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ORIGINAL ARTICLE

Nudix hydrolase 15 (*NUDT15*) loss-of-function variants in an Australian inflammatory bowel disease population

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Key words
NUDT15, inflammatory bowel disease, thiopurine, Crohn disease, myelosuppression, ulcerative colitis.

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Abstract

Background: Thiopurine-related adverse events such as leukopenia, liver dysfunction and pancreatitis are associated with variants in the *NUDT15* gene. Loss-of-function (low or no enzyme activity) alleles are more common in Asian and Hispanic populations. The prevalence of these variants in the Australian inflammatory bowel disease (IBD) population has not yet been reported.

Aim: To evaluate the presence of *NUDT15* loss-of-function alleles *2,*3,*9 in the Australian IBD population.

Methods: The *NUDT15* screening cohort included 423 IBD patients from Brisbane, Australia. Study patients were recruited by: (i) retrospective review of clinical charts for thiopurine-related severe adverse events; (ii) pathology data (white blood cell (WBC) and neutrophil counts). *NUDT15* genotyping was performed using polymerase chain reaction (PCR)-high-resolution melt (HRM), TaqMan genotyping and Sanger sequencing.

Results: *NUDT15* mutation R139C (allele *3) was identified in 8 of 423 (1.9%) IBD patients. Seven of eight patients were R139C heterozygous (C/T) and one patient was R139C homozygous (T/T). One of the C/T group and the T/T patient developed thiopurine-induced myelosuppression (TIM) within 60 days of dosing. One patient in the C/T group developed TIM after 60 days of thiopurine dosing. The remaining five patients in the C/T group did not show TIM; however, other thiopurine-related events could not be ruled out and therefore careful monitoring over a long period is recommended.

Conclusions: This is the first study to report the frequency of *NUDT15* haplotypes *2,*3,*9 in an Australian IBD population. The most common variant detected was the R139C mutation. PCR and Sanger sequencing are efficient and cost-effective approaches for *NUDT15* genotyping.

Introduction

Thiopurine drugs are used to treat both benign (inflammatory bowel disease (IBD)) and malignant (acute lymphoblastic leukaemia (ALL)) disorders. Fifty percent of IBD patients receive thiopurines (azathioprine (AZA), mercaptopurine (MP)) and thioguanine[1,2] and up to one-third of patients discontinue therapy due to thiopurine intolerance or toxicity.[3,4] Intolerance to thiopurines can include gastrointestinal symptoms, hepatotoxicity, nodular regenerative hyperplasia, pancreatitis, lymphoma, infections, intolerance or life-threatening haematoxicity (leukopenia and myelosuppression).[4–7]

Haematoxicity or thiopurine-induced myelosuppression (TIM) manifests when a severe adverse reaction is elicited due to excessive amounts of 6-thioguanine nucleotides (6-TGN), thioguanine metabolites, being produced causing a reduction in a haemopoietic lineage. Patients with genetic variants in the thiopurine S-methyltransferase (*TPMT*) and nudix hydrolase 15 (*NUDT15)* genes are at increased risk of developing TIM following standard thiopurine dosing.[8] *TPMT* variants are more common in individuals with European ancestry and rare in individuals of Asian descent. Conversely, *NUDT15* variants are

Conflict of interest: None.

more frequent in Asian and African populations and only present in approximately 2% of individuals of European ethnicity.[9]

Loss-of-function alleles in nudix hydrolase 15 (*NUDT15*) gene have been associated with thiopurine intolerance.[10,11] *NUDT15* plays a pivotal role in the catalytic conversion of 6-cytotoxic thioguanine triphosphate (6-TGTP) metabolites to 6-thioguanine monophosphate (6-TGMP; Fig. 1). *NUDT15* variants with no or low enzymatic activity lead to reduced conversion to the less toxic form (6-TGMP), allowing for greater levels of cytotoxic (6-TGTP) metabolites.[8] Up to 16 coding region variants in *NUDT15* have been identified[11] and designated the star (*) allele nomenclature. Three variants (*2,*3,*9) exhibit the strongest evidence for loss-of-function and thus greatest risk to thiopurine intolerance. The function of the remaining coding variants (*4-*8, *10-*19) currently remains uncertain.

Most recently, the Clinical Pharmacogenetics Implementation Consortium (CPIC) included *NUDT15* genetic information in their guidelines for thiopurine dosing recommendations.[10] Here, we report the frequencies of *NUDT15* genetic variants in an Australian IBD patient population of predominantly European ancestry. High-resolution melt (HRM) technique and custom TaqMan assay were used to genotype the *NUDT15* variants. Both the HRM technique[12] and TaqMan[13] methods have been shown to be reliable methods for genotyping of the *NUDT15* variants. In line with recommendations by CPIC *NUDT15*, we evaluated the effects of key variants *2,*3 and *9 on haematological parameters including white blood cell (WBC) count, neutrophil count (NEU), platelets, mean corpuscular volume (MCV) and haemoglobin levels.

Materials and methods

Patient recruitment

NUDT15 genetic screening of exons 1 and 3 was carried out in a retrospective cohort of 423 IBD patients of predominantly European descent. Study patients were recruited by: (i) retrospective review of IBD database and clinical charts for thiopurine exposure; and (ii) pathology data (6-thioguanine testing, absolute WBC and absolute NEU). Patients were considered negative for TIM based on pathology results when their minimum WBC and NEU within 60 days of commencement of thiopurine were higher than the thresholds listed below.

Two patient datasets were included in the study. The first dataset comprised 291 IBD patients with a history of thiopurine exposure and full blood cell count data. TIM-positive patients (affected) were defined as having a WBC $\leq 2.5 \times 10^9$/L or NEU $\leq 1.0 \times 10^9$/L (n = 12/291; 4.1%). A second dataset included 132 IBD patients who had undergone thiopurine methyltransferase (*TPMT*) activity testing alone.

Ethics

The Human Research Ethics Committee of QIMR Berghofer Medical Research Institute and Royal Brisbane and Women's Hospital (RBWH) approved the study (protocol: HREC/14/QRBW/323). All study participants were recruited from RBWH, Brisbane, Australia, and all gave informed written consent.

NUDT15 genotyping

Exons 1 and 3 of the *NUDT15* gene were genotyped using TaqMan assay and polymerase chain reaction (PCR)-HRM and Sanger sequencing as previously described.[8,14] Genotyping of *NUDT15* variant rs116855232 (R139C) was performed using a custom TaqMan assay (Thermo Fisher Scientific Inc.). Sanger sequencing was carried out using BigDye chemistry and ABI 3130 Genetic Analyser. Sequence analysis was conducted using Sequencher 5.0 (Gene Codes Corporation).

Statistical analysis

The Chi-squared test was used to assess differences in prevalence of myelosuppression for participants with known risk alleles (*1.05/*3, *1/*3 or *3/*3) versus all other alleles. A survival analysis performed to identify the percentage of participants who developed myelosuppression. A Cox proportional hazard model was used to assess differential survival between participants with and without risk alleles.

Results

Patient characteristics

The study cohort comprised 217 females and 206 males of predominantly European descent (95%) with a median age of diagnosis of 27 years. Of 423 patients in the study, 193 (46%) had CD, 211 (50%) had UC and 19 (4%) were classified as IBD-type unspecified (Table 1). Two-thirds (291/423; 69%) of the cohort had a history of thiopurine exposure. Of the 291 thiopurine-exposed patients, 12 (4%) were TIM-positive within 60 days of their initial 6-thiopurine (6TG) test. The remaining 279 (96%) of thiopurine-exposed patients were TIM-negative. Two of the eight patients with risk alleles experienced myelosuppression within 60 days of commencing thiopurine treatment. Of the remaining participants, 38 had alleles of unknown risk and 248 had the *1/*1 wild-type allele. Two participants with

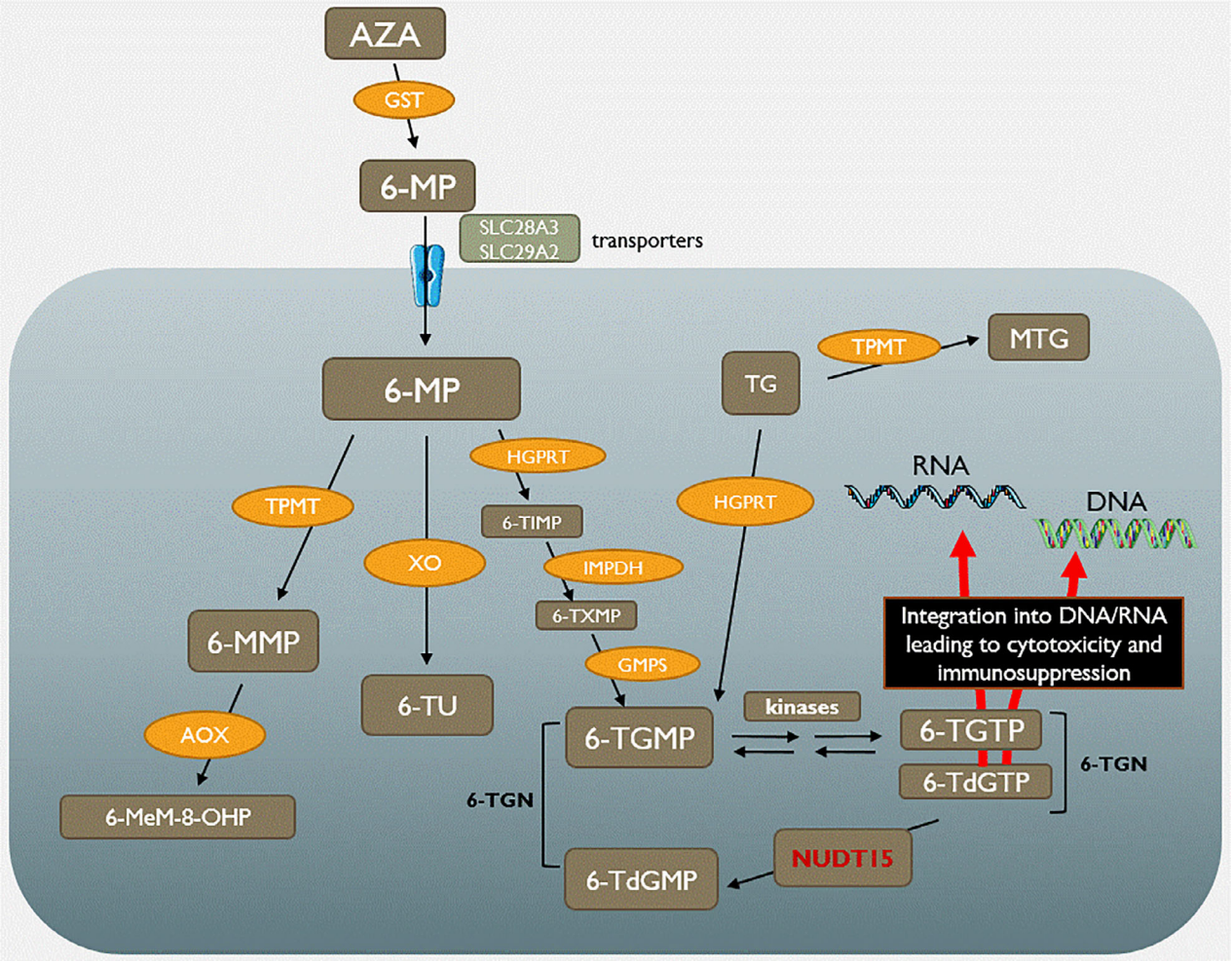

Figure 1 The role of *NUDT15* in thiopurine metabolism pathway. The drugs and metabolites are depicted using brown squares. The enzymes involved in the pathways are depicted using yellow ovals. SLC28A3 and SLC29A2 are cell membrane transporters. This schematic representation was created using images from https://smart.servier.com. 6-MeM-8-OHP, 6-methylmercapto-8-hydroxypurine; 6-MMP, 6-methylmercaptopurine; 6-MP, mercaptopurine; 6-TdGMP, 6-thio-deoxyguanine monophosphate; 6-TdGTP, 6-thiodoxyguanine triphosphate; 6-TGMP, 6-thioguanine monophosphate; 6-TGMP, 6-TdGMP, 6-TGTP and 6-TdGTP together form the 6-TGN; 6-TGN, thioguanine nucleotides; 6-TGTP, 6-thioguanine triphosphate; 6-TIMP, 6-thioinosine monophosphate; 6-TU, 6-thiouracil; 6-TXMP, 6-thioxanthosine monophosphate; AOX, aldehyde oxidase; AZA, azathioprine; GMPS, guanosine monophosphate synthetase; GST, glutathione-S-transferase; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; MTG, methylthioguanine; NUDT15, nudix hydrolase 15; TG, thioguanine; TPMT, thiopurine S-methyltransferase; XO, xanthine oxidase.

alleles of unknown risk (5.3%) and eight participants with the wild-type allele (3.3%) experienced myelosuppression within the same time period. A Chi-squared test revealed differences between myelosuppression prevalence between these groups ($\chi^2 = 16.90$; $P = 0.0002$). A *post-hoc* analysis identified this difference is driven by the differences in myelosuppression prevalence between known risk alleles and the wild-type allele ($P_{bon} = 0.039$). A Cox proportional hazard test indicated participants with a risk allele experienced myelosuppression at a significantly higher rate as compared with wild type (hazard ratio (HR): 15.35; 95% confidence interval (CI): 3.25–72.47; $P = 5.63 \times 10^{-04}$), but no difference was observed in participants with alleles of unknown influence compared with wild type (HR: 1.63; 95% CI: 0.34–7.72; $P = 0.53$).

Haematologic parameters

Of those who developed myelosuppression within 60 days of 6TG testing, six (50%) were myelosuppression positive within 2 days of 6TG testing. Nine (75%) participants were myelosuppression positive within 21 days and all patients who were myelosuppression positive within the first 60 days had developed myelosuppression within 48 days.

Figure 2 shows a survival analysis for myelosuppression within 60 days of 6-thioguanine testing.

NUDT15 variants

The loss-of-function *NUDT15* variant allele *3 (c.415C > T, rs116855232) was identified in 8 of 423 (1.9%) patients (Table 2). The eight cases with risk alleles in our cohort comprised four Caucasian, three Asian and one African ancestral backgrounds. Consistent with previously reported studies, we observed a higher frequency of *NUDT15* risk alleles in our Asian patients (3/8) compared with the patients from Caucasian (4/402) or African (1/6) ethnicity. Seven of the eight cases were heterozygous (diplotype *1/*3) and one patient was homozygous (diplotype *3/*3). No patients in the study had loss-of-function variant alleles *2 or *9. Additional *NUDT15* variants (of uncertain function) were detected by PCR-HRM in 51 of 423 (12.1%) patients (Table 2). All 51 additional variants were heterozygous changes. One of these additional variants includes a novel silent mutation in exon 1 (c.102G > A, R34R). Diplotypes *1/*1.02 (rs61746486, c.36A > C, P12P), *1/*1.03 (rs181638201, −79, G > A) and *1/*1.05 (rs61973267, *7 G > A) were detected in 1, 6 and 43 patients respectively. Eighty six percent of patients (364/423) carried the wild-type diplotype, *1/*1.

Table 1 Patient characteristics

Characteristic	*n* (%)
Gender	
Female	217 (51)
Male	206 (49)
Median age at diagnosis (SD) (years)	27 (16.1)
IBD type	
Crohn disease	193 (46)
Ulcerative colitis	211 (50)
IBD-TU	19 (4)
Thiopurine exposure	
Yes	291 (69)
No	132 (31)
TIM (yes)	12/291 (4)
TIM (no)	279/291 (96)
Ethnicity	
Caucasian	402 (95)
Asian	8 (1.9)
African	6 (1.4)
Middle Eastern	2 (0.5)
Central or South American	5 (1.2)

IBD-TU, inflammatory bowel disease unspecified type; SD, standard deviation; TIM, thiopurine-induced myelosuppression.

Performance of real-time PCR genotyping (HRM and TaqMan) versus sanger sequencing

All DNA samples were screened for *NUDT15* variants in exons 1 and 3 using PCR-HRM analysis and TaqMan technology respectively. Any sample displaying either a temperature-shifted melt curve profile or identified as being R139C heterozygous or R139C homozygous by TaqMan assay were sequenced (Supporting Information Figs S1, S2). Additionally, a further 8–10% exons 1 and 3 amplicons with normal (no mutation) profiles were also sequenced. We found 100% concordance between R139C TaqMan genotyping and Sanger sequencing results. Exon 1 indels (alleles *2, *9) were not detectable by either HRM or

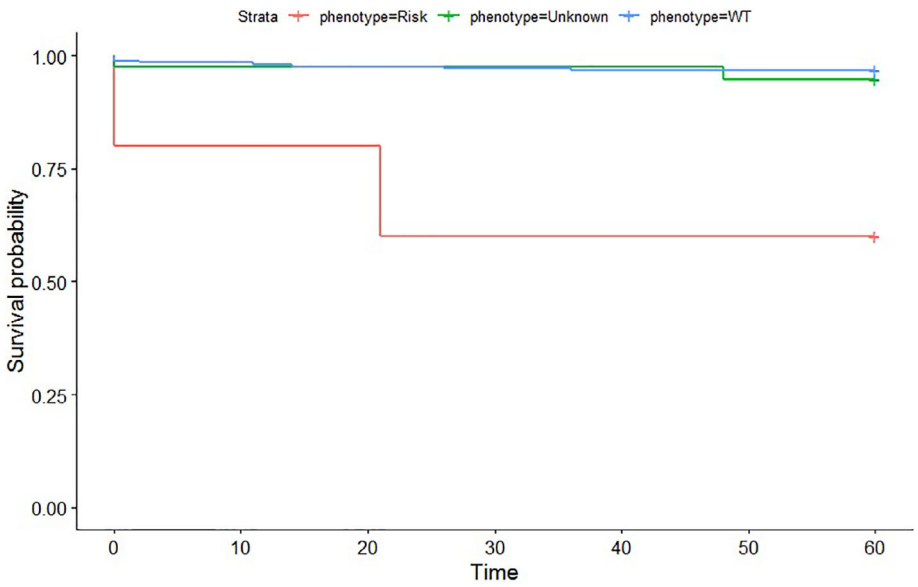

Figure 2 Kaplan–Meier survival plot for myelosuppression in thiopurine-exposed inflammatory bowel disease patients. A myelosuppression event was defined as having an absolute white blood cell count ≤2.5 $\times 10^9$/L or absolute neutrophil count ≤1.0 $\times 10^9$/L within 60 days post 6-thiopurine testing.

Table 2 Frequency of *NUDT15* variants associated with low or intermediate *NUDT15* activity

				Thiopurine exposure ($n = 291$)		No thiopurine exposure ($n = 132$)	
Diplotype	Nucleotide	Amino acid change	db SNP ID	No. patients	MAF	No. patients	MAF
*1/*2	c.55_56insGGAGTC; c.415C > T	V18_V19insGV; R139C	rs869320766; rs116855232	0	0.0000	0	0.0000
*1/*3	c.415C > T	R139C	rs116855232	4	0.0069	3	0.0114
*3/*3	c.415C > T	R139C	rs116855232	1	0.0034	0	0.0000
*1/*9	c.37_42delGGAGTC	G17_V18del	rs746071566	0	0.0000	0	0.0000

MAF, minor allele frequency.

Table 3 Composition of all *NUDT15* diplotypes in 423 inflammatory bowel disease patients

Diplotype	No. patients	Proportion (%)	Impact
*1/*1	364	86.1	Normal function
*1/*3	7	1.7	Intermediate metaboliser
*3/*3	1	0.2	Poor metaboliser
*1/*1.02	1	0.2	Unknown
*1/*1.03	6	1.4	Unknown
*1/*1.05	43	10.2	Unknown
Novel, c.102G > A	1	0.2	Unknown

Sanger sequencing. Interestingly, the PCR-HRM assay detected in one IBD patient, a somatic mosaicism at allele *1.05, rs61973267, was confirmed by sequencing analysis (data not shown; Table 3).

Discussion

NUDT15 variants play a significant role in thiopurine response. Recent guidelines by the CPIC recommend performing targeted *NUDT15* genotyping as a pre-emptive measure to avoid severe adverse events following thiopurine dosing. The 2018 updated CPIC guidelines identify in particular, three loss-of-function alleles of clinical importance (alleles *2, *3, *9).[10] However, the prevalence of *NUDT15* alleles differs among ancestral groups with populations of South and East Asian, African and Hispanic descents having the highest prevalence of *NUDT15* variants.[8] The no function allele most prevalent in the South and East Asian and European populations is the R139C missense mutation in exon 3. In a meta-analysis of eight IBD studies of South Asian descent, a significant association was found between R139C genotype and thiopurine-induced leukopenia (OR = 7.57; $P < 0.001$). Additionally, R139C was strongly associated with early (<8 weeks) and late (≥8 weeks) leukopenia (OR = 15.53, $P < 0.001$ and OR = 2.92, $P < 0.001$ respectively).[15]

Our study reports for the first time, the genetic variants of *NUDT15* present in a predominantly Caucasian (European) Australian IBD population. We observed a high prevalence of 38% (3/8) of *NUDT15* mutations in our patients of Asian ethnic background. In our IBD population, 364 (86%) were wild type for the *NUDT15* (diplotype *1/*1), 8 of 423 (1.9%) carried the severely reduced or no function allele *3 (R139C) diplotype of *1/*3 or *3/*3, respectively, and 51 patients carried *NUDT15* alleles of uncertain function (*1.02, *1.03, *1.05).[11] The majority of patients carrying an R139C mutation were genotype C/T (diplotype *1/*3), signifying intermediate metabolisers and at risk of a severe adverse event. One patient was R139C T/T genotype (diplotype *3/*3), a poor metaboliser and at high risk of a severe adverse event.[10] Early TIM (<60 days) was detected in 1 R139C C/T patient and the T/T patient leading to cessation of thiopurine therapy. Another R139C C/T patient experienced TIM after 60 days of commencing thiopurine therapy. Of note, *NUDT15* alleles *2 (6-bp in-frame insertion, V18_V19insGV and R139C) and *9 (6-bp in-frame deletion G17_V18del) were not detected in our IBD cohort.

TPMT is another crucial enzyme of thiopurine metabolism pathway that modulates clinical response in patients treated with the drug. Seven patients with severely reduced or no function alleles *3 (6 with diplotypes *1/*3 and 1 with *3/*3) in our cohort had normal *TPMT* status while we had no record for the *TPMT* status for one patient with R139C mutation of genotype C/T. Severe haematotoxicity in the two patients carrying no function/

severely reduced function alleles *3 and experiencing TIM within 60 days of starting treatment can be attributed to the *NUDT15* polymorphism given their normal *TPMT* status of 0.5 and 0.74 U/mL respectively. The outcomes of the other patients with risk alleles included drug withdrawal due to consistent pancytopenia in one patient, drug withdrawal due to cirrhosis with a persistent low platelets, haemoglobin levels, lymphopenia and macrocytosis in another. No thiopurine exposure history was recorded for one patient. Three patients with the *NUDT15* risk allele heterozygous genotype and normal *TPMT* levels had no adverse events to thiopurine treatment. However, one of these patients developed a gastric plasmablastic lymphoma following a 10-year period on AZA and a 3-year interval off this treatment.

While Sanger sequencing is the standard reference method for mutation screening and identification, performing an initial screen of Exon 1 indels of 6-bp by PCR-HRM and Exon 3 missense mutation (R139C) by TaqMan assay significantly reduced cost and turnaround time without compromising mutation detection accuracy. PCR-HRM is a powerful mutation screening technique and detected the novel heterozygous silent mutation, c.102 G > A (R > R), in one IBD patient and a somatic mosaicism for allele *1.05 in another IBD patient. Both mutations were confirmed by sequencing analysis (data not shown).

The low frequency of no function *NUDT15* mutations in the predominantly Caucasian (European) IBD patient cohort in this study was not unexpected. However, the genetic ancestry in Australia can vary by geographic location and is comprised of many ethnicities including Asian, African and Hispanic groups, all of which are known to have higher frequencies of *NUDT15* loss-of-function alleles. *NUDT15* genetic testing is therefore warranted in the Australian population to identify those individuals most at risk of a severe thiopurine-related adverse event. Genetic testing of the common variants of *NUDT15* has recently become available in Brisbane, Australia. We, in line with the CPIC guidelines, advocate a pre-emptive *NUDT15* testing and also testing in patients who have had a severe adverse reaction to thiopurine therapy. Severe adverse drug events contribute to increased patient morbidity and mortality, and significant hospitalisation costs to the health system. Multiple cost-effectiveness analyses studies for *NUDT15* testing have shown that adopting a targeted testing approach for *NUDT15* in the Asian population is a strategy that is able to justify the cost of testing.[16,17] Therefore, screening for *NUDT15* variants especially in patients of Asian, African or Hispanic descent before starting a regimen of thiopurines will reduce the risk of severe myelotoxicities and subsequent hospitalisations.

Conclusions

This is the first study to report the frequency of *NUDT15* haplotypes *2,*3,*9 in an Australian IBD population. The most common variant detected was the R139C mutation. The methods – PCR and Sanger sequencing – are efficient and cost-effective approaches for *NUDT15* genotyping.

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References

1 Beaugerie L, Brousse N, Bouvier AM, Colombel JF, Lémann M, Cosnes J *et al*. Lymphoproliferative disorders in patients receiving thiopurines for inflammatory bowel disease: a prospective observational cohort study. *Lancet* 2009; **374**: 1617–25.

2 Cosnes J, Nion-Larmurier I, Beaugerie L, Afchain P, Tiret E, Gendre JP. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. *Gut* 2005; **54**: 237–41.

3 Ansari A, Hassan C, Duley J, Marinaki A, Shobowale-Bakre EM, Seed P *et al*. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. *Aliment Pharmacol Ther* 2002; **16**: 1743–50.

4 Marinaki AM, Ansari A, Duley JA, Arenas M, Sumi S, Lewis CM *et al*. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). *Pharmacogenetics* 2004; **14**: 181–7.

5 Nielsen OH, Vainer B, Rask-Madsen J. Review article: the treatment of inflammatory bowel disease with 6-mercaptopurine or azathioprine. *Aliment Pharmacol Ther* 2001; **15**: 1699–708.

6 Schwab M, Schaffeler E, Marx C, Fischer C, Lang T, Behrens C *et al*. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism. *Pharmacogenetics* 2002; **12**: 429–36.

7 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* 2019; **321**: 773–85.

8 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.

9 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.
10 Relling MV, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM *et al*. Clinical Pharmacogenetics implementation consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. *Clin Pharmacol Ther* 2019; **105**: 1095–105.
11 Yang JJ, Whirl-Carrillo M, Scott SA, Turner AJ, Schwab M, Tanaka Y *et al*. Pharmacogene variation consortium gene introduction: NUDT15. *Clin Pharmacol Ther* 2019; **105**: 1091–4.
12 Kakuta Y *et al*. High-resolution melt analysis enables simple genotyping of complicated polymorphisms of codon 18 rendering the NUDT15 diplotype. *J Gastroenterol* 2020; **55**: 67–77.
13 Kojima Y, Hirotsu Y, Omata W, Sugimori M, Takaoka S, Ashizawa H *et al*. Influence of NUDT15 variants on hematological pictures of patients with inflammatory bowel disease treated with thiopurines. *World J Gastroenterol* 2018; **24**: 511–18.
14 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.
15 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.
16 Zarca K, Chansavang A, Loriot MA, Durand-Zaleski I, Pallet N. Cost-effectiveness analysis of pretreatment screening for NUDT15 defective alleles. *Pharmacogenet Genomics* 2020; **30**: 175–83.
17 Zeng D, Huang X, Lin S, Lin R, Weng X, Huang P. Cost-effectiveness analysis of genotype screening and therapeutic drug monitoring in patients with inflammatory bowel disease treated with azathioprine therapy: a Chinese healthcare perspective using real-world data. *Ann Transl Med* 2021; **9**: 1138.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Figure S1. Real-time PCR high resolution melt.
Figure S2. Flowchart of *NUDT15* genotype testing protocol.