SNP Communication


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Summary: A 62-year-old Chinese patient with recurrent pompholyx submitted his blood sample for pretreatment thiopurine S-methyltransferase (TPMT) pharmacogenetic profiling, and it was found to harbour a novel single nucleotide polymorphism (SNP). The novel SNP, detected by mRNA sequencing, was a c.2T>C (g.11018T>C) transition in the start codon, causing a Met1Thr amino acid change. This finding was confirmed on a subsequent blood sample from the same patient by DNA sequencing. The patient was genotyped as TPMT*1/*29, sequentially named as such following the latest TPMT SNP (TPMT*1/*28) at the time of writing. The novel SNP is expected to result in complete lack of protein translation, similar to the impact exerted by TPMT*14, another start codon SNP of the TPMT gene.

Keywords: thiopurine S-methyltransferase; single nucleotide polymorphism; genetic polymorphism

Introduction

Thiopurine S-methyltransferase (TPMT) is a cytoplasmic enzyme involved in the S-methylation of thiopurine compounds, which include anti-proliferative drugs such as azathioprine, 6-mercaptopurine, and 6-thioguanine. Owing to the relatively narrow therapeutic indices of thiopurine drugs, patients with low TPMT enzyme activity (poor metabolizers) may suffer potentially life-threatening toxicity when given a standard dose. Conversely, patients with high TPMT enzyme activity (extensive metabolizers) may be under-treated when a similar dosage is applied.

TPMT genotyping can provide predictive information about a person’s TPMT enzyme activity and therefore the thiopurine drug metabolism profile. Supported by early evidence for its clinical utility, an increasing number of physicians are requesting pre-treatment TPMT testing to help determine the optimum thiopurine dose for their patients.

Several PCR methods, which selectively target several common single nucleotide polymorphisms (SNPs), are available for this purpose. These methods may not detect other SNPs that are scattered throughout the TPMT gene, beyond the target regions of the assays. Complete genomic sequencing of the TPMT gene can overcome this limitation. An in-house mRNA-based TPMT sequencing assay, which covers the entire TPMT mRNA, was developed at the National University Hospital, Singapore. During routine application of this assay, a novel SNP was uncovered at the start codon of the TPMT mRNA of a patient, and this was confirmed by DNA sequencing, on a second sample. The discovery of the novel SNP underscores the importance of performing full genomic TPMT sequencing.

Materials and Methods

A 62-year-old Chinese man with diabetes and hypertension, which were satisfactorily controlled by oral medications, presented with recurrent pompholyx affecting his hands and feet. Initially, he was treated with oral prednisolone, to which he responded well. As there was concern about the impact of chronic steroid use on the patient’s diabetes and hypertension control, topical triam...
cinolone was given as an alternative. It did not adequately control his symptoms. Azathioprine was considered as next-line therapy and a pre-treatment TPMT genotyping was undertaken for this patient. During analysis of his specimen, a novel TPMT SNP located at the start codon was detected.

Total RNA was extracted from 3 mL of whole blood. cDNA, reverse transcribed from the extracted mRNA, served as the template for PCR amplification. Two sets of primers flanking exons 2 to 9, in 2 overlapping fragments, were designed to contain a single base mismatch at the 3’ end to discriminate against the TPMT pseudogene, using GenBank: NM_000367.2 as reference (Table 1).

The amplification was performed using HotStarTaq PCR reagents (Qiagen, Hilden, Germany), on the GeneAmp® PCR thermal cycler (Applied Biosystems, Foster City, CA, USA). Cycling conditions included an initial denaturation step (95°C, 15 min), followed by 40 cycles of the amplification step at 94°C for 10s, 58°C for 1 min, 72°C for 45 s, followed by a final extension step (72°C, 7 min).

The amplified products were sequenced bi-directionally with the amplification primers (Table 1), using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems). The DNA sequence of the purified product was analyzed on the ABI 3130xl genetic analyzer (Applied Biosystems), according to the manufacturer’s instructions.

In order to confirm the above finding of the novel mutation, an additional blood sample was obtained from the patient to perform direct DNA sequencing. A new set of primers flanking the start codon of the TPMT gene were designed using GenBank: NG_012137.1 as reference (Table 1). These primers were designed to contain mismatches at the 3’ end to prevent amplification of the TPMT pseudogene. The reverse primer was also used as the sequencing primer. The extracted genomic DNA was amplified and sequenced using the amplification/sequencing protocols described earlier. This study received exemption from the Institution Review Board of the National University Hospital, Singapore. Written informed consent was provided by the patient.

Results and Discussion

We found the novel SNP: SNP: 110607LeeCK001; GENE NAME: TPMT; ACCESSION NUMBER: NG_012137.1; LENGTH: 25 bases; OBSERVED: T/C; 5’-TCTCTGAAAACATATCGGATGGTACAAG-3’.

The TPMT gene carries a g.11018T>C transition in the start codon, resulting in a Met1Thr amino acid change, first discovered using an mRNA sequencing approach. To authenticate the finding of this mutation, it was confirmed by direct DNA sequencing in a follow-up sample received at a later date. The electropherograms of the novel TPMT SNP by mRNA and DNA sequencing are shown in Figure 1. This novel SNP was named TPMT*29, following the last described TPMT mutation (TPMT*28) at the time of writing.10 The 62-year-old Chinese patient carrying the novel TPMT mutation was genotyped as TPMT*1/*29.

This represents the second start codon SNP to be described in the TPMT gene, the first being TPMT*14.11 In an in-vitro functional study, TPMT*14 was associated with complete absence of TPMT enzyme expression.6 It is likely that the novel start codon mutation, TPMT*29, would display a similar lack of protein translation. Being heterozygous for the novel mutation, the patient probably has intermediate TPMT enzyme activity as described previously in a heterozygous TPMT*1/*14 individual.11

The likely thiopurine metabolic profile of a patient can be assessed by functional or genetic studies. TPMT enzyme activity is currently the more popular choice for pre-
treatment testing. However, it is susceptible to interference from blood transfusion and anaemia, as well as alteration of enzyme activity following certain medications. In one report, a patient with apparently normal TPMT enzyme activity developed myelotoxicity as a consequence of a hidden mutation uncovered with subsequent genotyping. TPMT genotype testing can circumvent some of the limitations of the functional study.

This case highlights the advantage of our proposed mRNA sequencing approach, which provides full coverage of all coding exons of the TPMT gene, and detects cryptic or hitherto unreported mutations that otherwise may be missed by targeted genotyping, with possible adverse clinical consequences. Moreover, this method minimizes the number of PCRs performed; although, precautions should be taken to avoid amplification of the TPMT pseudogene. While TPMT phenotyping and genotyping tests are useful diagnostic tools, they should not supersede routine monitoring of myelotoxicity by simple complete blood count. Finally, physicians should familiarize themselves with the non-TPMT causes of thiopurine toxicity.

References