Pharmacogenomic screening at Instituto Nacional de Câncer: *TPMT* and *NUDT15* genotyping for thiopurine chemotherapy

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INTRODUCTION

- Thiopurine drugs have a unique role in the treatment of several malignancies, including acute lymphoblastic leukaemia (ALL).
- The thiopurines undergo a series of enzymatic reactions to form 6-thioguanine nucleotides (6-TGNs), active metabolites that cause DNA damage. In addition, these drugs can be inactivated through the action of some enzymes such as xanthine oxidase, TPMT and NUDT15 (Figure 1).

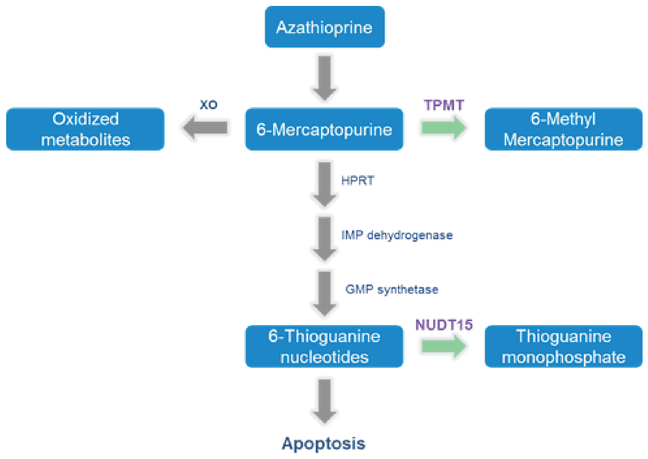


Figure 1. Thiopurine metabolism. The figure shows, in a simplified way, the thiopurine metabolism pathway. Some enzymes such as hypoxanthine guanine phosphoriboxyltransferase (HPRT), IMP dehydrogenase and GMP synthetase are responsible for transforming 6-Mercaptopurine into 'active metabolites' known as 6-Thioguanine nucleotides. In addition, 6-Mercaptopurine can be inactivated by S-methylation performed by TPMT or on oxidized metabolites by xanthine oxidase (XO). 6-Thioguanine nucleotides can be transformed into less toxic metabolites, such as Thioguanine monophosphate through the action of NUDT15.

- *TPMT* polymorphism is a major determinant of thiopurine adverse effects. More recently, *NUDT15* polymorphism (rs116855232) was also linked to thiopurine cytotoxicity (Figure 2).

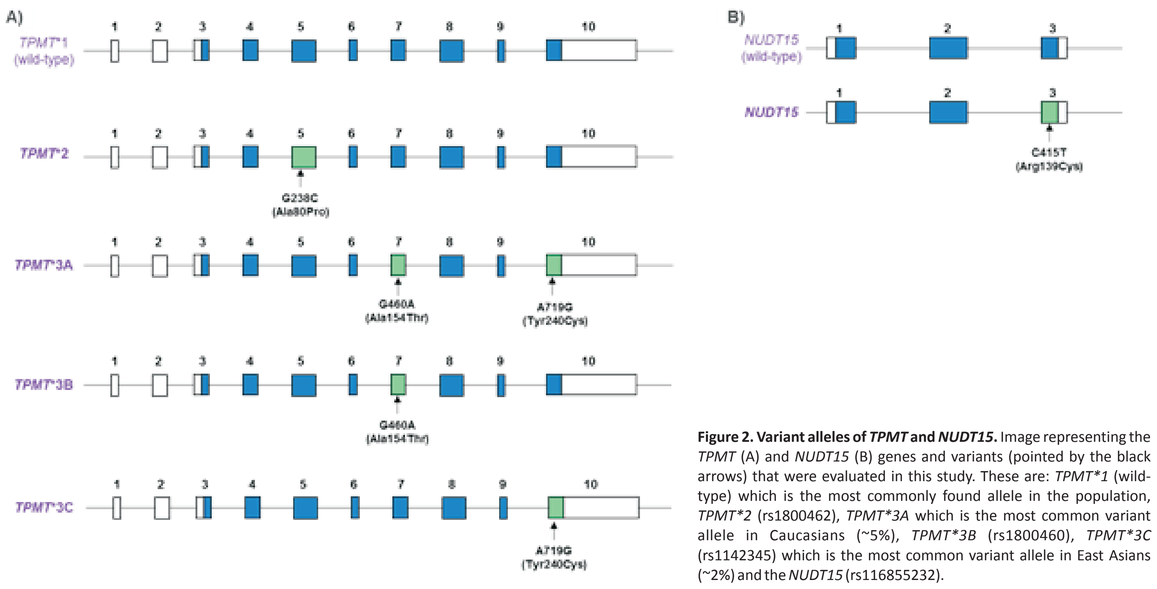
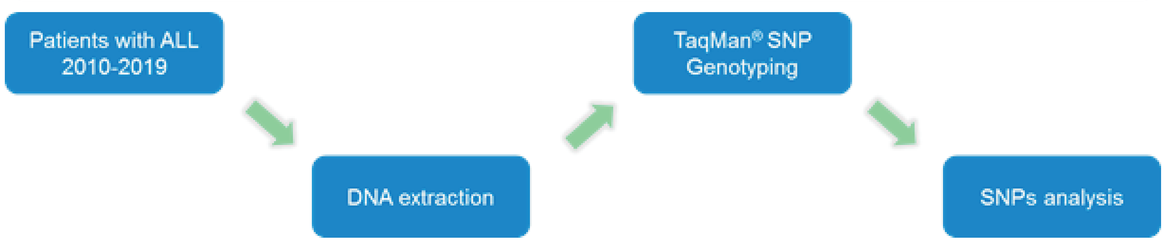


Figure 2. Variant alleles of *TPMT* and *NUDT15*. Image representing the *TPMT* (A) and *NUDT15* (B) genes and variants (pointed by the black arrows) that were evaluated in this study. These are: *TPMT*1* (wild-type) which is the most commonly found allele in the population, *TPMT*2* (rs1800462), *TPMT*3A* which is the most common variant allele in Caucasians (~5%), *TPMT*3B* (rs1800460), *TPMT*3C* (rs1142345) which is the most common variant allele in East Asians (~2%) and the *NUDT15* (rs116855232).

AIMS

Genotyping samples from ALL patients for SNPs in genes responsible for thiopurine metabolism.

STUDY-DESIGN



Funding Agencies

CNPq — FAPERJ — DECIT

METHODOLOGY

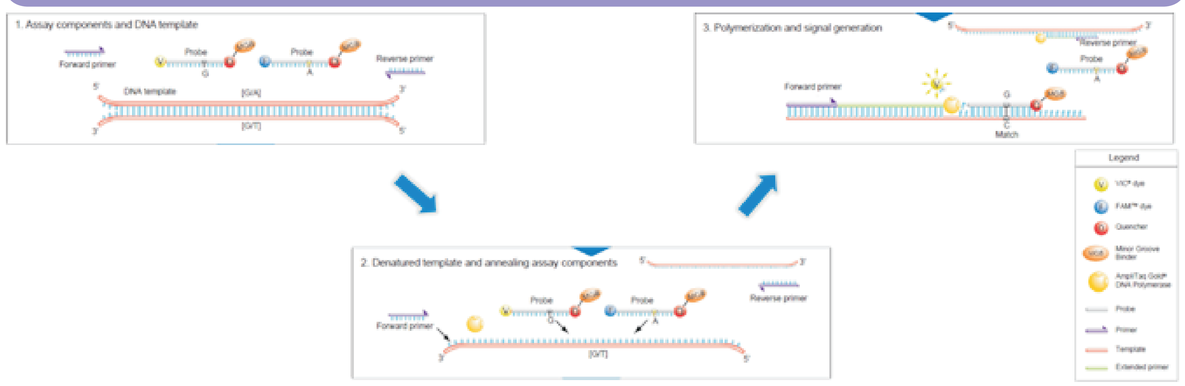


Figure 3. Allelic discrimination using TaqMan technology. This figure highlights the main reaction steps. Probes capable of discriminating which of the alleles are found in the sequence are used. Once the probe binds to the specific sequence, it emits a signal that will be detected. Allelic discrimination is achieved by the selective annealing of TaqMan® MGB probes. (PRODUCT BULLETIN TaqMan® SNP Genotyping Assays- Applied Biosystems).

RESULTS

- We have, so far, analysed 71 ALL cases diagnosed with either B-cell (n = 63) or T-cell subtype (n = 8).
- The *NUDT15* rs116855232 variant T allele was not detected in the samples.
- The *TPMT*2* and *3B haplotypes were also absent.
- Haplotypes *TPMT*3A* and *3C were detected with frequency of 4.5% and 3.0%, respectively.

Table 1. Clinical-demographic and laboratory characterisation of cases included in the present study.

Variables	n (%)
Age (years)[a]	
<10	35 (49.3)
10-21	20 (28.2)
>21	14 (19.7)
Gender	
Male	49 (69.0)
Female	22 (31.0)
WBC (x10⁹/L)[b]	
<50	41 (57.7)
≥50	27 (38.0)
ALL type	
B-ALL	63 (88.7)
T-ALL	8 (11.3)
***TPMT*1* (wild-type) [c]**	62 (92.5)
***TPMT*2*[c]**	0
***TPMT*3A*[c]**	3 (4.5)
***TPMT*3B*[c]**	0
***TPMT*3C*[c]**	2 (3.0)
***NUDT15* (wild-type) [c]**	67 (100.0)
***NUDT15* (rs116855232) [c]**	0
Total	**71 (100)**

a, for 2 cases the age was not informed; b, 3 cases lacked white blood cells (WBC) information; c, 4 cases could not be evaluated for SNPs due to insufficient biological material (DNA).

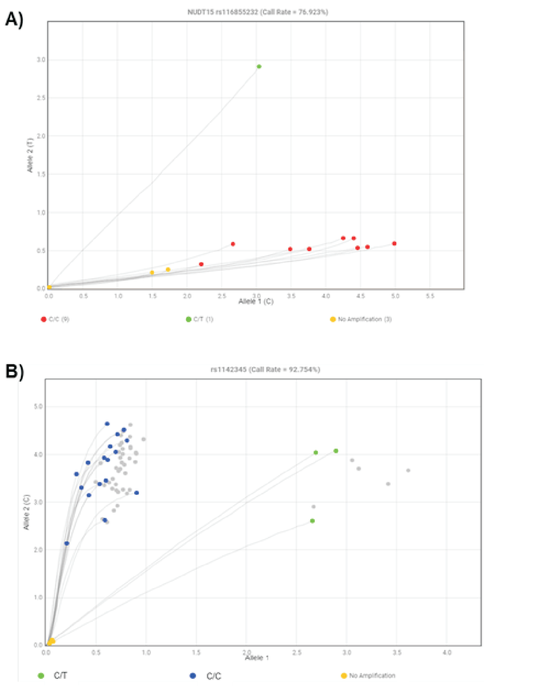


Figure 4. Genotyping performed for the *NUDT15* and *TPMT* gene. (A) These eleven cases represent the analyses for this SNP (rs116855232) in which 9 cases were homozygous (C/C). A heterozygous control (C/T) was included in this analysis. Cases represented in yellow correspond to samples not amplified in this run. (B) Twenty-one cases were analysed in this run for SNP (rs1142345). Fifteen samples were identified as homozygous (C/C) and 2 as heterozygous (C/T). One heterozygous (C/T) and 2 homozygous controls (C/C) were included in this analysis. Cases represented in yellow correspond to non-amplified samples. Cases represented in gray do not correspond to samples from this cohort.

CONCLUSION

On the basis of the inferred compound phenotypes (comprising both TPMT and NUDT15), the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines provide recommendations for adjustment of thiopurine dosing: accordingly, dose reduction was recommended for the 5 patients (7.5%) who are heterozygous for the *TMPT* polymorphisms investigated. The CPIC recommendation for drastic (~10-fold) dose reduction did not apply to the study cohort, since no patient was identified as poor metabolizer of either NUDT15 or TPMT.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

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