Supplemental Material

Table S1 was changed on May 24, 2011 to reflect bases being reported on the positive chromosomal strand.

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for thiopurine methyltransferase (TPMT) genotype and thiopurine dosing

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CPIC Updates:

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines are published in full on www.PharmGKB.org . Information will be reviewed and updated periodically on that website.

Literature Review:

We searched the PubMed database (1966 to May 2010 and Ovid MEDLINE (1950 to May 2010) for keywords ((TPMT) OR (thiopurine methyltransferase) OR thiopurine S-methyltransferase) AND (thiopurine OR mercaptopurine OR thioguanine OR azathioprine) for the contribution TPMT genotype had on predicting a thiopurine-related adverse drug event (ADE) or outcome. Definitive reviews (1-5) were relied upon to summarize much of the earlier literature.

To construct a *TPMT* minor allele frequency table (Supplemental Table S3) based on ethnicity, the following search criteria were used: (TPMT) OR (thiopurine) AND ((allele) OR (frequency) OR (genotype)). Studies were considered for inclusion if: (1) the ethnicity of the population was clearly indicated, (2) either allele frequencies or minor allele percentages for *TPMT* genotypes were reported, (3) the method by which *TPMT* was genotyped was reliable and proven (no proof-of-principle experiments), (4) the sample population consisted of at least 50 patients, and (5) the study represented an original publication (no reviews). The combined analysis included 8,676 Caucasians, 2,938 Mediterraneans, 1,028 South Americans, 1,146 Africans, 8,377 Asians, 600 South West Asians, 507 Mexicans, and 2,403 Middle Easterners. A similar search strategy was used to gather the body of evidence related to the use of thiopurines in specific disease states and the relative contribution TPMT genotype had on predicting a thiopurine-related adverse drug event (ADE).

Genetic Test Interpretation

The haplotype, or star (*) allele name, is determined by a specific SNP or a combination of SNPs that are interrogated in the genotyping analysis. The genotypes that constitute the haplotype, or star (*) alleles for *TPMT*, and the rs# for each of the specific genomic nucleotide alterations that define the alleles, are described in Supplemental Table S1.

The genotype results are generally reported as a diplotype, which includes one maternal and one paternal star allele (e.g. *1/*3A). The TPMT activity associated with each of the common * alleles is summarized in Supplemental Table S2. The most common inactivating allele among Caucasians for TPMT is designated as *3A; other alleles predominate in other ethnic/ancestral groups (Tables S3 and S4). The *3A allele designation for *TPMT* is assigned based on the SNP genotypes and the very strong linkage disequilibrium that has been established between two of the most common inactivating *TPMT* SNPs: Ala 154 Thr (rs1800460) and Tyr 240 Cys

(rs1142345); when the rare genotype is present at these two SNP positions in the heterozygous state, the assumption is that the rare genotypes are on the same allele (and the diplotype call is *1/*3A). However, each of these SNPs have been observed to exist on their own allele (*3B and *3C, respectively) in some populations (at the frequencies shown in supplemental Tables 3 and 4) with the rare genotypes present on their own); if these rare genotypes are present on opposite alleles in the same individual, the diplotype call should be that of a compound homozygote deficient diplotype (*3B/*3C)—a call consistent with homozygous TPMT deficiency. If one assumes that the frequency of the *3B allele is 0.000461, and of the *3C allele is 0.004207 (Table S3), the probability of finding such a compound homozygote deficient diplotype is estimated is 1 in 515,616 Caucasian individuals. To our knowledge, an individual with the *3B/*3C genotype has never been identified, but this is likely because both alleles are relatively rare. Phenotypic tests distinguish these two genotypes, which should be employed if a homozygous deficient genotype is suspected. One of the two phenotyping tests (measuring erythrocyte TPMT activity or thiopurine metabolites after thiopurine dosing) can differentiate a *1/*3A diplotype (heterozygote) from a very rare *3B/*3C diplotype (homozygous deficient). TPMT activity would be extremely low in the latter case and intermediate in the former case; erythrocyte thiopurine metabolites would indicate a low but detectable MeTIMP/TGN ratio for a *1/*3A diplotype and the *3B/*3C diplotype would be consistent with undetectable MeTIMP (or MeMPN) levels.

The frequencies of phenotypic groups (homozygous for one of any of the variant nonfunctional alleles, heterozygous (one wild-type and one variant allele), and homozygous for wild-type alleles that are cited in the CPIC guidelines is based on the assumption that the frequency of the wild-type allele are between 0.925 and 0.984, and the frequency of a nonfunctional allele is between 0.016 and 0.075, depending upon ethnicity/race. Assuming Hardy-Weinberg equilibrium, this leads to the estimates of 1 in 178 to 1 in 3736 being homozygous for a nonfunctional allele (depending upon ethnicity and ancestry), 3% to 14% being heterozygote, and ~ 86-97% being homozygous for wild-type alleles. These estimates of phenotypic group frequencies differ among race/ethnic groups, as per the allele frequency estimates in Tables S3 and S4.

Available Genetic Test Options

Commercially available genetic testing options change over time. Genetic tests registries include www.genetests.org. Below is some information that may assist in evaluating options. Many commercially available tests include only *2, *3A, *3B and *3C, although the rare *4 allele is also inactivating.

Examples include Clinical Laboratory Improvement Amendments (CLIA) certified testing offered via Prometheus Laboratories, San Diego, CA (*Prometheus™ TPMT Genetics*) and Specialty Laboratories, Valencia, CA (*TPMT GenotypeR™*) for *TPMT*2, *3A, *3B and *3C.

While there are no specific Current Procedural Terminology (CPT) codes for a *TPMT* genotyping test, the CPT code modifier 9A has been reserved for *TPMT* and should be used by clinicians once *TPMT* genotyping has been deemed medically necessary. The CPT codes for an individual *TPMT* genotyping test, if requested from Prometheus Laboratories, should include: 83891 – Isolation or extraction of highly purified nucleic acid (x1), 83898 – Amplification of patient nucleic acid for three different DNA sequences (x3), 83896 – Nucleic acid probe (x6), and 83912 – Interpretation and report (x1). However, it should be noted that while CPT codes are designed to be universal, it is the jurisdiction of the healthcare and service providers and that will determine proper coding.

Several health insurance companies cover testing for *TPMT* genotypic or phenotypic assays (6). For example, "Aetna considers *TPMT* gene mutation assays (e.g., PRO-Predict^{R} *TPMT*) or TPMT phenotypic assays (TPMT enzymatic activity, e.g., PRO-Predict^{R} EnzAct) medically necessary prior to initiation of 6-mercaptopurine or azathioprine therapy" for inflammatory bowel disease.(7) Some clinical laboratories provide healthcare professionals with template letters for physician appeal, medical necessity and pre-authorization to assist in obtaining reimbursement for *TPMT* genotyping. However, not all organizations support reimbursement for TPMT testing.

Levels of Evidence linking genotype to phenotype

Multiple grading scales for evidence were evaluated. (8-10) Ultimately, we chose a simple scale of high, moderate, or weak to grade the levels of evidence, based on criteria previously published.(9)

> High: Evidence includes consistent results from well-designed, well-conducted studies.
> Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.
> Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

We have focused on presenting evidence from well-done studies, and it is the interpretation of the results from these studies that provide the framework for the strength of the dosing recommendations in Table 2.

Strength of Dosing Recommendations

Multiple rating schemes for strength of recommendations in a number of clinical guidelines were evaluated. (8, 10, 11) Ultimately, we chose to use a slight modification of a transparent and simple system(10) for just 3 categories for recommendations: strong, where "the evidence is high quality and the desirable effects clearly outweigh the undesirable effects"; moderate, in which

"there is a close or uncertain balance" as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects; and optional, for recommendations in-between strong and weak where there is room for differences in opinion as to the need for the recommended course of action. CPIC's dosing recommendations are based on weighting the evidence from a combination of preclinical functional and clinical data (Table S5), as well as on some existing disease-specific consensus guidelines. (5) Some of the factors that are taken into account in evaluating the evidence supporting dosage recommendations include: *in vivo* clinical outcome data for thiopurines, *in vivo* pharmacokinetic and pharmacodynamic data for thiopurines, *in vitro* enzyme activity of expressed wild-type or variant-containing TPMT (with thiopurines as substrate), *in vitro* TPMT enzyme activity from tissues isolated from individuals of known *TPMT* genotypes, *in vivo* pre-clinical pharmacokinetic and pharmacodynamic studies, and *in vitro* studies of TPMT protein stability or enzyme activity.

Overall, the dosing recommendations are simplified to allow rapid interpretation by clinicians. They have been adopted from the rating scale for evidence-based therapeutic recommendations on the use of retroviral agents. (8)

A: Strong recommendation for the statement
B: Moderate recommendation for the statement
C: Optional recommendation for the statement

Other considerations

Complementary clinical laboratory tests are available to measure thiopurine metabolites in erythrocytes: TGNs (for mercaptopurine, azathioprine, and thioguanine) and MeMPNs (or MeTIMP) for those on mercaptopurine or azathioprine. These tests can be useful to confirm TPMT phenotype, and to test for patient adherence with oral medication regimens, but the values are dependent upon the prior thiopurine dosing. TPMT activity phenotype can also be assessed by measuring erythrocyte TPMT activity; however, activity measures must be interpreted with caution because TPMT activity increases after exposure to thiopurines, and will be spuriously altered from baseline if the patient has recently received allogeneic erythrocyte transfusions.(2, 5, 12-14) Furthermore, since TPMT activity is similar to other erythrocyte enzymes that decrease during the red cells' life-span, the erythrocyte TPMT activity in a wild-type patient with bone-marrow insufficiency (e.g. as is true at diagnosis of ALL) may be within the expected range of a healthy TPMT heterozygote patient, and a TPMT heterozygous patient with a rapid red cell turn-over (e.g. as seen during hemolysis) may have erythrocyte TPMT activity within the TPMT wild-type range.(15)

Conflicts between phenotype and genotype results (e.g. a wild-type TPMT activity in an individual with a homozygous or heterozygous low-activity genotype) may be resolved with repeat sampling and testing. Because most commercial genotyping assays test only the three most common inactivating SNPs, if a rare inactivating (and untested-for) SNP is present, a spurious wild-type genotype assignment could be made although phenotype tests indicate low TPMT activity or low MeTIMP/TGN ratio. Another rare possibility, would be that two inactivating SNP variants are mistakenly assumed to reside on the same allele, when they in fact reside on opposite alleles; phenotypic tests can distinguish these two possibilities.

One caveat to thiopurine use is that some serious long-term adverse effects (secondary tumors) have been associated with defective TPMT activity (16-19) without necessarily causing serious acute myelosuppression; whether capping doses of thiopurines in those with a TPMT defect will decrease the risk of the late effect of secondary cancer is not known. Moreover, some adverse reactions to thiopurines, such as pancreatitis and hepatotoxicity, are not related to low TPMT activity. (20)

Supplemental Table S1. Genotypes that constitute the * alleles for *TPMT*	
Allele	Constituted by genotypes at:
*1	C at rs#1800462; C at rs#1800460; T at rs#1142345; C at rs#1800584
*2	C>G at rs#1800462
*3A	C>T at rs#1800460 and T>C at rs#1142345
*3B	C>T at rs#1800460
*3C	T>C at rs#1142345
*4	C >T at rs#1800584
See www.pharmgkb.org for updates on *TPMT* alleles and nomenclature. Bases reported on the positive chromosomal strand from Golden Path.	

Supplemental Table S2. Association between allelic variants[1] and TPMT enzyme activity (21-31)

Functional Status	Alleles
Functional / normal activity/ wild-type[2]	*1
Non-functional, variant, or mutant / no activity	*2, *3A, *3B, *3C, *4
Probable Reduced-function / decreased activity (these alleles are very rare and most have shown reduced rather than absent activity)	*6, *9, *10, *11, *12,*13, *16, *17, *18

[1]: see www.pharmgkb.org for updates on *TPMT* allelic variants and nomenclature

[2]: an important caveat for all genotyping tests is that the decision to assign an allele a "wild-type" status is based upon a genotyping test that interrogates only the most common and already-proven sites of functional variation. In human DNA, it is always possible that a new, previously undiscovered (and therefore un-interrogated) site of variation may confer loss-of-function in an individual, and thus lead to the rare possibility of a non-functional allele being erroneously called as "wild-type"

Supplemental Table S3. Frequencies[1] of alleles in major race/ethnic groups[2]

Allele	Caucasian	Mediter-ranean	South American	African	Middle Eastern	Mexican	Asian	South West Asian
*1	**0.95671**	**0.96081**	**0.95233**	**0.94284**	**0.96987**	**0.92500**	**0.98364**	**0.97837**
*2	0.00190	0.00408	0.00876	0.000873	0.00749	0.00592	0	0.00250
*3A	0.0354	0.0254	0.0287	0.00218	0.0114	0.0533	0.000119	0.00583
*3B	0.000461	0.00426	0.000486	0	0.00562	0.00690	0	0
*3C	0.004207	0.00545	0.00924	0.0480	0.00562	0.00888	0.0157	0.0133
*4-*26	0.0000576 (*7) 0.00115 (*9) 0.0000576 (*11) 0.0000576 (*12)	N/A	0.000486 (*4)	0.00611 (*8)	N/A	N/A	0.000537 (*6)	N/A

[1]Average frequencies are reported based on the average from the actual numbers of subjects with each allele reported in multiple studies. See Supplemental Table S4 for details and references.
[2]Race/ethnic group designations correspond to those indicated in Supplemental Table S4

Supplemental Table S4. *TPMT* minor allele frequency

Pooled grouping	Ethnicity	TPMT minor allele frequency (%)					Total patients	TPMT minor alleles observed					Total alleles
		*2	*3A	*3B	*3C	*4–26		*2	*3A	*3B	*3C	*4–26	
Caucasian	British (32)	0.5	4.5	0	0.3	–	199	2	18	0	1	–	398
Caucasian	Bulgarian (33)	0.2	2.2	0	0.2	–	313	1	14	0	1	–	626
Caucasian	Caucasian (34)	0.1	2.9	0	0.3	–	390	1	23	0	2	–	780
Caucasian	Czech (35)	0.1	4.5	0.1	0.9	–	696	2	62	1	12	–	1392
Caucasian	Czech (36)	0	3.4	0	0	–	87	0	6	0	0	–	174
Caucasian	Danish (37)	0	3.3	0	0.3	–	200	0	13	0	1	–	400
Caucasian	Dutch (38)	0	2.6	0.5	0.5		190	0	10	2	2	–	380
Caucasian	Dutch (39)	0	2.1	0	1.4	–	72	0	3	0	2	–	144
Caucasian	Estonian (40)	0.6	4.9	0	0.6	0.3 (*9,*12)	154	2	15	0	2	1 (*9 & *12)	308
Caucasian	European (41)	0.5	5.8	0	0.8	0.3 (*7)	191	2	22	0	3	1 (*7)	382
Caucasian	German (42)	0	2.9	0	0.5	0	105	0	6	0	1	–	210
Caucasian	German (23)	0.2	4.3	0	0.4	–	1209	6	105	0	9	–	2418
Caucasian	German (24)	0.2	2.9	0	0.6	0.1 (*9, *11)	814	4	48	0	9	1 (*9 &*11)	1628
Caucasian	Greenlandic (37)	0	8.1	0	0	–	142	0	23	0	0	–	284
Caucasian	Irish (43)	0	3.4	0.1	0.4	–	407	0	28	1	3	–	814
Caucasian	Polish (44)	0.4	2.7	0	0.1	–	358	3	19	0	1	–	716
Caucasian	Polish (45)	0.6	4.2	0	0.6	–	180	2	15	0	2	–	360
Caucasian	Polish (46)	–	5.2	0	0.6	–	87	–	9	0	1	–	174
Caucasian	Portuguese (47)	1	2.4	0	0.7	–	143	3	7	0	2	–	286
Caucasian	Portuguese (48)	0	2.8	0	0	–	334	0	19	0	0	–	668
Caucasian	Russian (49)	0.1	2.3	0	0.4	–	995	2	45	0	8	–	1990
Caucasian	Serbian (50)	0.3	3.3	0.5	0	–	200	1	13	2	0	–	400
Caucasian	Swedish (51)	0.1	3.8	0.1	0.4	–	800	1	60	2	7	–	1600
Caucasian	Swedish (52)	0	4.1	0	0.8	–	61	0	5	0	1		122
Caucasian	Swiss (53)	0.2	2.9	0	0.6	–	240	1	14	0	3	–	480
Caucasian	White/Danish (54)	0	5.5	0	0	–	109	0	12	0	0	–	218
Total Caucasian							**8676**	**33**	**614**	**8**	**73**	**1 (*7) 2 (*9) 1 (*11) 1 (*12)**	**17352**

Mediterranean	French (55)	0.8	4	0	0.4	–	468	7	28	0	4	–	936
Mediterranean	Greek (56)	3.1	2.6	4.6	1	–	97	6	5	9	2	–	194
Mediterranean	Italian (57)	0.7	2.9	0	0	–	70	1	4	0	0	–	140
Mediterranean	Italian (58)	0.5	3.9	0	1	–	103	1	8	0	2	–	206
Mediterranean	Italian-Caucasian (59)	0	2.2	0.3	0.3	–	943	0	41	6	6	–	1886
Mediterranean	Sardinian (60)	1.7	0.6	0.4	0.8	–	259	9	3	2	4	–	518
Mediterranean	Slovenian (61)	0	4.1	0.3	0.5	–	194	0	16	1	2	–	388
Mediterranean	Slovenian (62)	0	3.4	0	0	–	313	0	21	0	0	–	626
Mediterranean	Spanish (38)	0	2.9	1.2	1.8	–	169	0	10	4	6	–	338
Mediterranean	Spanish (63)	0	2.5	0.7	1	–	216	0	11	3	4	–	432
Mediterranean	Turkish (64)	0	0.9	0	0.9	–	106	0	2	0	2	–	212
Total Mediterranean							**2938**	**24**	**149**	**25**	**32**	–	**5876**
South American	Argentinean (65)	0.7	3.1	0	0	0.3 (*4)	147	2	9	0	0	1 (*4)	294
South American	Bolivian (66)	0	6.5	0	0	–	115	0	15	0	0	–	230
South American	Brazil European (67)	0.8	1.6	0	2.1	–	306	5	10	0	13	–	612
South American	Brazilian (68)	2.2	1.5	0.2	1	–	204	9	6	1	4	–	408
South American	Brazilian (69)	0.4	3.9	0	0.9	–	116	1	9	0	2	–	232
South American	Colombian (70)	0.3	3.6	0	0	–	140	1	10	0	0	–	280
Total South American							**1028**	**18**	**59**	**1**	**19**	**1 (*4)**	**2056**
African	Afro-American (71)	0.4	0.8	0	2.4	0.2 (*8)	248	2	4	0	12	1 (*8)	496
African	Black (72)	0	0	0	3.5	–	227	0	0	0	16	–	454
African	Ghanaian (73)	0	0	0	7.6	–	217	0	0	0	33	–	434
African	Cabindanian (74)	0	0	0	3.9	2.4 (*8)	103	0	0	0	8	5 (*8)	206
African	Mozambiqueian (75)	0	0.2	0	3.8	1.6 (*8)	250	0	1	0	19	8 (*8)	500
African	Kenyan (76)	0	0	0	10.9	–	101	0	0	0	22	–	202

Total African							**1146**	**2**	**5**	**0**	**110**	**14**	**2292**
Asian	Chinese (77)	0	0	0	1.5	–	462	0	0	0	14	–	924
Asian	Chinese (32)	0	0	0	2.3	–	192	0	0	0	9	–	384
Asian	Chinese (78)	0	0	0	1.4	–	271	0	0	0	15	–	542
Asian	Chinese (79)	0	0.1	0	1	–	701	0	1	0	14	–	1402
Asian	Chinese (80)	0	0	0	1.3	–	278	0	0	0	7	–	556
Asian	Chinese (81)	0	0	0	3.2	–	332	0	0	0	21	–	664
Asian	Chinese (82)	0	0	0	2.3	–	150	0	0	0	7	–	300
Asian	Chinese (83)	0	0	0	1.3	–	225	0	0	0	6	–	450
Asian	Chinese (84)	0	0.3	0	1.6	–	160	0	1	0	5	–	320
Asian	Filipino (85)	0	0	0	1	–	100	0	0	0	2	–	200
Asian	Japanese (86)	0	0	0	0.3	–	151	0	0	0	1	–	302
Asian	Japanese (87)	0	0	0	1.6	–	522	0	0	0	17	–	1044
Asian	Japanese (88)	0	0	0	0.8	–	192	0	0	0	3	–	384
Asian	Japanese (89)	0	0	0	1.4	0.7 (*6)	71	0	0	0	2	1 (*6)	142
Asian	Japanese (90)	0	0	0	1.7	–	242	0	0	0	8	–	484
Asian	Japanese (91)	0	0	0	2.6	–	236	0	0	0	12	–	472
Asian	Japanese (92)	0	0	0	0.5	–	111	0	0	0	1	–	222
Asian	Japanese (93)	0	0	0	0.9	–	279	0	0	0	5	–	558
Asian	Japanese (94)	0	0	0	1	–	147	0	0	0	3	–	294
Asian	Korean (95)	0	0	0	0.9	0.25 (*6)	400	0	0	0	7	2 (*6)	800
Asian	Korean (96)	0	0	0	1.8	0.7 (*6)	342	0	0	0	12	5 (*6)	684
Asian	Korean (97)	0	0	0	1	–	812	0	0	0	17	–	1624
Asian	Korean (98)	0	0	0	1.2	–	286	0	0	0	7	–	572
Asian	Malay (78)	0	0	0	1.2	0.3 (*6)	217	0	0	0	10	1 (*6)	434
Asian	Taiwanese (85)	0	0	0	0.6	–	249	0	0	0	3	–	498
Asian	Taiwanese (99)	0	0	0	0.4	–	526	0	0	0	4	–	1052
Asian	Thai (85)	0	0	0	1	–	100	0	0	0	2	–	200
Asian	Thai (100)	0	0	0	5.3	–	75	0	0	0	8	–	150
Asian	Thai (101)	–	–	–	3.2	–	139	–	–	–	9	–	278
Asian	Thai (102)	0	0	0	5	–	200	0	0	0	20	–	400
Asian	Tibetan (66)	0	0	0	2	–	50	0	0	0	1	–	100
Asian	Vietnamese (95)	0	0	0	2.8	0 (*6)	159	0	0	0	9	0	318
Total Asian							**8377**	**0**	**2**	**0**	**261**	**9 (*6)**	**16754**

South West Asian	British Asian (103)	0	0.6	0	2.4	–	85	0	1	0	4	–	170
South West Asian	British SW Asian (32)	0	1	0	0	–	99	0	2	0	0	–	198
South West Asian	Indian (104)	2.1	0.7	0	2.1	–	71	3	1	0	3	–	142
South West Asian	Indian (78)	0	0.5	0	0.8	–	200	0	2	0	3	–	400
South West Asian	Indian (105)	0	0.3	0	2.1	–	145	0	1	0	6	–	290
Total South West Asian							**600**	**3**	**7**	**0**	**16**	–	**1200**
Mexican	Mexican adults (106)	1.4	4.4	1.7	1.7	–	147	4	13	5	5	–	294
Mexican	Mexican newborns (107)	0.3	5.7	0.3	0.6	–	360	2	41	2	4	–	720
Total Mexican							**507**	**6**	**54**	**7**	**9**	–	**1014**
Middle Eastern	Egyptian (108)	0	0.3	0	1.3	–	194	0	1	0	5	–	388
Middle Eastern	Iranian (109)	2.2	1.7	1.6	0.5	–	832	36	28	27	9	–	1664
Middle Eastern	Israeli (110)	0	1.2	0	0.4	–	881	0	22	0	6	–	1762
Middle Eastern	Jordanian (111)	0	0.6	0	0.3	–	169	0	2	0	1	–	338
Middle Eastern	Kazak (112)	0	0.3	0	0.9	–	327	0	2	0	6	–	654
Total Middle Eastern							**2403**	**36**	**55**	**27**	**27**	–	**4806**

"-" indicates that an allele frequency was not provided

Supplemental Table S5. Evidence linking genotype with phenotype			
Type of experimental model (in vitro, in vivo preclinical, or clinical)	**Major findings**	**References**	**Level of evidence***
In vitro	MP's catabolism to methylmercaptopurine absent in human erythrocytes, lymphocytes, liver, and kidneys from *TPMT* homozygous deficient individuals	**(28, 113-115)**	High
In vitro	TG's catabolism to methylthioguanine	**(116)**	High
In vitro	Mechanisms of functional inactivation for *TPMT* *2, *3A, *3B, *3C, *4 demonstrated by expression of specific variant alleles	**(31, 117, 118)**	High
In vitro	Heterologous expression of *TPMT* catabolizes mercaptopurine to methylmercaptopurine, thioguanine to methylthioguanine, and TIMP to methylTIMP	**(119, 120)**	High
preclinical	*TPMT* knock-out mice have more morbidity and mortality from thioguanine and mercaptopurine than wild type mice; heterozygotes were at intermediate risk.	**(121)**	High
clinical	*TPMT* wild-type patients with ALL have higher risk of hematologic relapse than those with at least one variant *TPMT* allele, particularly in regimens that are primarily antimetabolite-based; wild-type patients with IBD have higher risk of treatment failure	**(122-124)**	High
clinical	*TPMT* homozygous deficient individuals have life-threatening toxicity (myelosuppression) from normal doses of MP, TG, and azathioprine; toxicity can be minimized with substantially decreased doses	**(13, 125-138)**	High

clinical	Increased risk of myelosuppression in *TPMT* heterozygotes receiving normal doses of MP	**(13, 62, 122, 127, 129, 139)**	High
clinical	Higher level of residual leukemia in *TPMT* wild-type patients than in heterozygous/homozygous deficient patients with ALL after 10 days of fixed-dose TG but not in absence of thiopurines	**(24)**	High
clinical	No change in relapse risk for heterozygous patients with ALL who receive MP doses adjusted downward for *TPMT* defective patients	**(140-142)**	Moderate
clinical	No increase in acute toxicity in heterozygous compared to homozygous wild-type patients with ALL who received MP doses adjusted downward for TPMT defective patients	**(14, 139, 143)**	High
clinical	Increased risk of secondary leukemia in those with low TPMT activity and in those with high thiopurine active metabolites when dosed independently of TPMT status	**(16-19, 144)**	Moderate
clinical	*TPMT* genotyping is useful in predicting myelosuppression and likelihood of clinical response to AZA/6-MP in IBD	**(94, 98, 130-133, 145-149)**	Moderate
clinical	*TPMT* genotyping is useful in predicting myelosuppression and likelihood of clinical response to AZA in CD	**(129, 146, 150-152)**	Moderate
clinical	*TPMT* genotype-based dosing reduced toxicity while maintaining drug efficacy in trial of AZA for moderate-severe atopic eczema	**(153)**	Moderate
clinical	*TPMT* genotyping is useful in predicting myelosuppression from AZA in RA	**(63, 154-156)**	Moderate
clinical	*TPMT* genotyping is useful in predicting myelosuppression from AZA in transplant recipients	**(134-136, 157)**	High

*Rating Scheme for Quality of Evidence as per (9)
ALL = acute lymphoblastic leukemia; AZA = azathioprine; CD = Crohn's disease; RA = rheumatoid arthritis; IBD = inflammatory bowel disease; MP = mercaptopurine; TG = thioguanine; TPMT = thiopurine methyltransferase.

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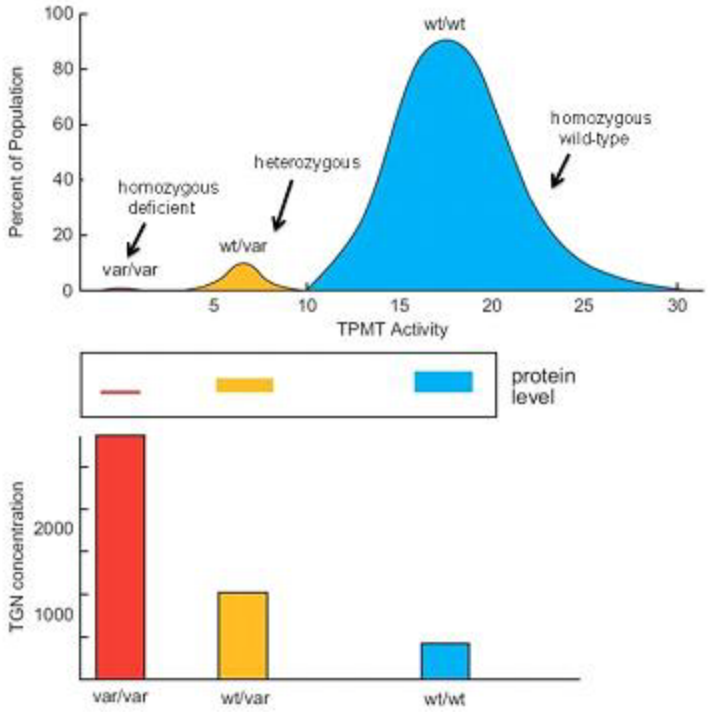

Figure Legend

Figure S1 Idealized depictions of TPMT activity in erythrocytes from a normal, healthy, non-transfused population. TPMT activity displays a trimodal frequency distribution (top) that corresponds to monogenic inheritance. Activity is generally directly related to TPMT protein levels, and inversely related to concentrations of active TGN (thioguanine nucleotide) metabolites.