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TPMT and *NUDT15* Variants Predict Discontinuation of Azathioprine for Myelotoxicity in Patients with Inflammatory Disease: Real-World Clinical Results

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Abstract

Azathioprine is used frequently to treat several inflammatory conditions. However, treatment is limited by adverse events—in particular, myelotoxicity. Thiopurine-S-methyltransferase (TPMT) and nudix hydrolase-15 (NUDT15) are enzymes involved in azathioprine metabolism; variants in the genes encoding these enzymes increase the risk for azathioprine myelotoxicity. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has recommended dose adjustments based on the results of *TPMT* and *NUDT15* genotyping. However, little is known about the importance of this genetic information in routine clinical care. We hypothesized that in patients with inflammatory diseases, *TPMT* and *NUDT15* genotype data predict the risk of discontinuing azathioprine due to myelotoxicity. This was a retrospective cohort study in 1,403 new adult azathioprine users for the management of inflammatory conditions for whom we had genetic information and clinical data. Among patients who discontinued azathioprine, we adjudicated the reason(s). Genotyping was performed using the Illumina Infinium® Expanded Multi-Ethnic Genotyping Array plus custom content. We used CPIC guidelines to determine TPMT and NUDT15 metabolizer status; patients were grouped as either: (1) poor/intermediate, or (2) normal/indeterminate metabolizers. We classified 110 patients as poor/intermediate, and 1,293 patients as normal/indeterminate metabolizers. Poor/intermediate status was associated with a higher risk for azathioprine discontinuation due to myelotoxicity compared to normal/indeterminate metabolizers (HR=2.90, 95%CI: 1.58–5.31, p=0.001). This association remained significant after adjustment for race, age at initiation, sex, primary indication, and initial daily dose of azathioprine (aHR=2.67, 95% CI: 1.44–4.94, p=0.002). In conclusion, TPMT and NUDT15 metabolizer status predicts discontinuation due to myelotoxicity for patients taking azathioprine for inflammatory conditions.

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AUTHOR CONTRIBUTIONS

A.L.D. and C.P.C. wrote the manuscript. A.L.D., L.L.D., C.M.S., and C.P.C. designed the research. A.L.D., L.L.D., J.Z., T.R., P.A., and W.Q.W. performed the research. A.L.D., L.L.D, J.Z., W.D.P., G.L., K.A.B., V.K., W.D.D., A.M.H., Q.F., C.M.S., and C.P.C. analyzed the data. J.Z., W.D.P., W.Q.W., G.L., N.J.C., W.D.D., and Q.F. contributed new reagents/analytical tools.

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Keywords

immunosuppressants; adverse events; toxicity; pharmacogenomics; precision medicine; personalized medicine

INTRODUCTION

Azathioprine is a thiopurine frequently used to treat several inflammatory conditions, such as Crohn's disease, ulcerative colitis, vasculitis, and systemic lupus erythematosus (SLE). It is among the most widely-prescribed immunosuppressants globally, and accordingly, it is listed as an essential drug by the World Health Organization.(1) Despite its importance, treatment with azathioprine is often limited by adverse events—in particular, myelotoxicity, which is potentially life-threatening.(2–6) Multiple factors can influence the risk of myelotoxicity in patients taking azathioprine; because myelosuppression is dose-dependent, one of the most significant considerations is the pharmacokinetics of azathioprine and its metabolites.(7) The activity of the enzymes involved in the thiopurine metabolic pathway has an important role on drug concentrations and therefore on the risk of myelotoxicity.

Azathioprine is converted to 6-thioguanine nucleotides (6-TGNs), one of the active metabolites that suppress the immune system by disrupting DNA and RNA synthesis.(8, 9) Excessive 6-TGN concentrations are toxic and can cause myelotoxicity.(10, 11) Buildup of 6-TGN is mitigated by both thiopurine-S-methyltransferase (TPMT) and nudix hydrolase-15 (NUDT15), which inactivate intermediate compounds in the metabolism of thiopurine drugs.(12) Therefore, absent or limited function of these enzymes leads to increases in 6-TGNs and a higher risk for myelotoxicity. Several single nucleotide polymorphisms (SNPs) in *TPMT* and *NUDT15* reduce or eliminate enzymatic activity and increase the likelihood of azathioprine-related myelotoxicity.(13–21)

Efforts to translate this information into clinical practice have led to clinical dosing guidelines for particular genotypes. The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an international group of experts whose shared goal is to facilitate the interpretation of the results of pharmacogenomic testing in routine patient care.(22) The most recent CPIC guidelines for azathioprine recommend adjusting starting doses of azathioprine if *TPMT* and *NUDT15* genotype information is known in order to decrease the risk of azathioprine-related myelotoxicity.(10, 23) Nonetheless, genotyping for *TPMT* and *NUDT15* has not been adopted universally, and some have questioned whether there is sufficient data on the effect of genotype information and subsequent clinically important outcomes among patients with chronic inflammatory diseases.(24) Results from real-world clinical practice can provide data relevant to understanding the effects of medicines as actually prescribed by physicians and taken by patients, including how outcomes among users are impacted by genotype in routine clinical care.(25) To define the association between *TPMT* and *NUDT15* genotypes and providers' decision-making to maintain or discontinue azathioprine use, we tested the hypothesis that metabolizer status predicted azathioprine discontinuation due to myelotoxicity in patients with a wide range of inflammatory diseases in real-world clinical practice.

METHODS

This study was conducted using BioVU, a clinical practice-based biobank at Vanderbilt University Medical Center. This biobank contains DNA samples linked to a database of de-identified electronic health records (EHR), the Synthetic Derivative.(26, 27) Diagnostic and procedure codes, demographic characteristics, clinical care notes, patient history, problem lists, and medications are available for research.(28) This study was approved by the Vanderbilt University Medical Center's Institutional Review Board (IRB# 180498).

Study population:

Using natural language processing, we identified 10,271 potential azathioprine users in the Synthetic Derivative. Of these, 5,064 had DNA successfully genotyped by BioVU that passed initial quality control. Following post-imputation quality control, 4,949 potential users remained (see further details below). We reviewed the EHRs of these patients while blinded to genotype assignment, and we removed an additional 18 patients from the cohort for a previous medical history that indicated potentially compromised genetic material (e.g., mention of stem cell transfusions). From the remaining 4,931 potential users, we found 1,403 patients who were new users of azathioprine (i.e., had no previous record of treatment with azathioprine or mercaptopurine in the EHRs), were at least 18 years of age at initial dose, and had been prescribed azathioprine for any of the following indications: SLE; other rheumatic conditions such as vasculitis; rheumatoid arthritis (RA) and other connective tissue disorders; or inflammatory bowel disease (Crohn's disease or ulcerative colitis) (Figure 1). These 1,403 patients comprised the study cohort.

Genotyping data, imputation, and quality control:

Genotyping for all patients was completed using the Illumina Infinium® Expanded Multi-Ethnic Genotyping Array plus custom content platform (VUMC BioVU MEGA^{EX}). Our project was part of a larger initiative at Vanderbilt; after VUMC BioVU MEGA^{EX} genotyping, over 100,000 samples, including HapMap controls, were clustered and then filtered for the following reasons: variants with a call rate<0.95, unexplained relatedness, lack of concordance in a HapMap Mendel/Concordance Evaluation, and gender discrepancies. For the smaller group of individuals with genetic information that were possible azathioprine users, including 5,064 individuals and 1,832,777 variants (Figure 1), we applied additional criteria for quality control. We removed SNPs if their call rate was less than 0.95 (0 removed) or if their minor allele frequency was less than 0.01 (899,095 removed). Also, individuals were removed if their call rate was less than 0.9 (0 removed).

We then prepared the data for imputation using the McCarthy Tools(29) and imputed additional genetic variants using the Michigan Imputation Server(30) with the HRC version r1.1 reference panel and phasing with Eagle.(31) Along with one directly genotyped *TPMT* SNP, the imputation produced an additional 8 (of a total possible 43) *TPMT* SNPs and 3 (of a total possible 19) *NUDT15* SNPs included in the CPIC guidelines. These 12 SNPs account for the vast majority of population variation within *TPMT* and *NUDT15*, particularly for patients of European ancestry (i.e., the SNPs available for our study

represent approximately 98.89% of *TPMT* diplotypes and 99.00% of *NUDT15* diplotypes among patients of European descent; see Tables S1 and S2).(15)

After imputation, we removed variants with an imputation $R^2 \leq 0.3$, individuals with sex inconsistencies ($F > 0.3$ for female, $F < 0.8$ for male; F=inbreeding coefficient estimate; n=8), individuals with high relatedness (removing one individual for any pair with a $\hat{\pi} > 0.20$ and both individuals for any pair with a $\hat{\pi} > 0.95$; $\hat{\pi}$=identical by descent estimate; n=107), and SNPs with a $p < 10^{-6}$ for their Hardy-Weinberg Equilibrium.(32, 33) In total, we filtered 115 individuals and 4 SNPs, resulting in 1,403 individuals who also met the inclusion criteria for our cohort (Figure 1) and the following relevant SNPs: rs1142345, rs56161402, rs6921269, rs1800460, rs200220210, rs151149760, and rs1800462 for *TPMT*, and rs116855232 for *NUDT15* (Table S3).

We completed genotyping quality control steps using PLINK version 1.9, PLINK version 2.0, and R version 3.6.2.(32, 34)

Metabolizer status:

We used CPIC dosing guidelines to assign metabolizer status—normal, indeterminate, intermediate, or poor—for each of *TPMT* and *NUDT15* based on the available SNPs after imputation and quality control (Tables S1 and S2). In concordance with CPIC guidelines, (23) which makes parallel dosing recommendations based upon the lowest metabolizer status of either TPMT or NUDT15, we next designated the poorer of the two statuses as a patient's overall metabolizer status, with indeterminate falling between normal and intermediate. Next, due to the relatively small number of patients classified as poor or indeterminate metabolizers, we grouped the patients as either (1) poor/intermediate metabolizers or (2) normal/indeterminate metabolizers.

Follow-up:

Patients entered the cohort on the date of their first prescription for azathioprine in the EHRs. Follow-up ended on the first of the following dates: (1) day of azathioprine discontinuation; (2) loss of follow-up; (3) the end of the study—12/31/2018; (4) 90 days after the last confirmed azathioprine dose; or (5) day of death.

Outcome:

The primary study outcome was discontinuation of azathioprine attributed to myelotoxicity in the clinical setting. We determined whether patients discontinued azathioprine before the end of the study by reviewing the de-identified medical records for all patients. If patients discontinued, we further reviewed their EHR—particularly clinician notes—to ascertain the rationale(s) for discontinuation at that time. If no reason was provided at the time of discontinuation, we continued to search forward in the clinical notes for mentions of azathioprine and the reason(s) for discontinuation. We allowed for multiple reasons for discontinuation among thirty-seven standardized possibilities, including the following five ascribed to myelotoxicity: leukopenia, neutropenia, thrombocytopenia, pancytopenia, and anemia. We attributed all patients who had multiple reasons cited for discontinuation which included myelotoxicity as achieving the outcome, even if the chart cited additional reasons

(e.g., fever and malaise) (Notes S1). All reviewers remained blinded to genotype data throughout the process of chart review and quality control.

Clinical variables:

We gathered information for sex, reported race, age at initial dose, initial daily dose of azathioprine, baseline white blood cell count (WBC) (i.e., the closest measure on or during the 365 days before the initial dose date), baseline weight (i.e., the closest measure 365 days before to 3 days after the initial dose date), last daily dose of azathioprine, and whether patients were monitored/tested for *TPMT* genetics or enzyme activity before azathioprine exposure from the EHRs (Notes S1). To replicate the clinical decision-making environment, we used self-identification or attribution of race in the clinical record, rather than a genetic determination of ancestry. There is a strong correlation between reported race and genetic ancestry in BioVU.(35, 36)

Statistical analyses:

We present demographic and clinical characteristics data as number and percentage for categorical variables, as median and interquartile range (IQR) for follow-up time, and as mean and standard deviation (SD) for other continuous variables. We used Fisher's exact tests to compare binary categorical variables, Pearson's chi-squared tests to compare polytomous categorical variables, and Wilcoxon's rank sum tests to compare continuous variables.

We estimated the incidence rates of azathioprine discontinuation by metabolizer status and used Cox proportional-hazard models to assess the association between metabolizer status and discontinuation due to myelotoxicity, first unadjusted and then adjusted for the most relevant clinical variables: race, age at initial dose, sex, primary indication, and initial daily dose of azathioprine.

Additionally, we conducted a series of sensitivity analyses. First, we removed individuals with an indeterminate metabolizer status for either TPMT or NUDT15 and reanalyzed the data, both unadjusted as well as adjusted for age at initial dose, reported race, sex, and initial daily dose. Second, we included patients <18 years of age at initiation of azathioprine. Third, since clinicians regularly consider weight in adjusting azathioprine doses for patients (e.g., 2 mg/kg/day), we completed a sensitivity analysis that included an additional adjustment for the baseline weight of patients. Fourth, because we were able to impute more information for *TPMT* variants that have been associated with impact on azathioprine metabolism, we categorized patients based on TPMT metabolizer group alone and reanalyzed the data, both unadjusted and adjusted. Fifth, we stratified our cohort by reported race and reanalyzed the data (unadjusted and adjusted analyses). Finally, to account for differences that may occur in clinical practice based on available data regarding metabolizer status, we stratified the cohort based on whether patients had prior *TPMT* genotyping or monitoring (*NUDT15* genotyping was not routinely available before the end of the study). We performed Wilcoxon rank-sum tests to compare initial doses between the groups and then completed both unadjusted and adjusted analyses of the association between metabolizer status and discontinuation due to myelotoxicity. Based on those results,

we completed a secondary analysis to assess the association between discontinuation due to myelotoxicity and both metabolizer status and prior testing. We first examined the rates of testing prior to initiation over the course of our study and then used a Cox proportional-hazard model grouped by both metabolizer status and prior testing (unadjusted and adjusted).

All analyses were conducted using STATA version 16.0.(37)

RESULTS

We studied 1,403 new adult users of azathioprine followed over a median of 19.1 [3.1–61.7] months. Their mean age was 46.1±15.8 years, 68% were female, and 87% were identified as being of European ancestry. A total of 110 patients were classified as poor/intermediate metabolizers, and 1,293 were classified as normal/indeterminate metabolizers. Table 1 shows the baseline characteristics of the patients included in the study by metabolizer status. Poor/intermediate metabolizers did not differ significantly in age, sex, reported race, baseline weight, baseline WBC, length of follow-up, or whether testing was completed prior to initiation compared to normal/indeterminate metabolizers. The two groups did differ significantly in initial daily dose of azathioprine, with poor/intermediate metabolizers starting on a lower mean dose (p=0.001). The groups also differed significantly by indication (p=0.03), with a greater proportion of patients taking azathioprine for inflammatory bowel disease among normal/indeterminate metabolizers.

A total of 70 patients stopped azathioprine treatment due to myelotoxicity (13 patients were poor/intermediate and 57 were normal/indeterminate metabolizers). The unadjusted incidence of azathioprine discontinuation due to myelotoxicity was 4.1/100 person-years for poor/intermediate TPMT or NUDT15 metabolizers and 1.4/100 person-years among normal/indeterminate metabolizers. Poor/intermediate TPMT or NUDT15 metabolizer status was associated with a higher risk to stop azathioprine due to myelotoxicity compared to normal/indeterminate (HR=2.90, 95%CI: 1.58–5.31, p=0.001). This association remained significant after adjustment for reported race, age at initial dose, sex, primary indication, and initial daily dose of azathioprine (aHR=2.67, 95%CI: 1.44–4.94, p=0.002) (Table 2). Figure 2 shows the cumulative incidence of azathioprine discontinuation due to myelotoxicity over time by metabolizer phenotype category.

Table 2 shows the results of the sensitivity analyses. First, we performed an analysis after removing indeterminate metabolizers (n=7), and the results remained robust in unadjusted (HR=2.94, 95%CI: 1.61–5.39, p<0.001) and adjusted analyses (aHR=2.71, 95%CI: 1.46–5.02, p=0.002). After expanding the cohort to include patients <18 years of age at initial dose, the findings were concordant with the main analysis. In a sensitivity analysis among 1278 patients with available weight at baseline, in both the unadjusted model and the multivariate model including weight-adjusted initial dose, the results remained significant (unadjusted: HR=2.29, 95%CI: 1.19–4.37, p=0.013; adjusted: aHR=2.12, 95%CI: 1.10–4.08, p=0.025). When grouped by TPMT metabolizer status alone, there were 100 poor/intermediate metabolizers and 1,303 normal/indeterminate. The unadjusted (HR=2.95, 95%CI: 1.58–5.50, p=0.001) and adjusted (aHR=2.74, 95%CI: 1.45–

5.18, p=0.002) associations were significant (not shown in Table 2). When stratified by race, the unadjusted association was significant for patients of reported European ancestry (n=1,225; HR=2.41, 95%CI: 1.14–5.11, p=0.022), as well as when adjusted (aHR=2.34, 95%CI:1.10–5.00, p=0.028). For patients of reported African ancestry, we observed a similar trend for elevated risk, although not meeting the threshold for statistical significance (n=149; HR=3.01, 95%CI: 0.92–9.77, p=0.067, unadjusted). The sample size was too small to draw any conclusions for other races.

After stratifying the cohort by whether or not patients had clinical testing for *TPMT* (either genetically or by enzyme activity) before initiating azathioprine, we first compared initial daily doses between these two groups to confirm that testing had an impact on clinical behavior. As anticipated, among patients with prior testing (n=713), poor and intermediate metabolizers were initiated on a statistically significant lower dose compared to normal and indeterminate metabolizers, with a mean of 69±44 versus 90±56 mg/day (p=0.003), respectively. Correspondingly, patients without prior testing (n=690) were started on relatively similar doses (64±32 versus 72±38 mg/day, p=0.123) for the two genotype groups. All groups showed evidence of titration, with increased mean final azathioprine doses compared to initial dose: prior testing-poor/intermediate metabolizer status (91±49 mg/day), prior testing-normal/indeterminate metabolizer status (115±56), no prior testing-poor/intermediate metabolizer status (88±42), and no prior testing-normal/indeterminate metabolizer status (99±52). The hazard ratios between metabolizer status and discontinuation for myelotoxicity remained significant for both groups of patients in unadjusted (with prior testing: HR=2.73, 95%CI: 1.21–6.20, p=0.016; without prior testing: HR=3.11, 95%CI: 1.27–7.65, p=0.013) and adjusted analyses (with prior testing: aHR=2.49, 95%CI: 1.06–5.83, p=0.035; without prior testing: aHR=2.68, 95%CI: 1.08–6.66, p=0.034).

As a secondary analysis, we compared outcomes based on a combination of prior testing and phenotype. We first assessed the rates of testing by calendar year to confirm when testing began in our cohort. Testing began in 2003 and increased steadily through the course of the study (Figure 3); we next divided users into three-year groups to adjust our analysis for trends over time, along with the adjustments made in our primary and sensitivity analyses. We then split the cohort into four groups based on prior testing and phenotype for patients initiated on azathioprine during or after 2003: (1) prior testing-normal/indeterminate metabolizer status (n=655; 32 events), (2) prior testing-poor/intermediate metabolizer status (n=58; 7 events), (3) no prior testing-normal/indeterminate metabolizer status (n=578; 23 events), and (4) no prior testing-poor/intermediate metabolizer status (n=50; 5 events). Figure 4 shows the cumulative incidence of azathioprine discontinuation due to myelotoxicity over time by metabolizer phenotype and prior testing category. With the prior testing-normal/indeterminate metabolizers as a reference, the only significant association in unadjusted and adjusted analyses was in the prior testing-poor/intermediate metabolizer group (HR=2.72, 95%CI: 1.20–6.17, p=0.017; aHR=2.40, 95%CI: 1.04–5.54, p=0.041). Within the two metabolizer status groups, patients who had been tested had slightly higher risk than patients without prior testing, but the results were not statistically significant.

DISCUSSION

To the best of our knowledge, this is the first study examining the association between TMPT and NUDT15 metabolizer status and azathioprine discontinuation due to myelotoxicity in routine clinical practice to define the usefulness of genetic testing on clinical decision making for patients with inflammatory diseases in the real-world. Our results indicate that (1) patients with poor or intermediate metabolizer status experienced higher risk for azathioprine discontinuation due to myelotoxicity, (2) that risk was persistent over time, (3) most patients who discontinued azathioprine for possible myelotoxicity were classified as normal TPMT and NUDT15 metabolizers, and (4) pre-genotyping may not mitigate the risk of azathioprine discontinuation for myelotoxicity.

Prior studies examining leukopenia associated with the use of azathioprine have focused on patients with specific diseases such as inflammatory bowel disease or vasculitis.(30, 38) Our approach—which by design included different azathioprine indications, a range of medication doses, variability in genetic testing prior to drug initiation, and many clinical variables seen in real-world practice—aimed to improve our understanding of the role of implementing these guidelines among a broad population of patients with chronic inflammatory conditions. In addition, the use of a clinically relevant outcome defined by a clinical action—drug discontinuation—rather than a laboratory result is novel.

If genotype is known, CPIC guidelines recommend adjusting the initial azathioprine dose based on *TPMT* and *NUDT15* genotype information to prevent myelotoxicity.(15) *NUDT15* testing is new, but *TPMT* testing has been clinically available for years. Despite long-recognized evidence regarding the impact of these enzymes on blood cell counts in patients receiving azathioprine as well as data that there is interindividual variability in both TPMT and NUDT15 activity,(39) testing prior to azathioprine use is not universal. While the rates of genetic testing and subsequent dose adjustments have increased over the last 10 years, there are still barriers, including conflicting data on cost-effectiveness and doubt over the usefulness of genetic testing in clinical practice.(40) As a result, some have urged for more high-quality evidence.(24)

This study provides real-world evidence for the usefulness of genetics to improve risk stratification in patients with inflammatory conditions who receive azathioprine in routine clinical practice. Patients with poor/intermediate metabolizer status discontinue azathioprine due to myelotoxicity at a significantly higher level than patients with normal/indeterminate metabolizer status. The results remain consistent across a wide spectrum of patients and across time.

Our secondary analysis suggests that the practice of testing did not lower the likelihood of discontinuation due to myelotoxicity for patients that initiated azathioprine (although metabolizer status remains predictive regardless of testing). Indeed, testing may be impacting clinical behavior for several potential reasons, which in turn, affect the likelihood of discontinuation for myelotoxicity: (1) for known poor/intermediate metabolizers, clinician tolerance for dropping white blood cell counts may be lower, leading to earlier discontinuation; (2) for known normal/indeterminate metabolizers, clinicians may feel

comfortable with relatively higher doses, leading to greater risk. Within this study, we observed lower initial doses in known poor/intermediate metabolizer patients relative to known normal/indeterminate metabolizers. In contrast, non-tested patients in both groups started at doses similar to those among known poor/intermediate metabolizers; and (3) just as testing has increased over time, likewise awareness of myelotoxicity risks as well as medication/treatment options have increased over the course of this study, allowing clinicians more flexibility to discontinue medications at early signs of potential side effects in more recent years. Additionally, individual clinicians' tolerance levels differ, and we were not able to assess that with de-identified information.

More significantly, among those with and without previous genetic testing, most patients who discontinued due to myelotoxicity were normal metabolizers. The fact that most cases of azathioprine discontinuation attributed to myelotoxicity occurred despite normal TPMT and NUDT15 metabolizer status underscores the need for further studies to elucidate the role of genetic and non-genetic determinants of myelotoxicity. For example, variants in other genes encoding enzymes involved in the thiopurine pathway such as *XDH*, *AOX1*, *ABCC4*, *ITPA*, and *GST* are potential candidates.(41)

Although the breadth of sensitivity analyses indicate that our results are robust, this study has limitations. First, some clinical information (e.g., drug adherence) is not included regularly in EHRs. Moreover, data regarding prescription fills are not standardized, limiting our ability to include dose changes in time-dependent analyses. Second, we were not able to genotype or impute all SNPs used to estimate phenotypes according to the CPIC guidelines; indeed, the number of SNPs available to determine NUDT15 metabolizer status, in particular, were limited and dependent solely on imputation. However, the omitted SNPs are primarily rare variants.(15) Third, while drug discontinuation is an important clinical outcome, the decision could be influenced by several additional factors including practitioners' or patients' preference as well as the availability of alternative treatments. Fourth, although we included all patients regardless of their self-reported race, patients of reported European descent predominated, and the number of patients from other races was small. Fifth, we did not have a large enough sample to run comparisons among individuals with poor and intermediate metabolizer status, and there was no overlap between poor/intermediate TPMT metabolizers and poor/intermediate NUDT15 metabolizers, so we could not detect differences among various more discrete comparisons (e.g., poor NUDT15 versus poor TPMT metabolizers or intermediate/intermediate versus intermediate/poor). Finally, while we would not anticipate differential use based on metabolizer status group, we did not include concurrent medications at baseline in our analysis.

Despite these limitations, this analysis supports the idea that TMPT and NUDT15 intermediate and poor metabolizers have a higher risk of azathioprine discontinuation due to possible myelotoxicity. Moreover, our findings highlight the need to define the role of other genes affecting the metabolism of azathioprine, as we strive to reduce the number of serious side effects associated with the use of this and other immunosuppressants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STUDY HIGHLIGHTS

What is the current knowledge on the topic?

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends adjustments to azathioprine dosing based on the results of *TPMT* and *NUDT15* genotyping. However, little is known about the role of this genetic information to predict azathioprine discontinuation due to myelotoxicity in routine clinical care.

What question did this study address?

Does *TPMT* and *NUDT15* genotype information predict azathioprine discontinuation due to myelotoxicity in routine clinical care?

What does this study add to our knowledge?

Poor or intermediate TPMT or NUDT15 metabolizers had a 2.9-fold increased risk for azathioprine discontinuation due to myelotoxicity compared to normal or indeterminate metabolizers in the real world.

How might this change clinical pharmacology or translational science?

This study indicates that TPMT and NUDT15 poor and intermediate metabolizers are have an increased risk of myelotoxicity based on real-world clinical practice.

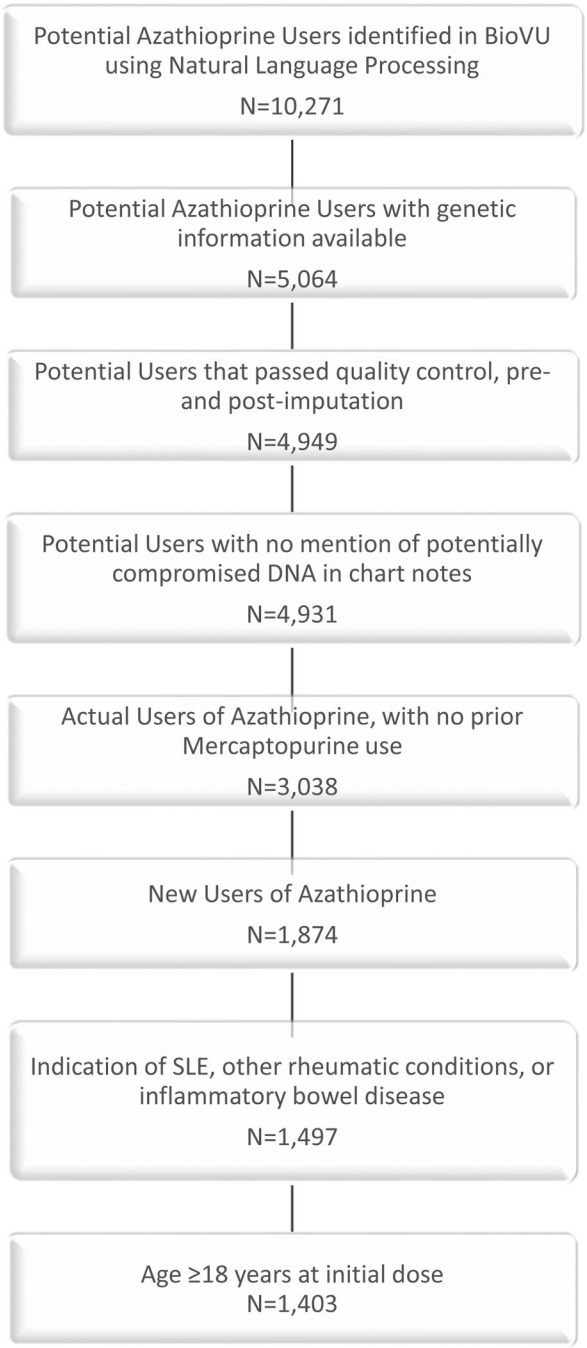

Figure 1:
Inclusion criteria

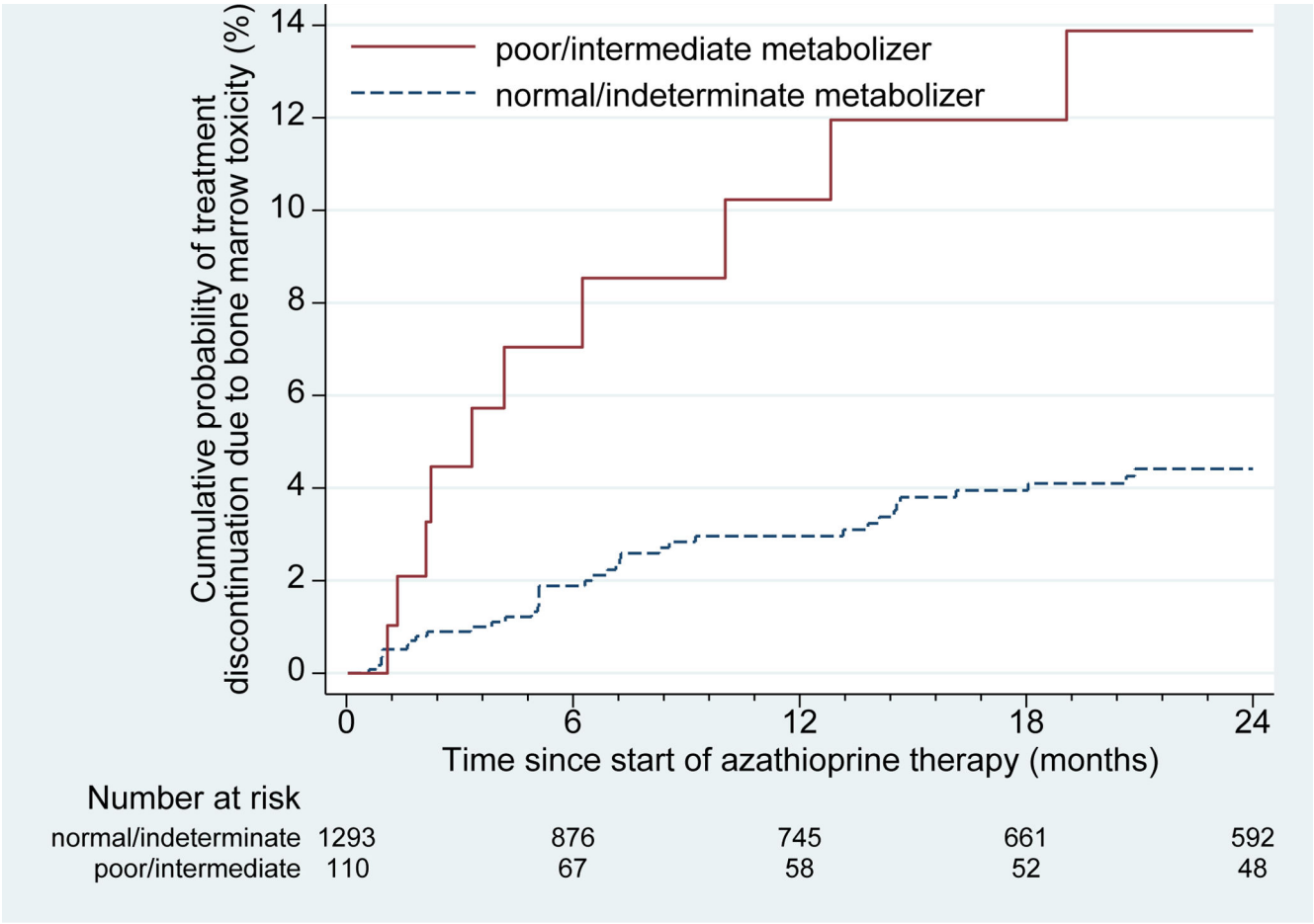

Figure 2:
Probability of azathioprine discontinuation for possible myelotoxicity by TPMT or NUDT15 metabolizer phenotype

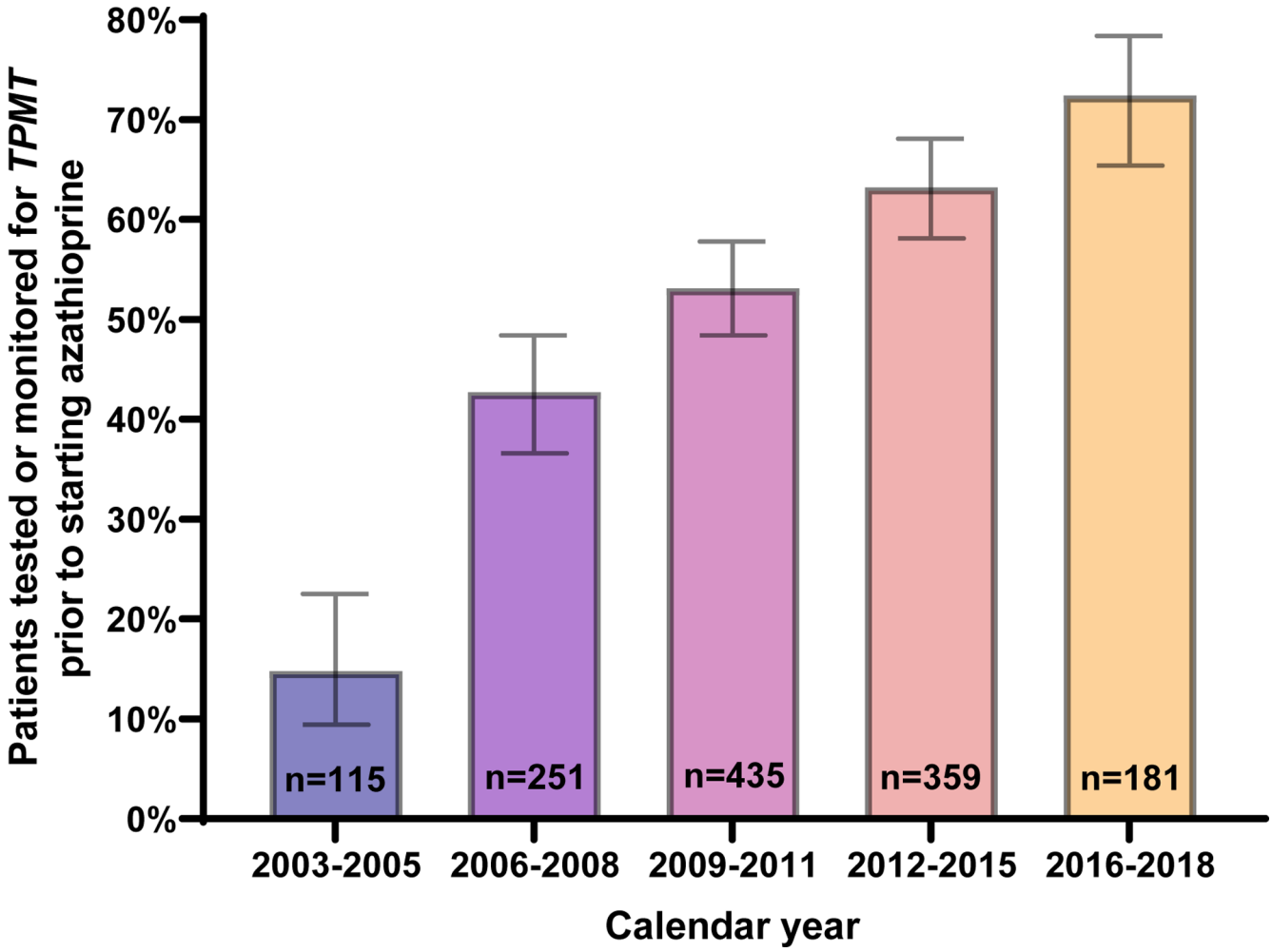

Figure 3:
Proportion of patients tested or monitored for *TPMT* prior to starting azathioprine

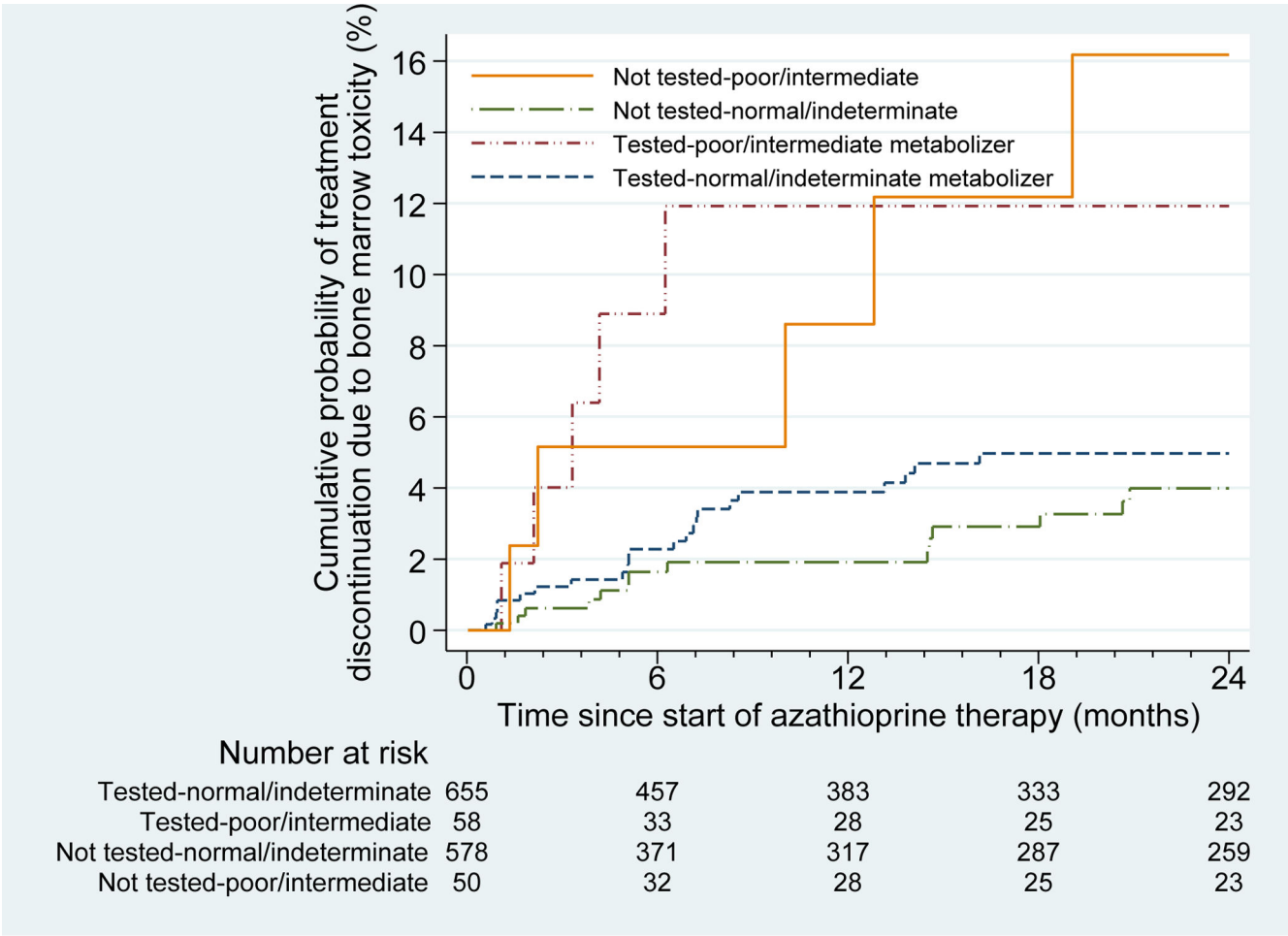

Figure 4:

Probability of azathioprine discontinuation by TPMT or NUDT15 metabolizer phenotype and prior testing status during or after 2003

Table 1:

Baseline characteristics by TPMT or NUDT15 phenotype

	NUDT15 or TPMT poor or intermediate metabolizers n=110	NUDT15 or TPMT normal or indeterminate metabolizers n=1293	p-value
Age in years, mean±SD	46.8±15.2	46.0±15.8	0.61
Female sex, n (%)	73 (66.4%)	884 (68.4%)	0.67
Reported European ancestry, n (%)	91 (82.7%)	1134 (87.7%)	0.14
Indications			0.03
Systemic lupus erythematosus (SLE)	16 (14.6%)	145 (11.2%)	
Inflammatory bowel disease (IBD)	25 (22.7%)	452 (35.0%)	
Inflammatory condition other than SLE or IBD	69 (62.7%)	696 (53.8%)	
Initial daily dose (mg/day), mean±SD	67.0±38.9^	81.1±48.6^^	0.001
Baseline weight (kg), mean±SD	81.47±21.1*	80.9±22.0**	0.62
Baseline white blood cell count (K/μL), mean±SD	8.87±4.18+	8.55±3.54++	0.77
Follow-up time (months), median [IQR]	15.6 [2.3–70.2]	19.2 [3.2–60.6]	0.55
Tested prior to initiation of azathioprine, n (%)	58 (52.7%)	655 (50.7%)	0.69

^ n=110,

^^ n=1288

* n=105,

** n=1173

+ n=100,

++ n=1144

Table 2:

Risk of possible myelotoxicity associated with azathioprine in poor/intermediate and normal/indeterminate TPMT and NUDT15 metabolizers

	Unadjusted			Adjusted*		
	At risk	**Events**	**HR**	**At risk**	**Events**	**HR**
Overall	1403	70	HR=2.90 (95%CI: 1.58–5.31) p=0.001	1385	70	aHR=2.67 (95%CI: 1.44–4.94) p=0.002
Excluding indeterminate metabolizers	1396	69	HR=2.94 (95%CI: 1.61–5.39) p<0.001	1378	69	aHR=2.71 (95% CI: 1.46–5.02) p=0.002
Including <18 years of age at initial dose	1497	72	HR=2.85 (95% CI:1.56–5.20) p=0.001	1479	72	aHR=2.65 (95% CI: 1.44–4.89) p=0.002
Patients with available baseline weight	1278	66	HR=2.29 (95%CI: 1.19–4.37) p=0.013	1262	66	aHR=2.12** (95%CI: 1.10–4.08) p=0.025
Stratified by reported race:						
European ancestry	1225	54	HR=2.41 (95%CI: 1.14–5.11) p=0.022	1220	54	aHR=2.34 (95%CI:1.10–5.00) p=0.028
African ancestry	149	15	HR=3.01 (95%CI: 0.92–9.77) p=0.067	149	15	aHR=2.99 (95%CI: 0.88–10.22) p=0.081
Asian ancestry	6	0	NA	6	0	NA
Other & Unknown	23	1	NA	10	1	NA
Stratified by testing before initiation:						
Tested for TPMT or NUDT15 before	713	39	HR=2.73 (95%CI: 1.21–6.20) p=0.016	706	39	aHR=2.49 (95%CI: 1.06–5.83) p=0.035
Not tested before	690	31	HR=3.11 (95%CI: 1.27–7.65) p=0.013	679	31	aHR=2.68 (95%CI: 1.08–6.66) p=0.034

*Adjusted for reported race (except in analyses stratified by race), age at initial dose, sex, primary indication, and initial daily dose of azathioprine.

**Adjusted for reported race, age at initial dose, sex, primary indication, and ratio of initial daily dose of azathioprine to baseline weight.