Retrospective Data Analysis of the Influence of Age and Sex on TPMT Activity and Its Phenotype–Genotype Correlation

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Background: Therapeutic efficacy and toxicity of thiopurine drugs (used as anticancer and immunosuppressant agents) are affected by thiopurine S-methyltransferase (TPMT) enzyme activity. *TPMT* genotype and/or phenotype is used to predict the risk for adverse effects before drug administration. Inosine triphosphate pyrophosphatase (ITPA) is another enzyme involved in thiopurine metabolism. In this study, we aimed to evaluate (*a*) frequency of various TPMT phenotypes and genotypes, (*b*) correlations between them, (*c*) influence of age and sex on TPMT activity, and (*d*) distribution of *ITPA* variants among various TPMT subgroups.

Methods: TPMT enzyme activity was determined by LC-MS/MS. *TPMT* (*2,*3A–C) and *ITPA* (rs1127354, rs7270101) genotypes were determined using a customized TaqMan® OpenArray®.

Results: TPMT enzyme activity varied largely (6.3–90 U/mL). The frequency of low, intermediate, normal, and high activity was 0.5% (n = 230), 13.1% (n = 5998), 86.1% (n = 39448), and 0.28% (n = 126), respectively. No significant difference in TPMT activity in relation to age and sex was found. Genotype analysis revealed the frequency of variant *TPMT* alleles was 6.73% (*3A, n = 344), 0.05% (*3B, n = 2), 2.22% (*3C, n = 95), and 0.42% (*2, n = 19). Analysis of paired phenotype and genotype showed that TPMT activity in samples with variant allele(s) was significantly lower than those without variant alleles. Lastly, an equal distribution of *ITPA* variants was found among normal and abnormal TPMT activity.

Conclusions: This retrospective data analysis demonstrated a clustering of variant *TPMT* genotypes with phenotypes, no significant influence of age and sex on TPMT activity, and an equal distribution of *ITPA* variants among various TPMT subgroups.

IMPACT STATEMENT

The influence of age and sex on thiopurine S-methyltransferase (TPMT) enzyme activity has not been well studied. Results from this study demonstrated a clustering of variant *TPMT* genotypes with phenotypes and no significant influence of age and sex on TPMT activity. Furthermore, an equal distribution of inosine triphosphate pyrophosphatase, another crucial enzyme involved in thiopurine metabolism, among various TPMT subgroups was found. Data presented here can be used to improve therapeutic efficacy and reduce adverse drug effects in patients treated with thiopurine drugs.

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Thiopurine prodrugs such as azathioprine (AZA)[5], 6-thioguanine, and 6-mercaptopurine (6-MP) are commonly used for the treatment of patients with various malignancies (e.g., acute lymphoblastic leukemia), inflammatory disorders (e.g., inflammatory bowel disease), and autoimmune disorders (e.g., Crohn disease and rheumatoid arthritis). Variation in therapeutic efficacy and toxicity of these drugs is largely affected by the activity of thiopurine S-methyltransferase (TPMT) (*1*). TPMT is an enzyme found in many organs, including kidney, liver, and red blood cells (RBCs), that is involved in the metabolism of the thiopurine drugs. TPMT catalyzes the primary inactivation pathway (Fig. 1) in which approximately 90% of thiopurines are converted to an inactive metabolite, 6-methylmercaptopurine (6-MMP). Despite good therapeutic efficacy of thiopurine drugs, adverse effects such as myelotoxicity and gastrointestinal and allergic effects have been documented when the standard dose is administered to individuals with impaired TPMT activity because of accumulation of active drug metabolites (e.g., 6-thioguanine nucleotide) (*2*, *3*). *TPMT*[6] genotype and/or phenotype has been used to identify patients at risk for adverse effects before drug administration to minimize the incidence of toxicity and improve dose selection (*4*). In whites, approximately 90% of individuals present with normal enzyme activity, 10% show intermediate activity, and 0.3% have low enzyme activity (*5*). Currently, >30 *TPMT*-deficient alleles are known (*6*), but the *2 (238G>C at rs1800462), *3A (460G>A at rs1800460 and 719 A>G at rs114234), *3B (460G>A at rs1800460), and *3C (719G>A at rs1142345) alleles account for 80% to 95% of low to intermediate activity (*7*–*9*). The *TPMT**3A allele is the most prevalent mutant allele in white (frequency, 2%–5%) and Mediterranean (frequency, 2%–5%) individuals, whereas *3C is the most common mutant allele in black (frequency, 2.5%) and Asian (frequency, 0.5%–3%) populations (*8*–*12*). Thirty percent to 60% of all cases of thiopurine intolerance can be attributed to *TPMT* genetic status (*13*, *14*); the remaining of those cases cannot be explained by a pharmacogenetics basis.

Variants in the gene that codes for inosine triphosphate pyrophosphatase (ITPA) are involved in the response to thiopurine drugs as well (Fig. 1). ITPA is a cytosolic enzyme found in RBCs and many tissues, and it catalyzes the conversion of inosine triphosphate (ITP) to inosine monophosphate to prevent the accumulation of ITP and deoxy-ITP in normal cells (*14*). The association of *ITPA* variants in the occurrence of adverse events of AZA has been studied over the past decade. It has been reported that low ITPA activity is associated with toxicity in patients treated with thiopurine drugs because of the accumulation of 6-thio-ITP intermediate. Two *ITPA* alleles (i.e., rs1127354 and rs7270101) were identified to be significantly associated with decreased enzyme activity exhibiting a frequency of 6% and 13%, respectively, in whites (*15*). However, several contradictory results have been reported. In several publications, no significant association between ITPA activity and adverse events of AZA was discovered (*16*).

In this study, we aimed to evaluate the frequency distribution of TPMT phenotype, genotype, and correlations between them. Sex and age differences in the distribution of TPMT activity and distribution of *ITPA* variants among samples with various subgroups of TPMT activity were also evaluated.

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DOI: 10.1373/jalm.2018.027276

[5] **Nonstandard abbreviations:** AZA, azathioprine; 6-MP, 6-mercaptopurine; TPMT, thiopurine S-methyltransferase; RBC, red blood cell; 6-MMP, 6-methylmercaptopurine; ITPA, inosine triphosphate pyrophosphatase; ITP, inosine triphosphate.
[6] **Human Genes:** *TPMT*, thiopurine S-methyltransferase; *ITPA*, inosine triphosphatase.

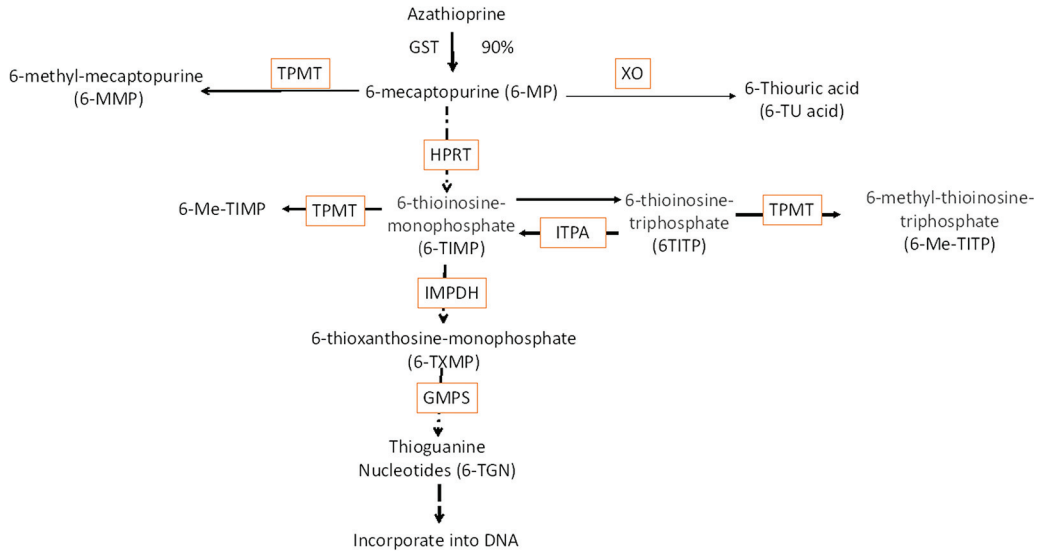


Fig. 1. Schematic diagram of the metabolic steps mediated by TPMT and ITPA.
GST, glutathione *S*-transferase; XO, xanthine oxidase; HPRT, hypoxanthine phosphoribosyl transferase; GMPS, guanosine-monophosphate-synthase.

MATERIALS AND METHODS

Chemicals and reagents

All solvents were reagent grade or better and purchased from VWR International or Thermo Fisher Scientific, and they included methanol, isopropanol, and acetonitrile. Acetic acid and ammonium acetate were purchased from Sigma-Aldrich. The internal standards were purchased from Cerilliant: 6-MP, 6-MMP, and *S*-adenosyl-L-methionine iodide. Type I water was generated using a Barnstead Nanopure Infinity ultrapure water system (Thermo Fisher Scientific).

TPMT phenotype/activity by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Whole blood specimens collected in EDTA, sodium heparin, or lithium heparin anticoagulants were subjected to LC-MS/MS analysis. The specimens were mixed well on a rocking table for 5 min before centrifuging at 3000 rpm (2113*g*) for 10 min. The plasma and white cell layer were discarded using a transfer pipette. A displacement pipette was used to pipet 400 µL of packed RBCs into a tube with 1.2 mL of clinical laboratory-grade water. The tubes were placed on an ice bath and mixed by inverting them a few times. Once the RBCs were lysed, the tubes were removed from the ice bath and vortex-mixed for 2 min at room temperature. The lysate was then centrifuged at 13000 rpm (16060*g*) at 4 °C for 15 min to remove cell debris. Four hundred microliters of the lysate supernatant was transferred to a 2-mL tube. Then 160 µL of 0.2 M sodium phosphate buffer (ph 7.4) was added along with 4 mmol/L 6-MP and 640 µmol/L *S*-adenosyl-L-methionine iodide, then the sample incubated at 37 °C for 60 min. *S*-adenosyl-L-methionine iodide was used as a methyl donor for the conversion of 6-MP to 6-MMP by TPMT. The reaction was stopped by heat (95 °C), and samples were centrifuged. Internal standards containing 2-chloroadenosine and 6-MP-^{13}C2, 15N-1 were

added to the supernatant. The concentration of 6-MMP produced from the enzyme reaction in the lysate was converted from nanograms per milliliter to nanomoles per 1 mL of packed RBCs (U/mL) to assess TPMT activity.

An LC-MS/MS method was developed and validated for quantification of 6-MMP. An API 5500 mass spectrometer (AB Sciex) with a TurboIonSpray electrospray ion source coupled to an Agilent 1260 HPLC system (Agilent) was used. Liquid chromatography separation was conducted using a Phenomenex Synergi 4-µm Fusion reversed-phased column [8.0 nm, liquid chromatography column (50 × 2 mm)]. Mobile phase A (2.5 mmol/L ammonium acetate in Nanopure water, pH 3.0) and mobile phase B (methanol) were used to develop a gradient: 5% B for 0.50 min, step from 5% B to 25% B, hold at 25% B for 2.5 min, step from 25% B to 95% B, and hold at 95% B for 0.5 min, followed by reequilibration to initial condition for 0.5 min. The liquid chromatography flow rate was 0.5 mL/min. The injection volume was 10 µL, and the column was held at 25 °C. Mass spectra were acquired using polarity switching. Positive polarity was used for 6-MP; negative polarity was used for 6-MMP. For each analyte and its internal standard, both a quantitative and a qualitative mass transition were selected. Analytical peaks were positively identified by combination of retention time, 2 multiple reaction monitoring transitions, and an ion ratio. For each compound, each ion ratio was calculated by dividing peak area of the qualifier ion by peak area of the quantifier ion. Quantification was performed by comparison with a calibration curve using QuanLynx software and the instrument's microprocessor. Calibrators and controls were carried through the same processes as the specimens being testing. The results were converted to units of activity per milliliter of lysed packed RBCs (U/mL). The TPMT activity cutoffs for correct prediction of different phenotypes have been studied previously using phenotype–genotype correlation of 1214 healthy blood donors [395 women, 827 men with a mean age of 38.1 (range, 18–69) years] (*17*). Our laboratory verified the cutoffs for low, intermediate, normal, and high TPMT activity from an in-house genotype–phenotype study performed on 157 patient samples. The results >44 U/mL were determined to be high enzyme activity, indicating patients may be at low risk for bone marrow toxicity and myelosuppression from standard thiopurine dosing, but may be at risk for therapeutic failure because of excessive inactivation of thiopurine drugs. The results between 24.0 and 44.0 U/mL were defined as normal activity, indicating patients may be at low risk of bone marrow toxicity following standard thiopurine therapy. The results between 17.0 and 23.9 U/mL were determined to be intermediate enzyme activity, indicating patients may be at intermediate risk of bone marrow toxicity and myelosuppression after standard thiopurine therapy. The results <17.0 U/mL were determined to be low enzyme activity, indicating patients may be at high risk of bone marrow toxicity and myelosuppression.

TPMT and *ITPA* genotypes

Genomic DNA was extracted from whole blood using Chemagen M-PVA magnetic Bead Technology and Chemagic MSM I instrument (PerkinElmer). DNA concentration was determined (A260/280), and samples were normalized to 50 ng/µL. *TPMT* and *ITPA* genotypes were determined using a custom TaqMan® OpenArray® on the QuantStudio™ 12K Flex system (Thermo Fisher Scientific). Genotyping experiments were performed according to instructions provided by the manufacturer. The TaqMan genotyping assays detected the *TPMT**3C (719A>G), *TPMT**3B (460 G>A), *TPMT**3A (460G>A and 719A>G), and *TPMT**2 (238G>C). Absence of the detection of variant alleles defines the *1 allele (normal or no-risk allele). Haplotype was not determined. The *ITPA* genotyping assays target the single-nucleotide variants rs1127354 and rs7270101. After generating an end point read on the QuantStudio, data were

Table 1. Distribution patterns of TPMT activity in RBC in all ages.

	Age, years	Low % (n)	Intermediate % (n)	Normal % (n)	High % (n)
Female	<18 (n = 1855)	0.70 (13)	15.17 (281)	83.98 (1558)	0.16 (3)
(Total = 27 414)	18–65 (n = 21 073)	0.52 (109)	14.13 (2979)	85.15 (17 944)	0.19 (41)
	≥65 (n = 4486)	0.55 (25)	12.72 (571)	86.49 (3880)	0.22 (10)
Male	<18 (n = 1872)	0.43 (8)	12.82 (240)	86.49 (1619)	0.27 (5)
(Total = 18 388)	18–65 (n = 13 541)	0.45 (62)	11.86 (1606)	87.29 (11 821)	0.38 (52)
	≥65 (n = 2975)	0.43 (13)	10.78 (321)	88.26 (2626)	0.50 (15)
Female + male (Total = 45 802)		0.5 (230)	13.1 (5998)	86.13 (39 448)	0.28 (126)

imported into TaqMan® Genotyper software (Thermo Fisher Scientific) and were plotted by the instrument software to generate allele calls.

Historical data analysis

Three archived data sets were retrieved retrospectively and deidentified at ARUP Laboratories (Salt Lake City, UT), according to protocols approved by the University of Utah Institutional Review Board. Data set 1 included results from 45 802 specimens, representing all routine laboratory TPMT activity testing (phenotype). The data available included sex, age, and enzyme activity. Data set 2 included results from 4441 specimens, representing all routine laboratory *TPMT* genotype testing. The data were imported into Microsoft Excel to perform analysis. Data set 3 included paired results, representing TPMT genotype and phenotype from a single patient and were available for 408 specimens. Ethnicity data were not available. This study considered (*a*) distribution patterns of TPMT activity and *TPMT* genotypes, (*b*) correlations between genotypes and phenotypes, (*c*) the influence of sex and age on TPMT activity, and (*d*) distribution of *ITPA* polymorphisms among abnormal and normal TPMT activity specimens. Excel and GraphPad prism software (GraphPad Software) were used for all calculations. Kruskal–Wallis statistics with Dunn comparison test were used to compare the TPMT activity in relation to age or sex or genotype. In all tests, *P* values ≤0.05 were considered statistically significant.

RESULTS

Distribution patterns of TPMT activity in RBCs and the influence of age and sex on TPMT activity

The entire study group (n = 45 802) consisted of 27 414 females (59.85%) and 18 388 males (40.15%) with ages ranging from 0 to 90 years. The data were classified into 4 different subgroups with low, intermediate, normal, and high TPMT activities as described in Materials and Methods. The distributions of TPMT activity among the sexes are shown in Fig. 2, A and B. In contrast with the previous publication (*17*), the distribution of the TPMT activity does not show bimodality; distribution patterns of TPMT activity in males and females were similar. The results are summarized in Table 1. Females: This group, based on age, was further configured into 3 subgroups: subgroup 1 consisting of 1855 females 0 to 17 years of age; subgroup 2 consisting of 21 073 females 18 to 65 years of age; and subgroup 3 consisting of 4486 women 65 to 90 years of age. TPMT activity levels among females varied greatly, ranging from 6.3 to 90 U/mL. In subgroup 1, 0.7% of individuals (n = 13) had low TPMT activity; 15.17% of individuals had intermediate TPMT activity; 83.98% of individuals showed normal TPMT activity; and 0.16% of individuals

presented with high TPMT activity. Similar distribution patterns were observed for subgroups 2 and 3. The frequencies of low, intermediate, normal, and high activity in subgroup 2 (subgroup 3) were 0.52% (0.55%), 14.13% (12.72%), 85.15% (86.49%), and 0.19% (0.22%). Males: Similarly, this group was configured into 3 subgroups based on age: subgroup 1 consisting of 1872 males 0 to 17 years of age; subgroup 2 consisting of 13541 males 18 to 65 years of age; and subgroup 3 consisting of 2975 men 65 to 90 years of age. Compared with females, the frequency of low TPMT activity was slightly lower: 0.42% in subgroup 1, 0.45% in subgroup 2, and 0.43% in subgroup 3. No dramatic difference in the frequency of low TPMT activity among these 3 subgroups was observed. The frequencies of intermediate, normal, and high TPMT activity in subgroup 2 (subgroup 3) were 11.86% (10.78%), 87.29% (88.26%), and 0.38% (0.50%). There were no significant differences in TPMT activity in relation to age and sex in any of the 3 subgroups (Fig. 2C). TPMT activity within subgroup 1 (both female and male) was further distributed according to age with an interval of 1 year (Fig. 2D). Median, mean, and standard deviation (SD) of TPMT activity in each age-group (1-year intervals) within subgroup 1 are summarized in Table 2. Again, no significant differences were observed in TPMT activity among and between subgroup 1 with a higher age resolution ($P > 0.05$). Further breakdown of TPMT activity within adults is illustrated in Table 1 and Fig. 1 of the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol3/issue5.

Frequency of *TPMT* genotype

Genotype results from 4441 individuals were used for analysis for the common alleles *2, *4, and *3A to *3C. The frequencies of variant alleles were 7.74% (*3A, n = 344), 0.05% (*3B, n = 2), 2.22% (*3C, n = 95), and 0.42% (*2, n = 19). The total number of heterozygous individuals was 392 (*3A/*1, 6.73%, n = 300; *3B/*1, 0.02%, n = 1; *3C/*1, 2.04%, n = 91; *2/*1, 0.43%, n = 19). Thirteen of 4441 individuals were identified as carriers of 2 variant alleles, 8 in a homozygous variant status (*3A/*3A) and 5 as compound heterozygotes (*3A/*3C, n = 4; *3A/*3B, n = 1) (Fig. 3).

Phenotype–genotype correlation

To correlate *TPMT* genotype with phenotype, 408 deidentified paired genotype and phenotype results were retrieved from the database and used to study the correlation of phenotype and genotype. The distribution of TPMT activity among 408 results in relation to their genotypes is summarized in Fig. 4. The frequencies of genotypes were 8.06% (n = 33, 3A*/*1), 1.96% (n = 8, *3C/*1), 0.5% (n = 2, *2/*1), and 0.2% (n = 1, *3A/*3A). Note that no *3B variant allele was identified in this data set. Compared with the activity in specimens without variant alleles (n = 364), TPMT activity in specimens with variant allele(s) was significantly lower (Fig. 4). TPMT activity [mean ± SD] was 29.4 ± 3.7 U/mL, 22.1 ± 2.8 U/mL, 23.7 ± 3.9 U/mL, and 23.3 ± 0.7 U/mL for *TPMT**1/*1, *3A/*1, *3C/*1, and *2/*1, respectively. TPMT activity in 1 specimen with *TPMT**3A/*3A genotype was 14.2 U/mL. No significant difference in TPMT activity in relation to sex in the intermediate phenotype group (female, 23.1 ± 2.7 U/mL vs male, 21.4 ± 3.3 U/mL) was observed.

Distribution of *ITPA* variants among normal and abnormal TPMT phenotype and genotype

ITPA is another enzyme involved in thiopurine metabolism. To evaluate distribution of *ITPA* polymorphisms among various groups of TPMT activity (i.e., low, intermediate, and normal), 95 residual blood specimens that had been previously tested for TPMT enzyme activity were collected for genotype analysis as described above. TPMT activity among these specimens was in the range of 15 to 35 U/mL. Association of *ITPA* polymorphisms and *TPMT* genotypes in this sample set is illustrated in Table 3 here and Table 2 in the online Data Supplement.

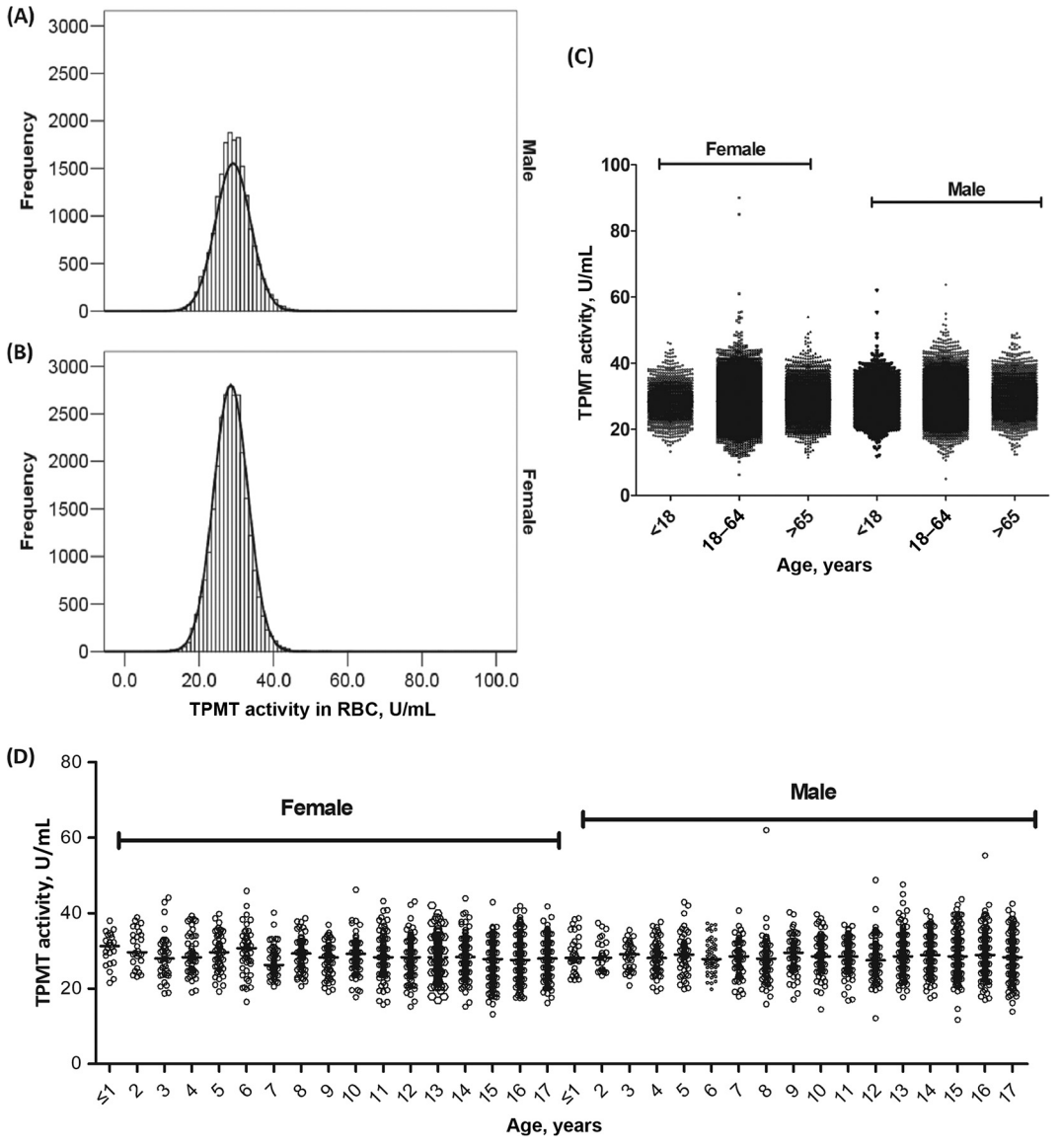


Fig. 2. Distribution patterns of TPMT activity in (A) males and (B) females, (C) the influence of age and sex on the enzyme activity in all ages, and (D) in pediatrics.
Kruskal–Wallis statistic with Dunn comparison was used to compare the TPMT activity in relation to age or sex. No significant difference were observed ($P > 0.05$).

Briefly, no *TPMT* variants were detected in 69 of 95 blood specimens tested, with the normal enzyme activities ranging from 20.4 to 35 U/mL. Among the group with normal TPMT activity, 53.6% of samples (n = 24) had at least 1 *ITPA* variant allele (A>C at rs1127354, 17.4%, n = 12; C>A at rs7270101, 17.4%, n = 12; rs1127354 together with rs7270101, 5.8%, n = 4). The most common variant *TPMT* alleles (*3B and *3C) were found in 27.3% of specimens (n = 24) with low to intermediate TPMT enzyme activity

Table 2. Distribution patterns of TPMT activity in RBC in relation to age and sex in pediatric population (0–17 years old).

	Female																
Age, years	**≤1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Total specimens, n	24	26	43	50	50	51	59	71	70	106	124	144	143	179	203	249	263
Minimum, U/mL	21.5	23.1	18.7	19.0	19.2	16.5	20.5	20.6	19.2	17.7	15.8	15.2	16.9	15.2	13.2	17.5	16.2
25% percentile, U/mL	27.4	26.7	24.1	24.8	26.0	27.0	24.6	26.2	25.2	27.3	25.4	25.8	25.1	25.1	24.8	24.7	25.4
Median, U/mL	31.3	29.7	28.0	28.3	29.7	30.7	26.3	29.4	28.3	29.2	28.4	28.4	28.3	28.4	27.8	27.6	28.0
75% percentile, U/mL	34.1	34.4	31.3	32.4	33.9	33.5	29.8	31.7	30.9	31.3	31.7	30.7	31.4	31.3	30.7	31.0	30.5
Maximum, U/mL	38.0	38.9	44.1	39.3	39.8	45.9	40.1	38.6	36.9	46.2	43.2	43.1	42.0	43.9	42.9	41.9	41.8
Mean, U/mL	30.6	30.6	28.4	28.9	29.6	30.1	27.3	29.2	28.0	29.1	28.6	28.3	28.4	28.4	27.6	27.9	28.0
SD[a]	4.2	4.9	5.6	5.1	4.9	5.9	3.9	4.1	4.0	4.1	5.0	4.4	4.9	4.4	4.7	4.7	4.0
	Male																
Age, years	**≤1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Total specimens, n	32	26	32	58	50	52	74	76	80	109	127	114	166	180	217	248	231
Minimum, U/mL	22.3	23.4	20.8	19.3	19.8	19.8	17.9	15.9	17.1	14.5	16.8	12.1	17.7	17.5	11.7	17.0	13.9
25% percentile, U/mL	25.8	24.9	25.2	25.3	25.9	25.6	25.4	23.9	26.0	25.8	26.0	25.4	26.0	26.0	25.6	25.9	25.4
Median, U/mL	28.1	28.2	29.1	28.1	29.1	27.8	28.6	28.0	29.5	28.6	28.5	27.5	28.6	28.9	28.6	28.9	28.3
75% percentile, U/mL	32.3	32.0	31.8	31.3	32.3	31.8	31.1	30.2	31.8	31.7	31.3	30.6	32.0	31.3	31.7	31.7	31.3
Maximum, U/mL	38.7	37.4	35.5	37.7	42.9	37.3	40.7	62.0	40.2	39.7	36.9	48.8	47.6	40.5	43.7	55.3	42.5
Mean, U/mL	29.2	28.9	28.7	28.4	29.5	28.6	28.2	27.7	29.1	28.7	28.6	27.9	29.2	28.9	28.9	29.0	28.4
SD[a]	4.8	4.1	3.7	4.4	5.3	4.5	4.4	5.9	4.3	4.3	3.8	4.7	4.9	4.2	5.0	4.8	4.6

[a] SD of TPMT activity in each age-group.

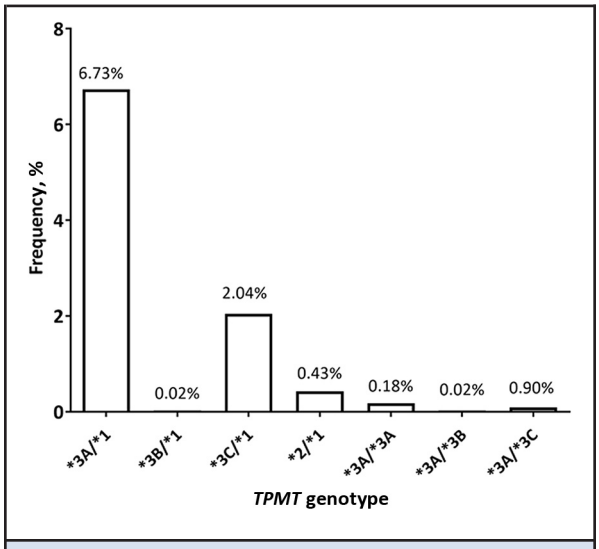
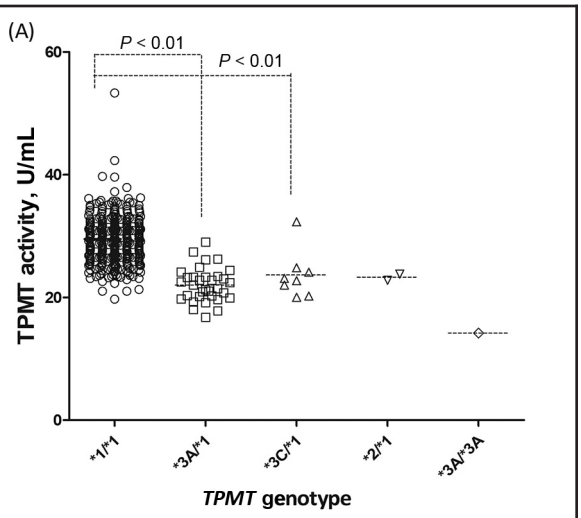


Fig. 3. The frequency distribution of genotype results from 4441 individuals.

ranging from 15 to 24.8. Fifteen specimens harbored the *3A allele (i.e., *3A/*1); 9 specimens harbored the *3C allele (i.e., *3C/*1); and 2 specimens harbored the *3A and*3B alleles (i.e., *3A/*3B). At least 1 *ITPA* variant was detected in combination with the *TPMT**3A or *3C, whereas 2 specimens with both *ITPA* polymorphisms were detected in specimens that harbored *TPMT**3A and *3B alleles. The association of *ITPA* polymorphisms and TPMT activity is illustrated in Fig. 5. No significant difference in TPMT activity was observed between *ITPA* variant alleles and nonvariant alleles. In summary, an equal distribution of *ITPA* variants was found among normal and abnormal *TPMT* phenotype and genotype.

DISCUSSION

TPMT plays important roles in the metabolism of thiopurine drugs, including AZA, 6-thioguanine,



(B)

	*1/*1	*3A/*1	*3C/*1	*2/*1	*3A/*3A
TPMT activity	29.4 ± 3.7 (n = 364)	22.1 ± 2.8 (n = 33)	23.65 ± 3.89 (n = 8)	23.3 ± 0.71 (n = 2)	14.2 (n = 1)

Fig. 4. (A), The distribution of TPMT activity in relation to their genotypes, and (B) mean ± SD of TPMT activity.

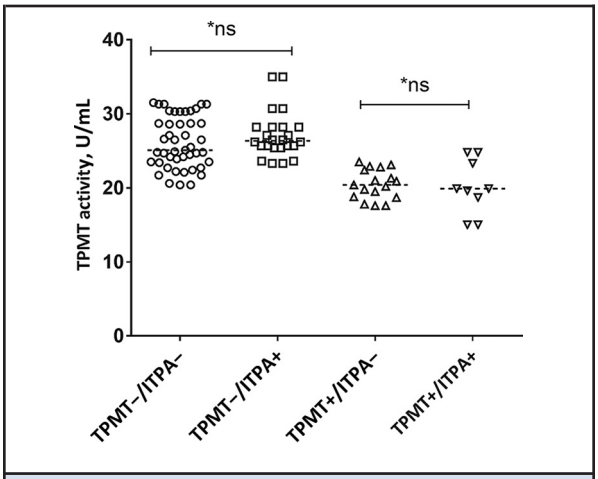


Fig. 5. Association of *ITPA* polymorphisms and TPMT activity.

TPMT+ represents *TPMT* gene found positive for variants allele; *TPMT*− represents *TPMT* gene with no variant alleles detected; *ITPA*+ represents *ITPA* gene found positive for variant alleles; *ITPA*− represents *ITPA* gene with no variants detected. *ns, not significant ($P > 0.05$).

and 6-MP. Therefore, it is important to detect impaired TPMT activity or loss of function *TPMT* variants before thiopurine drug administration, as advocated by clinical guidelines and drug labeling to identify patients at risk for dose-related toxicity, to facilitate appropriate dosing. In this study, we evaluated (*a*) distribution patterns of TPMT phenotypes (activity) and *TPMT* genotypes, (*b*) correlations between genotype and phenotypes, (*c*) the influence of sex and age on TPMT activity, and (*d*) distribution of *ITPA* polymorphisms among low, intermediate, and normal TPMT activity specimens.

The distribution of TPMT activity in packed RBC lysate is similar to previous studies (*6*), wherein 0.5% of samples showed low, 13.1% intermediate, 86.1% normal, and 0.28% high activity levels. However, one limitation related to this portion of the finding is the unavailability of clinical data. We do not know whether those individuals have recently had red cell transfusions, as TPMT activity is influenced by red cell transfusion. Moreover, it is unknown whether these patients were taking

Table 3. Association of *ITPA* polymorphisms and *TPMT* diplotype.

		TPMT activity, U/mL				ITPA		
***TPMT* diplotype**	**Total number**	**Mean**	**Median**	**Maximum**	**Minimum**	**rs1127354 A/C % (n)**	**rs7270101 C/A % (n)**	**rs7270101 A/C and rs1127354 C/A % (n)**
*1/*1	69	24.2	25.7	35	20.4	12 (17.4%)	17.4% (12)	4 (5.8%)
*3A/*1	15	22.9	21	24.8	18.7	6.7% (n = 1)	26.7% (4)	—
*3C/*1	9	19.5	18.8	23.3	17.6	11% (n = 1)	11% (1)	—
*3A/*3B	2	15	—	—	—	—	100% (2)	—

medications, such as salicylic acid, trimethoprim, and diuretics, which can alter TPMT activity. Without clinical information, we are unable to exclude such interferences from our results (*18*, *19*). Additionally, diseases and disease-related treatment also influence TPMT activity (*20*). Furthermore, the results from our study showed the expected *TPMT* allele frequencies, with *3A as the most common variant allele (frequency, 6.73%), 0.02% frequency of *3B, 2.22% frequency of *3C, and 0.49% frequency of *2. Another limitation of this study was that race/ancestry was not known for this population.

The influence of age on TPMT activity has been previously studied (*21*–*24*). In one study, the authors reported that, in healthy populations, children have higher TPMT activities than elders, and younger children were associated with higher activities than older children, with healthy neonates having the highest activities (*21*, *24*). Our studies also examined the impact of age on the distribution of TPMT activity in RBCs. In contrast to the previous findings, no dramatic difference in TPMT activity in relation to age was observed within the pediatric population (age interval of 1 year) and adults (age interval of 10 years) and between them. As our samples were submitted for medical testing, it is presumed that the patients in our population were not healthy at the time of testing, which may explain the lack of an age-related trend in activity. The influence of sex on the enzyme activity has also been investigated previously (*21*, *22*, *25*). However, contradictory findings have been reported from no influence of sex on TPMT activity (*25*) to some influence of sex on TPMT activity (*22*). One study reported that wild-type TPMT infants <2 years of age have a higher activity in boys than girls (*21*). We have performed similar analysis, and no dramatic difference in TPMT activity between boys and girls was observed (Table 1 and Fig. 2). Again, we believe the discrepancy is likely because of the different sample population and/or using different methods to normalize the activities (use of packed red cells vs hemoglobin/hematocrit). In contrast, our results agreed with the findings reported by Klemetsdal et al. (*25*). In this previous publication (*25*), by examining TPMT activity in RBCs in a healthy population sample of children 1 to 10 years of age (87 boys and 71 girls), no significant differences were observed between boys and girls. However, in the same study, age was found to be negatively correlated with TPMT activity. Again, the discrepancy can be attributed to the factors discussed above.

Adverse drug reactions to thiopurine drugs occur in 15% to 28% of patients, but many cannot be explained by *TPMT* pharmacogenetics. The association of *ITPA* variants and the occurrence of adverse events or lack of response of AZA have been studied. In one study, the *ITPA* 94C>A (rs1127354) allele was significantly associated with flu-like symptoms, rash, and pancreatitis (*15*). However, several contradictory results have been reported (*16*, *26*). Our studies evaluated the distribution of *ITPA* variants among normal, intermediate, and low TPMT phenotypes, and revealed an equal distribution of *ITPA* variants among normal and abnormal TPMT phenotype and genotype. Moreover, our findings showed that TPMT activity is not significantly different between individuals with an *ITPA* mutation vs those without the mutation. Likely, a multigenetic signature of *TPMT*, *ITPA*, and genes that code for other proteins involved in thiopurine pharmacogenetics, such as glutathione *S*-transferase (*27*) and nucleoside diphosphate-linked moiety X motif 15 (*28*, *29*), is necessary to explain adverse effects in the patients that cannot explained by *TPMT* pharmacogenetics, and is likely to provide better dosing recommendations for thiopurines in the future.

In summary, this retrospective data analysis demonstrated (*a*) a clustering of variant *TPMT* genotypes with phenotypes, (*b*) insignificant influence of age and sex on distribution of TPMT activity in RBCs, and (*c*) an equal distribution of *ITPA* polymorphisms among normal and abnormal TPMT phenotype and genotype.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form.* **Employment or Leadership:** ARUP Laboratories. **Consultant or Advisory Role:** None declared. **Stock Ownership:** None declared. **Honoraria:** None declared. **Research Funding:** The ARUP Institute for Clinical and Experimental Pathology. **Expert Testimony:** None declared. **Patents:** None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: The authors thank Dave Davis from the IT department at ARUP for help with retrieving data from the database.

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