NUDT15 variant and thiopurine-induced leukopenia in Hong Kong

Felix CK Wong [1], MB, BS, MResMed, **Alex WK Leung** [2], MB, ChB, FHKAM (Paediatrics), **Jeffrey SS Kwok** [1], FHKCPath, FHKAM (Pathology), **Michael HM Chan** [1], FHKCPath, FHKAM (Pathology), **CK Li** [2], MD, FHKAM (Paediatrics), **YP Yuen** [1*], FHKCPath, FHKAM (Pathology)

Departments of [1]Chemical Pathology and [2]Paediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

* Corresponding author: lizyuenyp@cuhk.edu.hk

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Thiopurines, including azathioprine and 6-mercaptopurine (6-MP), are widely used in the treatment of autoimmune diseases and cancers, as well as prevention of rejection in organ transplantation. Azathioprine is a pro-drug that is converted to 6-MP, and subsequently undergoes extensive metabolism to the formation of 6-thioguanine nucleotides (6-TGNs). Such 6-TGNs exert their therapeutic effect by inducing apoptosis of T lymphocytes.[1] Myelosuppression, manifesting as a reduction in one or more of the haematopoietic lineages (most commonly leukopenia), is a serious adverse drug reaction related to the excessive generation of 6-TGNs.[2] Thiopurine S-methyltransferase (TPMT) diverts 6-MP from the formation of 6-TGNs by converting 6-MP into inactive metabolites. Thus, TPMT deficiency plays a causal role in the pathogenesis of thiopurine-induced leukopenia by shunting thiopurine metabolites towards the formation of excessive 6-TGNs. Genetic variants present in the *TPMT* gene result in TPMT deficiency and the trait is inherited in an autosomal co-dominant manner. *TPMT**1 represents the wild-type allele with normal TPMT activity while *2, *3A, *3B, *3C, and *8 account for approximately 95% of all *TPMT* variants known to result in TPMT deficiency.[3] With the conventional dose of thiopurines, individuals who have inherited two copies of the inactive *TPMT* allele (homozygous deficient) experience severe myelosuppression. A significant proportion (30%-60%) of individuals who have inherited one copy of the inactive *TPMT* allele (heterozygous deficient) develop moderate-to-severe myelosuppression. Those who carry two wild-type *TPMT* alleles have the least myelosuppression. Prospective *TPMT* genotyping has been recommended by the US Food and Drug Administration.[4,5] In addition, guidelines on *TPMT* genotype–based dosage recommendations are currently available that include a reduced thiopurine starting dose or use of an alternative non-thiopurine treatment in individuals who carry defective *TPMT* allele(s).[6,7]

In Hong Kong, many patients are prescribed thiopurine without prospective *TPMT* genotyping, largely because of the low frequency of *TPMT* variants in the Asian, including Chinese, population. The predominant *TPMT* variant in the Asian population is *3C (all other variants being exceedingly rare), with an allele frequency of 2.3%, in contrast to the higher allele frequency of *TPMT* variants in the Caucasian population (5.3% for all *TPMT* variants detected in one study).[8] Nevertheless, thiopurine-induced myelosuppression is more common in the Asian than Caucasian population.[9-11] Prospective *TPMT* genotyping can only identify a minor proportion of Asian patients who are at risk of thiopurine-induced myelosuppression. Moreover, the majority of Asian patients who are referred for *TPMT* genotyping after the occurrence of myelosuppression (called retrospective *TPMT* genotyping) do not carry any defective *TPMT* variant both in published studies[10,12,13] or in the experience of the authors' laboratory that has provided a *TPMT* genotyping service since 2013 (Table). There are clearly additional genetic and/or non-genetic factors that contribute to an increased risk of thiopurine-induced myelosuppression in Asians.

In 2014, the NM_018283.2:c.415C>T, p.Arg139Cys (R139C) variant in the *NUDT15* gene (dbSNP ID: rs116855232) was found to have a strong association with thiopurine-induced leukopenia in a large retrospective cohort of Koreans prescribed thiopurines for Crohn's disease.[14] Of those patients who carried one or two *NUDT15* R139C variants, 89.4% (59/66) developed leukopenia within the first 8 weeks of thiopurine therapy (defined as early leukopenia). In contrast, this risk allele was found in only 6.8% (43/632) of controls who did not develop leukopenia while on thiopurine therapy. Most strikingly, all patients (14/14) who were homozygous for the R139C variant developed early leukopenia. In addition, 25.6% (45/176) and 50% (88/176) of patients who were heterozygous for the R139C variant developed early and late leukopenia (occurrence of leukopenia after 8 weeks), respectively. A gene-dose effect was also seen as the number of risk alleles

TABLE. Summary of patients referred to our laboratory for *TPMT* genotyping. The ethnic origin of all patients listed is Chinese

Patient No.	Age at onset (years)	Gender	Indication for thiopurine treatment	Drug and maximum dosage	Nadir WCC (x 10^9 /L)	Nadir ANC (x 10^9 /L)	*TPMT* genotype	*NUDT15* genotype
1	23	F	SLE with recurrent myocarditis	AZA 50 mg daily	0.9	0.0	*1/*1[a]	Homozygous R139C
2	41	F	SLE and scleroderma, with cerebral lupus	AZA 100 mg daily	1.0	0.1	*1/*1	Homozygous R139C
3	21	F	Polyarteritis nodosa	AZA 50 mg daily	0.9	0.1	*1/*1	Homozygous R139C
4	54	F	Pemphigus vulgaris	AZA 50 mg daily	0.2	0.0	*1/*1	Homozygous R139C
5	61	F	SLE and lupus nephritis	AZA 75 mg daily	1.0	0.5	*1/*1	Heterozygous R139C
6	6	M	Acute lymphoblastic leukaemia	6-MP 25 mg once per week	0.8	0.0	*1/*6	Heterozygous R139C
7	44	F	SLE and lupus nephritis	AZA 150 mg daily	0.9	0.6	*1/*1	Wild-type
8	61	M	Henoch-Schönlein purpura with diffuse pulmonary haemorrhage	AZA 75 mg daily	0.3	0.1	*3C/*3C	Unknown[b]

Abbreviations: ANC = absolute neutrophil count; AZA = azathioprine; F = female; M = male; 6-MP = 6-mercaptopurine; SLE = systemic lupus erythematosus; WCC = white cell count

[a] *1/*1 represents the homozygous wild-type *TPMT* genotype

[b] The blood sample of this patient was discarded 2 years after *TPMT* genotyping, thus retrospective *NUDT15* genotyping could not be performed

increased, demonstrated by a lower thiopurine dose at which leukopenia occurred, a shorter time interval from the start of treatment till occurrence of leukopenia, and a higher grade of leukopenia. Overall, the presence of one or two of this risk allele had a sensitivity of 89.4% and specificity of 93.2% for early leukopenia. The association of *NUDT15* R139C with thiopurine-induced leukopenia has subsequently been demonstrated in Japanese patients with inflammatory bowel disease[15] and Taiwan Chinese patients with childhood acute lymphoblastic leukaemia (ALL).[16] *NUDT15* R139C is much more common than the *TPMT**3C variant in the Asian population, with an allele frequency of 16% in Southern Han Chinese.[17] Thus, *NUDT15* R139C testing is of greater diagnostic value than *TPMT* genotyping for prospective risk assessment of thiopurine-induced leukopenia in the local Chinese population. The exact role of *NUDT15* R139C in thiopurine toxicity remains unclear. NUDT15 is a nudix hydrolase that degrades 8-oxo-dGTP and 8-oxo-dGDP in vitro, suggesting that it prevents misincorporation of 8-oxo-2'-deoxyguanosine 5'-triphosphate (8-oxo-dGDP) into DNA in vivo.[3,18] In-vitro studies showed that treatment with 6-MP resulted in a higher percentage of apoptosis and necrosis in cells transfected with the *NUDT15* R139C construct compared with cells with the wild-type construct.[14]

We performed *NUDT15* R139C testing by polymerase chain reaction and bidirectional Sanger sequencing on all patient samples received by our laboratory for *TPMT* genotyping from August 2013 to November 2015. All patients were originally referred for retrospective *TPMT* genotyping. We received no requests for prospective *TPMT* genotyping during this period of time. The clinical history and test results are summarised in the Table. *NUDT15* R139C was identified in six (85.7%) of the seven patients referred to our laboratory for *TPMT* genotyping in whom a specimen was available for testing, while *TPMT* variants (*6 and *3C detected in this patient cohort) were identified in two (25%) of the eight patients. The *TPMT**6 variant is a rare variant with an allele frequency of 0.16% in the Chinese population.[19] Of the six patients who were positive for the *NUDT15* R139C, four were homozygous, one was heterozygous, and one patient was doubly heterozygous for *NUDT15* R139C and *TPMT**6. The identification of double heterozygosity is clinically relevant as one study showed that double heterozygotes required a substantially lower dose intensity of 6-MP in the treatment of childhood ALL compared with those with heterozygous genotype for only one of the two genes.[20] Although limited by the small number of cases, our results demonstrate the relevance of *NUDT15* R139C testing in local Chinese patients who develop thiopurine-induced leukopenia. In view of the close association of *NUDT15* R139C with early leukopenia and the relatively high carrier rate of this variant in the local Chinese population, prospective *NUDT15* R139C testing together with *TPMT* genotyping will likely become a necessary requirement for all patients in whom thiopurine therapy is indicated. Dose recommendations based on combined *NUDT15/TPMT* genotyping results may be feasible as more clinical data accumulate.

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