Genetic association analysis and frequency of NUDT15*3 with thiopurine-induced myelosuppression in patients with inflammatory bowel disease in a large Dutch cohort

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ARTICLE

Genetic association analysis and frequency of *NUDT15*3* with thiopurine-induced myelosuppression in patients with inflammatory bowel disease in a large Dutch cohort

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Thiopurine drugs are cornerstone treatment for patients with inflammatory bowel disease (IBD). The most common adverse drug reaction is thiopurine-induced myelosuppression (TIM), that may partly be explained by the genetic polymorphism *NUDT15*3*. The aim of this retrospective study was to determine the *NUDT15*3* polymorphism frequency and its association with TIM in an IBD patient population in the Netherlands. DNA from patients previously genotyped for *TPMT* was genotyped for *NUDT15*3*. In IBD patients treated with thiopurines association tests with TIM were conducted. Out of 988 included patients, 13 (1.3%) were heterozygous for *NUDT15*3*. Of all patients, 606 had IBD and received thiopurine treatment. In these patients, 8/606 (1.3%) were heterozygous polymorphic for *NUDT15*3* of which 50.0% developed TIM compared to 2.3% in the wild type patients ($p < 0.001$). The study results show a clinically relevant prevalence of *NUDT15*3* in the Dutch patient population. Its strong association with TIM suggests pre-therapeutic genotyping potentially clinically utile.

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INTRODUCTION

For almost 60 years thiopurines have been used in clinical practice as antimetabolite drugs. Since the nineties, they are most commonly used for the treatment of inflammatory bowel disease (IBD) [1]. Although thiopurines are effective drugs, a major limitation in its use is that up to 15–40% of patients withdraw from using thiopurine drugs due to adverse drug reactions [2–7]. One of these adverse drug reactions is thiopurine-induced myelosuppression (TIM), which occurs in 4–7% of patients using thiopurines [3, 8–10]. The cumulative incidence of severe is approximately 1%. Although most patients with severe TIM areasymptomatic, serious opportunistic infections may occur with an estimated risk of death of 1% in patients who develop severe TIM [3, 11].

The most widely used thiopurines are azathioprine (AZA), 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG). AZA is converted into 6-MP by the enzyme glutathione S-transferase (GST). Both AZA and 6-MP are prodrugs, and need to be activated intracellularly into active triphosphates. After absorption these prodrugs are partially inactivated either through thiopurine methyltransferase (TPMT) to 6-methyl mercaptopurine (6-MMP or 6-MeMP) or through xanthine oxidase (XO) into thiouric acid (Supplementary Fig. 1). To exert its drug activity, 6-MP is intracellularly activated through conversion to 6-thioguanine nucleotides (6-TGN). The main active intracellular metabolites are 6-thioguaninetriphosphate (6-TGTP) and 6-thiodeoxyguaninetriphosphate (6-TdGTP) which are incorporated into the RNA and DNA of cells respectively, causing cell damage and eventually apoptosis [12–16]. Nudix hydrolase 15 (*NUDT15*) is the primary enzyme responsible for the inactivation of the main active triphosphate metabolites 6-TGTP and 6-TdGTP [12–14, 17].

Since *NUDT15* inactivates the active metabolites 6-TGTP/6-TdGTP, its enzyme activity directly impacts efficacy and toxicity of thiopurines. Therefore, *NUDT15* is considered an important enzyme associated with development of thiopurine-induced adverse drug reactions [6]. Furthermore, it should be noted that there is no association between *NUDT15*3* and intracellular 6-TGN concentrations since in general, the entire pool of 6-TGN nucleotides is measured, in stead of the individual phosphorylated mono-, di-, and triphosphate nucleotides [7]. Various genetic polymorphism in the *NUDT15* gene are known to reduce its enzymatic activity. Several studies in patients of Asian ancestry have shown that the gene variants *NUDT15*2, NUDT15*3, NUDT15*4*, and *NUDT15*5* are risk factors for the development of TIM, with *NUDT15*3* being the most common genetic polymorphism in these patients [4–7, 9, 18–22]. In addition, meta-analysis showed that patients with a *NUDT15* genetic polymorphism (*NUDT15*2*, *NUDT15*3*, *NUDT15*5* or *NUDT15*6*) had a significantly increased odds risk of 11 and 16 to develop

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early onset leukopenia and neutropenia following treatment with thiopurine drugs, respectively [23].

The worldwide allele frequency of the *NUDT15*3* genetic polymorphism is 11.6% in Asians, whereas the prevalence in Caucasians is only 0.3% [24]. In the Netherlands, the prevalence of *NUDT15*3* has not yet been determined. Due to globalization and international industry, the composition of the population of some areas within the Netherlands has changed and is no longer almost exclusively of Caucasian origin. Thereby, the frequency of *NUDT15*3* may be higher in these areas compared with a pure Caucasian population. For example, many people from non-Caucasian roots are living due to their work in the Brainport Region Eindhoven, the Netherlands. Also, it is only poorly investigated yet whether the association between TIM and *NUDT15* polymorphism found in Asian studies can be extrapolated to patient populations of other ethnic origin, and for the understanding of genetic variants on Mendelian disorders it is important to assess genetic variation among multiple populations [25]. The aim of this study was therefore to determine the frequency of *NUDT15*3* in a typical Dutch patient population, and to determine the association of *NUDT15*3* with TIM in patients with IBD treated with thiopurine drugs. In addition, to gain more insight into the clinical effects of *NUDT15*3* on thiopurine treatment, the clinical course of treatment in *NUDT15*3* variant allele carriers was described.

MATERIALS AND METHODS

Study design

This was a retrospective pharmacogenetic association study that was conducted in patients who were treated in either one of the two participating hospitals (Catharina Hospital in Eindhoven and Elkerliek Hospital in Helmond). The primary objectives of this study were 1] to determine the allele frequency of the *NUDT15*3* gene variant in our patient population, and 2] to determine the association between *NUDT15*3* and thiopurine-induced myelosuppression in patients with inflammatory bowel disease. Thiopurine-induced myelosuppression was defined as white blood cell count (WBC) $< 3.0 \times 10^9$/L (leukopenia grade≥2) and/or absolute neutrophil count (ANC) $< 1.5 \times 10^9$/L (neutropenia grade≥2). Furthermore, a subgroup analysis for the primary endpoint was conducted for adult patients (≥18 years) and children (<18 years). The Medical Research Ethics Committee United (MEC-U, Nieuwegein, the Netherlands) approved the study protocol and declared the study not to be subject to the Medical Research Involving Human Subjects Act (MEC-U study registration number W22.216). Given the retrospective character of the study and the anticipated size of the patient population, a waiver was provided for informed consent.

Patient population

The study population for the *NUDT15*3* frequency analysis consisted of patients in whom *TPMT* genotyping was consecutively conducted in the years from 2018 until September 2022 as part of their routine clinical care. Pretherapeutic *TPMT* genotyping is standard of care in all patients intended to be treated with thiopurine drugs since at least 2017, and was centrally conducted for both study sites in the laboratory of the Catharina Hospital. From left-over DNA (that is routinely stored for a minimum of 15 years after genotyping) *NUDT15*3* was retrospectively determined. Patients were excluded when no DNA extract was available or when they were treated in another institute than either one of the participating centers. Subsequently, for the genetic association study, only patients were selected when they had IBD, and had actually received treatment with a thiopurine drug within the first 24 months after *TPMT* genotyping. All patients that were either intermediate or poor metabolizer for TPMT were treated with initially reduced thiopurine starting doses of 50 and 10%, respectively, according to international guidelines (www.pharmgkb.org), and were therefore not excluded for the association analysis.

Data extraction

Data of up to 24 months after *TPMT* genotyping was extracted from the electronic healthcare system (EHR) HiX (Chipsoft®, Amsterdam, the Netherlands) in order to collect the necessary characteristics of the included patients. The following characteristics were collected: date of birth, age at *TPMT* genotyping, *TPMT* genotype (*TPMT*2, *3 A, *3B* and *3 C*), diagnosis, type of thiopurine used, start and stop date of thiopurine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), estimated glomerular filtration rate (eGFR), WBC, ANC and use of co-medication allopurinol, mesalazine, and/or biologicals. Leukopenia and neutropenia were graded according to the National Cancer Institute Common Toxicity Criteria, Version 3.0 [26]. Shortly, a normal WBC is defined as grade 0; leukopenia grade 1, 2, 3 and 4 is defined as WBC count below lower limit of normal – 3.0×10^9/L; <3.0–2.0 $\times 10^9$/L; <2.0–1.0 $\times 10^9$/L, and <1.0 $\times 10^9$/L, respectively. Similarly, a normal ANC is defined as grade 0; grade 1, 2, 3 and 4 neutropenia is defined as absolute neutrophil counts below lower limit of normal – 1.5×10^9/L; <1.5–1.0 $\times 10^9$/L; <1.0-0.5 $\times 10^9$/L, and <0.5 $\times 10^9$/L, respectively. Patients with leukopenia and neutropenia with grade 2 or higher, having the diagnosis IBD, and being prescribed thiopurines, were considered as cases of TIM. Thereafter, the patient files were checked: if the patient file did not clearly state the diagnosis of TIM, the patients were double checked by a gastro-enterologist to confirm or refute the diagnosis of TIM. This way only adverse events that were probably or definitely related to the use of thiopurines were included. All laboratory values were automatically extracted from the EHR. Diagnosis, use of allopurinol, mesalazine or use of biologicals were manually extracted from the patient files. To rule out investigator bias all clinical data extraction and analysis was performed independently and prior to *NUDT15* genotyping.

NUDT15 genotyping

DNA was isolated from whole blood using the MagNA Pure 24 Total NA Isolation Kit (Roche Diagnostics®, Basel, Switserland) on the Magnapure 24 (Roche Diagnostics®, Basel, Switzerland) that was previously obtained for *TPMT* genotyping. *NUDT15*3* mutation was detected using the NUDT15 kit (TibMolBiol, Roche®, Basel, Switserland) according to the instruction for use, on a lightcycler 480 (Roche Diagnostics®, Basel, Switzerland).

Statistical analysis

The continuous data was checked for normality using Skewness and Kurtosis. For the association analysis a two-sided Chi-squared test was used. Adjustments for multiple comparison was not applicable. All statistical tests were conducted using IBM® SPSS Statistics for Mac, Version 29.0.0.0 (241).

RESULTS

Within the selected study period, a total of 1144 *TPMT* genotype results were extracted from the EHR. After exclusion of double patient values, ineligible patients due to unavailable DNA, or referall from another non-study centre, the final population for the frequency analysis included a total of 988 patients that were genotyped for *NUDT15*3*. For the genetic association analysis of *NUDT15*3* with TIM, a total of 382 patients were excluded for not having IBD and/or not having received thiopurine-based treated within the 24 months after *TPMT* genotyping, leaving a total of 606 patients that fulfilled the inclusion criteria. The flow chart of the study is provided in Fig. 1.

Frequency analysis

Table 1 provides the baseline patient characteristics for the patients included in the *NUDT15*3* frequency analysis. Patient biological gender was equally divided with 51% females and 49% males, with an average age at *TPMT* genotyping of 44 years.

The *NUDT15*3* genetic polymorphism was found heterozygously in 13 out of 988 patients (population frequency of 1.3%); none of the patients were homozygously polymorphic, resulting in an allele frequency of 0.66% (13/1976). The *NUDT15*3* polymorphism frequency was similar 1.3% (8/606 patients) when only IBD patients were selected. None of the *NUDT15*3* positive patients was positive for either of the tested *TPMT* polymorphisms.

Genetic association analysis

Baseline patient and treatment characteristics for the thiopurine-treated IBD population are shown in Table 1. Biological gender

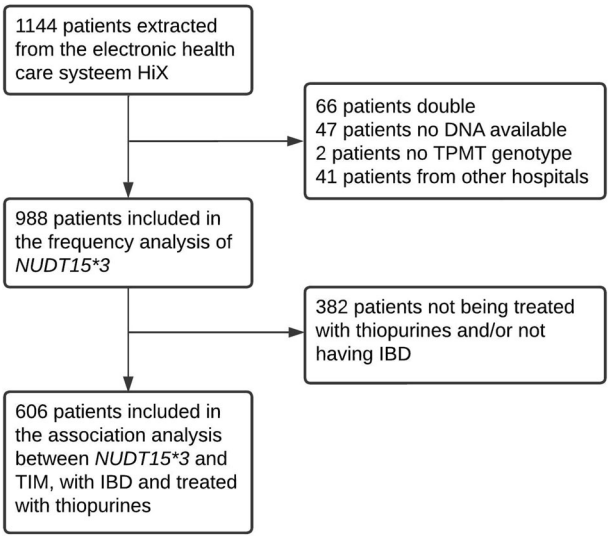

Fig. 1 Flow chart of the study.

was relatively equally divided with 53% females and 47% males with an average age of 40 years. The most common diagnosis was Crohn's disease (57%). A total of 121 patients (20%) had any grade leukopenia within the timeframe of 24 months after the start of thiopurine treatment, and 34 patients (6%) had any grade neutropenia (Table 2). Within the total IBD population, the primary endpoint – confirmed thiopurine-induced myelosuppression defined as leukopenia grade ≥2 and/or neutropenia grade ≥2 – occurred in 18 (3%) patients. Results of the genetic association analyses are provided in Table 3. Of the eight patients with the *NUDT15*3* genetic polymorphism four patients (50%) developed TIM compared to 14 out of 598 (2.3%) in the *NUDT15* wild type patients ($p < 0.001$). Subgroup analysis showed that the association was true and remained statistically significant in both children as well as adults (Table 3).

Table 4 describes the patient characteristics and the clinical course of treatment of the eight patients with the *NUDT15*3* variant allele. Just one of these *NUDT15*3* positive patients was from Caucasian ethnicity; all other patients were from Asian or central-American origin. Five (63%) of these eight patients developed clinical toxicity requiring either stop of treatment, early dose reduction, switch to another treatment, and in one case hospitalization with blood transfusion due to severe anemia. Two (25%) patients developed no toxicity, however, one patient was only on treatment for three weeks as he stopped all his IBD medication on patients' own decision, and the other patient was only treated for two months at a low dose.

Table 1. Baseline patient and treatment characteristics.

	***NUDT15*3* frequency population ($n = 988$)**	**Thiopurine-treated IBD association population ($n = 606$)**
Female, *n* (%)	500 (50.6)	321 (53.0)
Age, *y*, mean (SD)	44.2 (19.3)	39.7 (18.4)
eGFR, ml/min, median (IQR)	91 (82–91)	91 (85–91)
Diagnosis, *n* (%)		
Crohn's disease	428 (43.3)	342 (56.5)
Ulcerative Colitis	408 (41.3)	251 (41.4)
IBD-U	19 (1.9)	13 (2.1)
Other[a]	70 (7.1)	-
Unknown	63 (6.4)	-
TPMT phenotype, *n* (%)		
NM	878 (88.9)	544 (89.6)
IM	107 (10.8)	61 (10.0)
PM	3 (0.3)	1 (0.2)
Medication, *n* (%)		
azathioprine	184 (18.6)	139 (22.9)
6-mercaptopurine	148 (15.0)	144 (23.8)
tioguanine	52 (5.3)	49 (8.1)
azathioprine/6-mercaptopurine	7 (0.7)	7 (1.1)
azathioprine/tioguanine	101 (10.2)	98 (16.2)
6-mercaptopurine/tioguanine	169 (17.1)	166 (27.4)
azathioprine/6-mercaptopurine/tioguanine	3 (0.3)	3 (0.5)
None	324 (32.8)	-
Co-medication, *n* (%)		
allopurinol	55 (5.6)	39 (6.4)
mesalazine	302 (30.5)	159 (26.2)
biological agent	202 (20.4)	150 (24.8)
mesalazine + biological agent	174 (17.6)	142 (23.4)

[a]Other: autoimmune hepatitis (1.9%), autoimmune pancreatitis (0.8%), cholangiopathy (0.1%), chronic tubule-interstitial nephritis (0.1%), colon cancer (0.1%), granulomatous infection (0.2%), interstitial lung disease (2.1%), lupus (0.1%), myositis (0.1%), optic neuromyelitis (0.2%), kidney transplant (0.1%), perianal abscess/fistula (0.2%), vasculitis (1.0%).

DISCUSSION

This study analyzed the frequency of the *NUDT15*3* genetic polymorphism and its association with thiopurine-induced myelosuppression in a Dutch patient population with inflammatory bowel disease. In line with the hypothesis, the genotype frequency of 1.3% in this patient population was slightly higher than other reported frequencies in Caucasian populations. For example Yang et al. (2014) reported an allele frequency of 0.004%, Yang et al. (2015) an allele frequency of 0.2%, and the meta-analysis of Zhang et al. an allele frequency of 0.24% and a population genotype frequency of 0.49% [20, 27–30]. The higher allele frequency found in our study could potentially be explained due to the relatively high percentage of people from non-Caucausian ancestry in the area of Eindhoven in the Netherlands, given the fact that allele frequencies among Asian, south and central-American patients have been reported to range from 9.8% to 13.3% [27, 30, 31]. A smaller study in 102 Spanish patients revealed a comparable genotype prevalence with our study of 1% [32].

The observed association with thiopurine-induced myelosuppression in our patient cohort is consistent with other studies showing an association between TIM and *NUDT15* gene variants in other populations [11, 33]. Although the association has always shown to be of significance, the absolute incidence for developing TIM differs between studies, primarily as the result of using different definitions for clinically relevant leukopenia [4, 23, 34]. For example, our reported incidence of 50% is in accordance with the study by Chao et al. reporting that 46.3% of patients had *NUDT15*3* and TIM, compared to 16.2% of patients with wild type *NUDT15* and TIM [4]. Whereas the study by Walker et al. reported that 100% of patients polymorphic for *NUDT15*3* developed TIM, compared to 33% in patients with the wild type *NUDT15* genotype

Table 2. Laboratory toxicity during treatment of the thiopurine treated IBD patient population.

	Thiopurine-treated IBD association population ($n = 606$)
Leukopenia grade, *n* (%) [a]	
No leukopenia	479 (79.0)
CTCAE grade 1	95 (15.7)
CTCAE grade 2	22 (3.6)
CTCAE grade 3	3 (0.5)
CTCAE grade 4	1 (0.2)
Missing	6 (1.0)
Neutropenia grade, *n* (%) [a]	
No neutropenia	341 (56.3)
CTCAE grade 1	7 (1.1)
CTCAE grade 2	21 (3.5)
CTCAE grade 3	4 (0.7)
CTCAE grade 4	2 (0.3)
Missing	231 (38.1)
Thiopurine-induced myelosuppression grade ≥2, *n* (%)	
Yes	18 (3.0%)
No	588 (97.0%)
ALT, *n* (%)	
>2x ULN	90 (14.8)
≤2x ULN	512 (84.5)
Missing	4 (0.7)
AST, *n* (%)	
>2x ULN	56 (9.2)
≤2x ULN	544 (89.8)
Missing	6 (1.0)

ULN upper limit of normal.
[a]Leukopenia grade 1, 2, 3 and 4 is defined as WBC count below lower limit of normal – 3.0×10^9; $<3.0–2.0 \times 10^9$; $<2.0–1.0 \times 10^9$, and $<1.0 \times 10^9$, respectively. Grade 1, 2, 3 and 4 neutropenia is defined as absolute neutrophil counts below lower limit of normal – 1.5×10^9; $<1.5–1.0 \times 10^9$; $<1.0–0.5 \times 10^9$, and $<0.5 \times 10^9$, respectively.

Table 3. Association tests of *NUDT15*3* with thiopurine-induced myelosuppression.

	*NUDT15*3*	*NUDT15* wild type	*P* value
Overall population	$N = 8$	$N = 598$	<0.001
TIM	4 (50%)	14 (2.3%)	
No TIM	4 (50%)	584 (97.7%)	
Subgroup analysis			
Patients ≥18 years	$N = 6$	$N = 539$	0.007
TIM	2 (33.3%)	11 (2.0%)	
No TIM	4 (66.7%)	528 (98.0%)	
Patients <18 years	$N = 2$	$N = 59$	0.005
TIM	2 (100%)	3 (5.1%)	
No TIM	0 (0%)	56 (94.9%)	

TIM thiopurine-induced myelosuppression.

[11]. Chao et al. defined leukopenia as WBC $< 3.5 \times 10^9$/L and did not measure neutrophils, while Walker et al. defined TIM either as a decline in WBC to 2.5×10^9/L or less, or a decline in ANC to 1.0×10^9/L or less [4, 11]. Recently, Yu et al. have conducted a systematic review and meta-analysis regarding the prevalence of *NUDT15* genetic variants and incidence of thiopurine-induced leukopenia in patients with IBD [35]. This meta-analysis included a total of 20 studies comprising 5232 patients. Of all tested *NUDT15* polymorphisms, *NUDT15*3* was the most prevalent variant, and showed a pooled risk ratio for developing thiopurine-induced leukopenia of 4.12 (95%-confidence interval (CI) 2.87–5.91) in heterozygous carriers and 9.38 (95%-CI 5.17–17.01) for homozygous variant allele carriers as compared to wild type patients. For a detailed overview of the individual study characteristics of the studies included in this meta-analysis we refer to Supplementary Table 3 of the study by Yu et al. [35].

It should be noted that there is no association between *NUDT15*3* and intracellular 6-TGN concentrations [7]. This can be explained by the fact that in general 6-TGN concentrations are measured as a mixture of mono, di and triphosphates, and thereby does not allow to differentiate between the three types of nucleotides. Thereby, it is not possible to distinct between normal and reduced *NUDT15* activity by regular 6-TGN measurements. This could potentially be overcome by a more selective 6-TGN measurement method that allows to quantify 6-TGMP, 6-TGDP and 6-TGTP separately [36].

The association between *NUDT15*3* and TIM was investigated in both children and adults. It showed that in children the absolute incidence of *NUDT15*3* and TIM was higher (100%) than in adults (33.3%). To the best of our knowledge, there are no previous reports published in literature data that have analyzed this association in children with IBD treated with thiopurines. The results of this study show that this association is also relevant to children in addition to adults.

Strengths of this study are the relatively large number of patients of a real world patient population, and very well and electronically documented patient and treatment characteristics. Therefore, data could be reliably extracted. Second, the selected primary endpoints (leukocyte and neutrophil counts) are objective values, and were obtained routinely at regular and planned outpatient visits. Third, selection bias was prevented by conducting the TIM and genotyping analysis independently from each other and establishment of TIM was performed before *NUDT15* genotyping was done. Finally, information bias was as much as possibly prevented by excluding patients who had no information on diagnosis, use of thiopurine drug, or no registered *TPMT* genotype.

Several limitations may be noted to this study. First, the retrospective design, wherein patients were not followed over time according to protocol as would have been conducted in a prospective study. On the other hand, this allowed us to properly analyze the effect of aberrant *NUDT15* genotype in patients treated with the standard dose of thiopurine drug, without the treating phycisian being aware of the patient's *NUDT15* genotype. Thereby, this may also be considered a strength. In addition, thiopurine treatment was still protocollarized, and thus sufficient laboratory data were available in this real world patient population.

Second, the study did not analyze further treatment and clinical data such as thiopurine dose, infection prevalence, hospitalization, intensive care admissions and antibiotic usage for all patients. We only retrieved these treatment and clinical data in the *NUDT15* variant allele carriers as we were specifically interested in the clinical course of treatment in these patients. Given the relatively low frequency of *NUDT15*3*, wild type patients may be considered the "normal population". Data about infection and hospitalization prevalence would not have added new information since these

Table 4. Patient characteristics and clinical course of treatment description of the NUDT15*3 cases.

Case	Sex	Age	TPMT fenotype	Etnicity	Disease	Drug	Dose (mg/kg bodyweight)	Treatment duration	Clinical course of treatment
1	M	79	NM	Indian	M. Crohn	mercaptopurine	1.1	3 weeks Stop treatment	No toxicity during first weeks of treatment; stopped thereafter all IBD medication on patients own decision
2	M	65	NM	Curacao	UC	mercaptopurine tioguanine	0.7 0.3 0.6	3 months 2 months Ongoing	no toxicity during treatment
3	F	48	NM	Surinam	UC	mercaptopurine tioguanine	0.9 0.4	3 months 14 months Stop treatment	Malaise and abdominal pain → switched to tioguanine. → Fatigue, hair loss, nail toxicity. Signs of toxicity improved after stop treatment
4	M	40	NM	Caucasian	UC	mercaptopurine	0.6 1.2	2 months Ongoing	No toxicity during first 2 months dose. After dose increase grade 2 leukopenia, grade 2 neutropenia, mild nausea and diarrea
5	M	20	NM	Surinam	UC	tioguanine	0.1	2 months Stop treatment	2 months low dose treatment: no toxicity
6	M	17	NM	Antillean	IBD-U	azathioprine tioguanine	2.5 0.8 1.3 0.2 0.3	1 month 1 month 1 month 4 months Ongoing	Grade 2 leukopenia, grade 2 neutropenia plus nausea and fatigue at highest dose. Therefore switch tot tioguanine and no toxicity at that dose level
7	F	8	NM	Eastern	IBD-U	azathioprine	2.4 1.6 0.8	1 month 2 months ongoing	Grade 2 leukopenia, grade 2 neutropenia. Therefore dose reduction. No clinical toxicity
8	M	57	NM	Surinam	M. Crohn	azathioprine	1.9 2.4	1 month 2 months Stop treatment	Acute stop treatment due to grade 3 leukopenia and grade 3 anemia requiring hospitalisation and blood transfusion

F female, *IBD-U* inflammatory bowel disease unclassified, *NM* normal metaboliser, *M* male, *M. Crohn* Morbus Crohn, *UC* ulcerative colitis.

data are readily available and described in phase 3 trials or other population studies as thiopurines being already relatively old drugs. Similary, lacking data about thiopurine doses did not allow us to test for associations between dose and development of TIM, but again, this was largely out of the scope of this study of this *NUDT15* pharmacogenetic association study.

Third, low leukocytes and neutrophils were selected as primary endpoints in this study because these are primarily measured as standard laboratory values in our hospital [37]. Other studies have e.g. focused on the lymphocytes as primary outcome parameter for myelosuppression, as lymphopenia may increase the risk of serious opportunistic infections. We didn't take this laboratory parameter into account. However, as lymphocytes are part of total leukocytes and highly correlate with each other, we postulate that by including lymphocytes similar associations for *NUDT15* with TIM would have been observed.

Fourth, in this study we only conducted a subgroup analysis based on the patients' age. Further subgroup analyses were not considered to add usefull data given the total number of eight *NUDT15* variant allele carriers. This would need a larger number of patients. In addition, we did not test for additional polymorphisms within *NUDT15*. Other *NUDT15* genetic variants than *NUDT15*3*, including **5* and **6*, have also been demonstrated to be associated with thiopurine-induced toxicity, but showed a lower allele frequency (pooled prevalence of 2 and 7%, respectively, in mainly Japanese and Asian patients) [35]. Since these prevalences are lower as compared to *NUDT15*3*, we expect this also to be lower in the Caucasian population. Therefore, to properly assess this would require an even larger patient population.

In conclusion, we showed that the frequency of the *NUDT15*3* genetic polymorphism in this patient population in the Netherlands is higher than in typical Caucasian populations, and that there is an association between *NUDT15*3* and the development of TIM. In daily practice, *NUDT15* genotyping could be combined with genotyping for *TPMT*, using the same DNA extract. Hereby, the results can be obtained on the same working day as the *TPMT* genotyping results. As such, *NUDT15* genotyping has been implemented in our institution as routine pretherapeutic test. Although the study results suggest that pre-therapeutic genotyping for *NUDT15*3* could reduce the risk of thiopurine-induced myelosuppression in IBD patients treated with thiopurines, its clinical utility in patient populations with low *NUDT15* variant allele frequencies still needs to be demonstrated.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Derijks LJJ, Gilissen LPL, Hooymans PM, Hommes DW. Review article: thiopurines in inflammatory bowel disease. Aliment Pharmacol Ther. 2006;24:715–29.
2. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. Gut. 2002;50:485 LP–489.
3. Gisbert JP, Gomollón F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: a review. Am J Gastroenterol. 2008;103:1783–800.
4. Chao K, Wang X, Cao Q, Qian J, Wu K, Zhu X, et al. Combined detection of NUDT15 variants could highly predict thiopurine-induced leukopenia in Chinese patients with inflammatory bowel disease: a multicenter analysis. Inflamm Bowel Dis. 2017;23:1592–9.
5. Moriyama T, Nishii R, Perez-Andreu V, Yang W, Klussmann FA, Zhao X, et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet. 2016;48:367–73.
6. Zhu X, Wang XD, Chao K, Zhi M, Zheng H, Ruan HL, et al. NUDT15 polymorphisms are better than thiopurine S-methyltransferase as predictor of risk for thiopurine-induced leukopenia in Chinese patients with Crohn's disease. Aliment Pharmacol Ther. 2016;44:967–75.
7. Asada A, Nishida A, Shioya M, Imaeda H, Inatomi O, Bamba S, et al. NUDT15 R139C-related thiopurine leukocytopenia is mediated by 6-thioguanine nucleotide-independent mechanism in Japanese patients with inflammatory bowel disease. J Gastroenterol. 2016;51:22–9.
8. Chaparro M, Ordás I, Cabré E, Garcia-Sanchez V, Bastida G, Peñalva M, et al. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. Inflamm Bowel Dis. 2013;19:1404–10.
9. Lee YJ, Hwang EH, Park JH, Shin JH, Kang B, Kim SY. NUDT15 variant is the most common variant associated with thiopurine-induced early leukopenia and alopecia in Korean pediatric patients with Crohn's disease. Eur J Gastroenterol Hepatol. 2016;28:475–8.
10. Gearry RB, Barclay ML, Burt MJ, Collett JA, Chapman BA. Thiopurine drug adverse effects in a population of New Zealand patients with inflammatory bowel disease. Pharmacoepidemiol Drug Saf. 2004;13:563–7.
11. Walker GJ, Harrison JW, Heap GA, Voskuil MD, Andersen V, Anderson CA, et al. Association of genetic variants in NUDT15 with thiopurine-induced myelosuppression in patients with inflammatory bowel disease. JAMA J Am Med Assoc. 2019;321:753–61.
12. Moyer AM. NUDT15: a bench to bedside success story. Clin Biochem. 2021;92:1–8.
13. Zaza G, Meyling C, Krynetskaia N, Thorn C, Stocco G, Hebert JM, et al. Thiopurine Pathway [Internet]. *Pharmacogenetics and genomics*. 2010 [cited 2022 Sep 23]. Available from: https://www.pharmgkb.org/pathway/PA2040
14. Matsuoka K. NUDT15 gene variants and thiopurine-induced leukopenia in patients with inflammatory bowel disease. Intest Res. 2020;18:275–81.
15. KNMP. Algemene achtergrondtekst Farmacogenetica - NUDT15 [Internet]. 2018 [cited 2022 Sep 19]. Available from: https://www.knmp.nl/downloads/g-standaard/farmacogenetica/achtergrondtekst-Farmacogenetica-CYP2D6-feb2020.pdf/view
16. Derijks LJJ, Wong DR, Hommes DW, van Bodegraven AA. Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of inflammatory bowel disease. Clin Pharmacokinet. 2018;57:1075–106.
17. Kakuta Y, Kawai Y, Okamoto D, Takagawa T, Ikeya K, Sakuraba H, et al. NUDT15 codon 139 is the best pharmacogenetic marker for predicting thiopurine-induced severe adverse events in Japanese patients with inflammatory bowel disease: a multicenter study. J Gastroenterol. 2018;53:1065–78.
18. Dean L. Azathioprine therapy and TPMT and NUDT15 genotype [Internet]. Med Genet Summaries. 2012. Available from: https://www.ncbi.nlm.nih.gov/books/
19. Tanaka Y, Saito Y. Importance of NUDT15 Polymorphisms in Thiopurine Treatments. J Pers Med. 2021;11:778.
20. Yang SK, Hong M, Baek J, Choi H, Zhao W, Jung Y, et al. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. Nat Genet. 2014;46:1017–20.
21. Yin D, Xia X, Zhang J, Zhang S, Liao F, Zhang G, et al. Impact of NUDT15 polymorphisms on thiopurines-induced myelotoxicity and thiopurines tolerance dose. Oncotarget. 2017;8:13575–85.
22. Kakuta Y, Naito T, Onodera M, Kuroha M, Kimura T, Shiga H, et al. NUDT15 R139C causes thiopurine-induced early severe hair loss and leukopenia in Japanese patients with IBD. Pharmacogenomics J. 2016;16:280–5.
23. Khaeso K, Udayachalerm S, Komvilaisak P, Chainansamit SO, Suwannaying K, Laoaroon N, et al. Meta-Analysis of NUDT15 Genetic Polymorphism on Thiopurine-Induced Myelosuppression in Asian Populations. Front Pharmacol. 2021;12:784712.
24. PHARMGKB. rs116855232 [Internet]. [cited 2023 Jan 19]. Available from: https://www.pharmgkb.org/variant/PA166154759/overview.
25. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91.
26. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Blood/bone marrow [Internet]. 2006 [cited 2022 Sep 19]. Available from: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf.
27. Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol. 2015;33:1235–42.
28. Fan X, Yin D, Men R, Xu H, Yang L. NUDT15 polymorphism confer increased susceptibility to thiopurine-induced leukopenia in patients with autoimmune hepatitis and related cirrhosis. Front Pharmacol. 2019;10:1–8.
29. Liu Y, Meng Y, Wang L, Liu Z, Li J, Dong W. Associations between the NUDT15 R139C polymorphism and susceptibility to thiopurine-induced leukopenia in Asians: a meta-analysis. Onco Targets Ther. 2018;11:8309–17.
30. Zhang AL, Yang J, Wang H, Lu JL, Tang S, Zhang XJ. Association of NUDT15 c.415C>T allele and thiopurine-induced leukocytopenia in Asians: a systematic review and meta-analysis. Ir J Med Sci. 2018;187:145–53.
31. von Muhlenbrock C, Estay C, Covarrubias N, Miranda J, Venegas M. The c.415C>T polymorphism in NUDT15 is more frequent than the polymorphisms in TPMT in Chilean patients who use thiopurine drugs. Pharmacogenet Genomics. 2023;33:161–3.
32. Díaz-Villamarín X, Fernández-Varón E, Rojas Romero MC, Callejas-Rubio JL, Cabeza-Barrera J, Rodríguez-Nogales A, et al. Azathioprine dose tailoring based

on pharmacogenetic information: insights of clinical implementation. Biomed Pharmacother. 2023;168:115706.
33. Schaeffeler E, Jaeger SU, Klumpp V, Yang JJ, Igel S, Hinze L, et al. Impact of NUDT15 genetics on severe thiopurine-related hematotoxicity in patients with European ancestry. Genet Med. 2019;21:2145–50.
34. Akiyama S, Matsuoka K, Fukuda K, Hamada S, Shimizu M, Nanki K, et al. Long-term effect of NUDT15 R139C on hematologic indices in inflammatory bowel disease patients treated with thiopurine. J Gastroenterol Hepatol. 2019;34:1751–7.
35. Yu N, Sriranganathan D, Walker GJ, Sazonovs A, Wilding H, Roberts C, et al. Prevalence of NUDT15 genetic variants and incidence of thiopurine-induced leukopenia in inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis. 2023;17:1920–30.
36. Han J, Xu J, Sun N, Jin S, Mei D, Wang X, et al. Analysis of mono-, di-, and triphosphates of thioguanosine and methylthioinosine in children with acute lymphoblastic leukemia by LC-MS/MS. J Pharm Biomed Anal. 2022;217:114813.
37. Vögelin M, Biedermann L, Frei P, Vavricka SR, Scharl S, Zeitz J, et al. The impact of azathioprine-associated lymphopenia on the onset of opportunistic infections in patients with inflammatory bowel disease. PLoS ONE. 2016;11:e0155218.

AUTHOR CONTRIBUTIONS

MJD: conceptualization, data curation, methodology, project administration, resources, supervision, writing—original draft. AJvN: data curation, methodology, formal analysis, investigation, visualization, writing—original draft. JBB: data curation, investigation, writing—review and editing. MAvD: data curation, resources, validation, writing—review and editing. JMS: resources, writing—review and editing. LJJD: conceptualization, methodology, writing—review and editing. LPLG: conceptualization, investigation, methodology, resources, validation, writing—review and editing. BALMD: conceptualization, data curation, funding acquisition, methodology, resources, writing—review and editing. All authors approved the final version of the manuscript, including the authorship list. MJD acts as the guarantor of the article and takes responsibility for the integrity of the work as a whole.

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ADDITIONAL INFORMATION

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