. The reaction was allowed to proceed for 10 min at 37°C, and methylmercaptopurine was measured, as described (10). The same assay mixture with yeast cytosol expressing vector alone served as the blank, and the background values were subtracted from all values obtained. There was never a sufficient amount of catalytically active TPMT protein to permit these studies with either TPMT*+2* or TPMT*+3A*.

**Immunoprecipitation.** The radioactivity of chloroauratic acid-precipitable [35S]labeled protein was determined in the final filtrate. Portions of the filtrate containing equal amounts of the total acid-precipitable [35S] were processed for immunoprecipitation, with a polyclonal antibody to human TPMT (10). This antibody detected TPMT*+1*, TPMT*+2*, TPMT*+3A*, TPMT*+3B*, and TPMT*+3C* with similar intensity on Western blot after in vitro translation, demonstrating that this polyclonal antibody recognizes both wild-type and mutant TPMT proteins (data not shown). Antigen–antibody incubation proceeded on ice for 2 hr to overnight, protein A-Sepharose (Sigma) was then added, and the suspension was incubated on ice for an hour with vortex mixing every 3–5 min. The protein A-Sepharose pellets were washed three times with RIPA buffer (0.1% SDS/1% Triton X-100/15% sodium deoxycholate/0.15 M NaCl/1 mM EDTA/0.25 mM phenylmethylsulfonyl fluoride/50 mM Tris-HCl, pH 7.5), once with RIPA plus 0.5 M NaCl, and a final wash with 50 mM Tris-HCl (pH 7.5). The washed protein A pellets were then prepared for electrophoresis by the addition of 2× SDS–PAGE sample buffer. Electrophoresis was on 15% SDS–PAGE gels. Gels were fixed and treated with 1 M sodium salicylate; [35S]TPMT was quantitated with a PhosphorImager (Molecular Dynamics) and autoradiography was performed thereafter.

**TPMT Synthesis Rate.** TPMT synthesis experiments were performed in yeast as described above, with the following modifications. The synthesis of TPMT protein in yeast was measured by pulse labeling in the galactose-containing medium for 5–20 min. After electrophoresis, gel slices corresponding to the area of the expected mobility of the protein were digested overnight in Solvable (Packard) and radioactivity of [35S] was measured with the addition of scintillation fluid. The molar amount of TPMT was calculated by using the specific activity of [35S]methionine, based on four molecules of methionine in each TPMT protein and a counting efficiency of 95%. Total protein in yeast lysates was measured (18) and used to normalize the amount of TPMT synthesized. Translational efficiency was also measured in vitro in rabbit reticulocyte lysates by taking aliquots of the translated product during the linear phase of the reaction (5, 10, and 20 min) at 30°C and analyzing by SDS–PAGE and by PhosphorImager (Molecular Dynamics).

**Intrinsic Stability.** To assess intrinsic stability of TPMT activity, 5–20 µl of cytosol from yeast expressing recombinant human TPMT*+1*, TPMT*+3B*, or TPMT*+3C* were preincubated (37°C) in 250 µl of 0.1 M Tris-HCl (pH 7.5) buffer with or without 1 mM S-adenosylmethionine (SAM), for up to 4 hr. An aliquot of preincubation mixture was then assayed for TPMT activity with 2 mM 6-mercaptopurine and 1 mM SAM as substrates. The reaction was allowed to proceed for 10 min at 37°C, and methylmercaptopurine was measured, as described (10). The same assay mixture with yeast cytosol expressing vector alone served as the blank, and the background values were subtracted from all values obtained. There was never a sufficient amount of catalytically active TPMT protein to permit these studies with either TPMT*+2* or TPMT*+3A*.

**Modeling and Statistics.** A one-compartment first-order model was fit to the amount of labeled TPMT*+1*, TPMT*+3A*, TPMT*+3B*, and TPMT*+3C*, for up to 24 hr after pulse labeling of these proteins in yeast. A two-compartment model was used for fitting TPMT*+2* amount, since biphasic degradation was observed. Weighted least squares as implemented in ADAPT II software was used to fit the model to the data and estimate the degradation rate constants and degradation half-lives for each protein. Synthesis rates were estimated by fitting a one- or two-compartment first-order model to the amount of pulse-labeled TPMT protein from 5 to 20 min (see above) with degradation rates fixed at the rate measured from pulse–chase experiments with each protein. The 95% confidence intervals for the parameter estimates calculated in ADAPT II software were used to determine whether TPMT proteins half-lives...
differed significantly. The relation between TPMT activity and TPMT protein levels in RBC lysates was assessed by the Spearman rank correlation coefficient ($r_s$).

**RESULTS**

Western blots of human liver cytosol, human RBC lysates (except TPMT-deficient patients), and lysates from yeast or COS-1 cells expressing recombinant human wild-type TPMT revealed a band of approximately 32.5 kDa that was detected by the polyclonal TPMT antibody and migrated with in vitro-translated TPMT in purified TPMT (Fig. 1A). No TPMT protein was detected in controls using plasmids without TPMT cDNA inserts (Fig. 1A, lanes 3, 5, and 11). As expected, slower mobility was observed with 6×His-tagged TPMT, due to six additional histidine residues (Fig. 1A, lane 1). Densoitometric quantitation of TPMT bands on Western blots revealed a significant correlation ($r = 0.99; P < 0.001$) between TPMT activity and TPMT protein levels in RBC lysates from patients with TPMT activities ranging from <0.4 to 23 units/ml pRBC (Fig. 1B). Patients with two mutant alleles (TPMT2 and/or TPMT3A), were deficient in TPMT activity and had no detectable TPMT protein, whereas patients with heterozygous phenotypes (activity 5–10 units/ml pRBC) had intermediate levels of TPMT protein, and patients with homozygous wild-type phenotypes (>10 units/ml pRBC) had the highest level of TPMT protein.

Similar to patients with different TPMT genotypes, marked differences in TPMT protein levels were observed when wild-type and mutant TPMT cDNAs were expressed in yeast (Fig. 2); with the levels of TPMT2 and TPMT3A protein about 20-fold and more than 200-fold less than TPMT1, respectively. Yeast expressing the TPMT3B cDNA with only the G460A had 4-fold lower TPMT protein, and TPMT protein levels in yeast expressing TPMT3C (only A719G) were similar to wild type. Comparable results were obtained when these mutant cDNAs were expressed in COS-1 cells (for TPMT3A, see Fig. 1A, lane 13; data not shown for other mutants). TPMT mRNA levels were comparable in yeast expressing wild-type or any of the four mutant cDNAs (9, 10), indicating that these mutations did not affect transcription under these conditions, implicating post-transcriptional mechanisms as the basis for TPMT deficiency.

By using pulse–chase techniques, significant differences were found in half-lives of wild-type and mutant TPMT proteins in yeast, with a degradation half-life of 18 hr for TPMT1 and TPMT3C, 6 hr for TPMT3B, 0.2 hr ($\alpha$) and 14.8 hr ($\beta$) for TPMT2, and 0.25 hr for TPMT3A (Fig. 3). In contrast, synthesis rates were similar for the wild-type and mutant TPMT proteins expressed in yeast (Table 1). Similar translational rates were also observed when TPMT1, TPMT2, and TPMT3A were translated in vitro in rabbit reticulocyte lysates (Fig. 4). In yeast expressing wild-type or mutant TPMT cDNAs, the synthesis and degradation rates for each protein were in good agreement with relative levels of TPMT protein measured after 24 hr of culture (Fig. 2).

Two major proteolytic pathways are responsible for the degradation of proteins in eukaryotic cells; an ATP-dependent proteasomal pathway and an ATP-dependent lysosomal pathway (20, 21). To assess the requirement of ATP in the degradation of mutant TPMT proteins, 2-deoxyglucose and 2,4-dinitrophenol were used to inhibit ATP production and thus deplete intracellular ATP in yeast expressing mutant TPMT proteins (22, 23). As depicted in Fig. 5, the proteolysis of TPMT2 and TPMT3A was inhibited in the ATP-

![Fig. 1.](Image 49x196 to 288x566) Western blot of purified histidine-tagged human TPMT1 protein (0.2 ng, lane 1); TPMT1 generated from thrombin-treated purified glutathione S-transferase–TPMT fusion protein (2 ng, lane 2); in vitro-translated control (vector alone without TPMT cDNA insert) in rabbit reticulocyte lysate (RRL; 2 µl of 1:10 dilution, lane 3); in vitro-translated human TPMT1 in RRL (2 µl of 1:1 dilution, lane 4); yeast cytosol expressing vector alone (45 ng, lane 5); yeast cytosol expressing human TPMT1 (45 ng, lane 6); human liver cytosol (5 µg, lane 7); RBC lysate equivalent to $2 \times 10^6$ cells from patients with homozygous deficient (+2/+3A; 64.3 µg, lane 8), heterozygous (+1/+2; 87.8 µg, lane 9), or homozygous wild-type (+1/+1; 78.2 µg, lane 10) TPMT genotypes; COS-1 cells expressing vector alone (3.65 µg, lane 11); COS-1 cells expressing TPMT1 (5.01 µg, lane 12); and COS-1 cell lysate expressing TPMT3A (4.7 µg, lane 13).

![Fig. 2.](Image 316x78 to 549x213) Western blot of recombinant human TPMT protein in yeast cytosol expressing wild-type (+1) and mutant TPMT cDNAs (+2, +3A, +3B, and +3C) after a 24-hr culture in galactose-containing medium. The amount of total protein loaded in each lane is indicated at the top.
The polymorphism of TPMT activity was discovered more than 15 years ago (1), yet the genetic basis for this inherited trait was not known until recently (9, 10). Although these studies have established that TPMT*2 and TPMT*3A are mutant alleles associated with inheritance of TPMT deficiency (9, 10), the mechanism for loss of TPMT activity has not been previously elucidated.

The current study has established a significant relation between RBC TPMT activity and the level of TPMT protein in RBC lysates from patients with differing TPMT phenotypes (Fig. 1B). The presence of comparable TPMT mRNA levels in patients with TPMT deficiency and those with heterozygous or homozygous wild-type phenotypes (9, 10) and the absence of TPMT protein in TPMT-deficient patients indicate a post-transcriptional mechanism for TPMT deficiency. This is consistent with results in our heterologous expression system, wherein expression of wild-type and mutant TPMT cDNAs revealed comparable TPMT mRNA levels (9, 10), but yeast expressing either TPMT*2 or TPMT*3A had significantly lower TPMT protein levels compared with yeast expressing the wild-type cDNA (Fig. 2). This difference in TPMT protein was also evident when wild-type and mutant TPMT was heterologously expressed in mammalian COS-1 cells (Fig. 1A). Thus, both humans and yeast expressing TPMT*2 and TPMT*3A have markedly lower TPMT protein levels and catalytic activity, despite TPMT mRNA levels comparable to wild type. A second, invariant, lower molecular weight band was also observed in human RBC and monkey kidney COS-1 cell lysates. Direct sequencing of this second band in human RBC and monkey kidney COS-1 cell lysates indicates that it is the hemoglobin β-chain (data not shown), which may react with the antibody due to its abundance in RBC lysates. The second lower molecular weight band in COS-1 cells may reflect endogenous TPMT in these cells.

**Table 1. Half-lives and synthesis rates of wild-type and mutant TPMT proteins in yeast**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TPMT*1</th>
<th>TPMT*2</th>
<th>TPMT*3A</th>
<th>TPMT*3B</th>
<th>TPMT*3C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degradation $t_{1/2}$, hr</td>
<td>18 (0.4)</td>
<td>0.2α (43), 14.8β (1)*</td>
<td>0.25 (6)*</td>
<td>6.1 (0.3)*</td>
<td>18 (0.3)</td>
</tr>
<tr>
<td>Formation rate fmol per mg per hr</td>
<td>335 (8)</td>
<td>409 (9)</td>
<td>268 (14)</td>
<td>349 (8)</td>
<td>220 (12)</td>
</tr>
</tbody>
</table>

Data in parentheses are coefficient of variation percent.

* $P < 0.05$, when compared to TPMT *1.
Pulse–chase and *in vitro* translation experiments established that lower TPMT protein levels in yeast expressing mutant human TPMT*2* or TPMT*3A* cDNAs are due to differences in degradation rates of mutant proteins (Fig. 3) and not differences in synthesis rates (Fig. 2 and Table 1). These results indicate that the G238C, G460A, and A719G point mutations in TPMT cDNAs have little or no effect on transcription or translational efficiency but can have a significant effect on protein degradation. The G238C mutation (Ala-80 \(\rightarrow\) Pro) had the greatest effect on protein degradation (\(t_{1/2} = 0.2\) hr), and the G460A (Ala-154 \(\rightarrow\) Tyr) mutation had a modest effect (\(t_{1/2} = 6\) hr) and the A719G (Tyr-240 \(\rightarrow\) Cys) mutation alone had no effect on TPMT protein degradation in yeast. Models of the TPMT secondary structure using the Garnier algorithm (25), indicate that the Ala-80 \(\rightarrow\) Pro substitution produces an additional turn in the secondary structure of the TPMT protein, and the G460A mutation (Ala-154 \(\rightarrow\) Tyr) produces an additional turn and a loss of one \(\beta\)-sheet, but no changes are predicted for the A719G mutation. Thus, the G238C and G460A mutations may result in more disruption of the tertiary structure than the A719G mutation, consistent with more rapid degradation of TPMT*2* and TPMT*3B*. Moreover, when the G460A and A719G mutations are present together, as is the case for the most prevalent mutant allele in humans (TPMT*3A*), there was a dramatic increase in the rate of protein degradation (Fig. 3), suggesting that there is a greater effect on the tertiary structure of TPMT when both mutations are present, thus making it more susceptible to proteolysis.

Significant differences were also observed in the intrinsic stability of mutant proteins. When incubated at 37°C in Tris buffer at pH 7.5, proteins encoded by TPMT*3B* or TPMT*3C* (with either the G460A or A719G mutation alone, respectively) completely lost catalytic activity within 4 hr, without evidence of protein degradation, whereas proteins encoded by TPMT*1* retained \(\sim 80\%\) of its catalytic activity, demonstrating that intrinsic stability of proteins encoded by TPMT*3B* and TPMT*3C* are significantly different from wild type. The TPMT*1* proteins are more stable than the mutant proteins, indicating that the mutations may affect the structure of the TPMT protein and make it more susceptible to degradation.
addition of high concentrations of SAM (1 mM) partially stabilized TPMT*3C catalytic activity under these conditions (Fig. 8), which could be explained by stabilization of the tertiary structure in the presence of substrate, as has been reported for other enzymes (26). It remains to be determined whether TPMT*3C is stabilized by endogenous substrate(s) in yeast, thereby preventing its rapid degradation. However, it is clear from the present work that enhanced protein degradation is a primary mechanism for loss of TPMT activity with TPMT*2 and TPMT*3A, which represent >80% of mutant TPMT alleles in Caucasians (27).

The present work further indicates that the mechanism for proteolysis of mutant TPMT is via an ATP-dependent proteasome-mediated pathway, a mechanism known to degrade a number of intracellular proteins, including those with misfolded structures due to mutations (28, 29). Degradation of mutant TPMT proteins was not affected by the lysosomal inhibitor chloroquine (30, 31), but degradation was impaired in the mutant pre-1 yeast (15) (Figs. 7 and 8). It should be noted that some degradation of TPMT*2 and TPMT*3A proteins occurred in the pre-1 proteasome mutants, which lacks the “chymotrypsin-like” activity of the multi-catalytic protein complex, but retains trypsin-like and peptidyl-glutamylpeptide hydrolyzing activities (32). Thus, one of the remaining proteolytic activities in pre-1 proteasomes may also mediate degradation of mutant TPMT proteins or an additional non-lysosomal pathway may contribute to the degradation of these proteins.

It is clear from the current studies that TPMT deficiency inherited by TPMT*2 and TPMT*3A, the most prevalent mutant TPMT alleles in humans, is associated with lower cellular levels of TPMT protein and that the proteins encoded by these mutant alleles are degraded more rapidly by an ATP-dependent proteasome-mediated pathway. Although enhanced proteolysis of mutant proteins is not uncommon, there are many examples of mutations that lead to loss or gain of function without enhanced degradation [e.g., CFTR (33), steroid 21-hydroxylase (34), and p53 (35)]. An additional implication of enhanced proteolysis of mutant TPMT is that the absence of TPMT protein in those inheriting TPMT deficiency means these individuals will have decreased metabolism of all TPMT substrates, including as yet undiscovered environmental and endogenous substrate(s) for this polymorphic enzyme.

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