Two cases of 5-fluorouracil toxicity linked with gene variants in the DPYD gene

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Two cases of 5-fluorouracil toxicity linked with gene variants in the DPYD gene

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ABSTRACT

Objectives: Dihydropyrimidine dehydrogenase (DPD) is the initial rate-limiting enzyme in endogenous pyrimidine catabolism and is responsible for the reduction of the pyrmidine analog 5-fluorouracil (5-FU). DPD deficiency is known to cause potentially lethal toxicity in patients receiving 5-FU.

Design and Methods: Restriction fragment length polymorphism (RFLP) and DNA sequence analysis were performed on genomic DNA and mRNA.

Results: In 400 patients that were diagnosed with cancer and were eligible for 5-FU treatment, 14 patients were found to be heterozygous for the splice-site mutation DPYD IVS14+1G>A, which corresponds to a population frequency of 3.5%. Two novel variants in the DPYD gene were identified. The first case was heterozygous for DPYD c.1796T>C (p.M599T). In the second case, we observed heterozygosity for the splice-site mutation DPYD IVS14+17A>G.

Conclusions: We report two new DPYD gene variants, of which DPYD c.1796T>C is potentially pathogenic, whereas DPYD IVS14+17A>G is suggested as a variant without clinical significance.

Keywords: Dihydropyrimindine dehydrogenase, DPYD, 5-fluorouracil, toxicity, colon cancer

1. INTRODUCTION

5-fluorouracil and its prodrug capecitabine is one of the most commonly used anti-cancer drugs when treating cancers of the head and neck, breast, cervix and gastrointestinal tract. As previously reported, dihydropyrimidine dehydrogenase (DPD) is the rate-controlling enzyme of endogenous pyrimidine and fluoropyrimidine catabolism responsible for the elimination of approximately 80% of administered 5-fluorouracil (5-FU) [1]. Patients with a partial or complete enzyme deficiency may suffer from severe toxicity following 5-FU exposure.

DPD activity is highly variable in the population. Despite adjustable dosage schedules and modes of application, 3% of the patients that are given 5-FU demonstrate reduced enzymatic activity, leading potentially to severe 5-FU-related toxicity, i.e. WHO grades III-IV [2]. Common potentially severe side-effects related to 5-FU include diarrhea, mucositis/stomatitis [3], and leucopenia [4]. Cardiotoxicity is a relatively uncommon side-effect of 5-fluorouracil and capecitabine.

DPD enzyme deficiency has been reported to be responsible for about 50% of severe 5-FU-related toxicity. The most common DPD mutation is located in the splice site of intron 14 (IVS14+1G>A), resulting in the deletion of exon 14 and thereby creating a non-functional enzyme [5, 6]. It has been suggested that this mutation accounts for about 25 percent of 5-FU-associated toxicity [7]. Moreover, over 40 polymorphisms in the DPD gene have so far been described [8].

Considering the frequent use of 5-FU, it would be clinically beneficial to identify patients with complete or partial deficiency prior to treatment. However, to date, no detection strategy or handling procedure has been proposed and accepted clinically.

Moreover, the peripheral blood enzyme assay has not been sufficiently effective in predicting 5-FU clearance or severe toxicity to warrant routine use [9]. We now report two novel gene variants in the DPYD gene of which one, DPYD c.1796T>C (p.M599T), may be implicated in 5-FU-associated toxicity.

2. MATERIALS AND METHODS

A total of 240 samples were obtained from healthy donors in peripheral blood and 400 blood samples were collected from cancer patients before the start of 5-FU treatment.

2.1 Patients

Case 1: The first case was a 76-year-old woman that after a radical hemicolectomy for a T3N2 colon cancer began 6 months of adjuvant chemotherapy consisting of weekly bolus injections of 500 mg/m^2 5-FU and 20mg/m^2 Calcium folinate. She was asymptomatic through the first two series but on day 3-5 after the third cycle she experienced mild stomatitis and watering eyes. The symptoms rapidly became severe and she was admitted to hospital care for three weeks with grade 3-4 oral and intestinal mucositis and grade 3-4 diarrhea (CTCAE v4.0). After one month of convalescence, the treatment was reintroduced with a 50% dose reduction, a dose she could tolerate with only minor degree of toxicity at the end of the treatment period.

The second case was a 75-year-old woman who suffered from colon cancer. After surgery, she was planned to receive adjuvant treatment for 6 months with the Nordic schedule of 5-FU (500 mg/m^2 i.v. bolus) and folinic acid (60 mg/m^2 i.v. bolus) after 30 min, day 1 and 2, every 2 weeks. After two thirds of the treatment, the treatment was interrupted because of severe fatigue, grade 3 and grade 2 watering eyes (CTCAE v4.0), but no diarrhea.

2.2 Clinical genetic analysis

Genomic DNA was isolated from peripheral leucocytes using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota, USA). Exon 14 in the DPYD gene was amplified and subjected to cleavage by a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP). 20 µl of mix containing HF buffer, dNTP, HF-MgCl and Taq primer polymerase were prepared. The PCR primer sequences were 5’TCCTCTGCAAAAATGTGAGAAGGGACC-3’ and 3’TCACCAACTTATGCCAATTCTC-5’ [5].

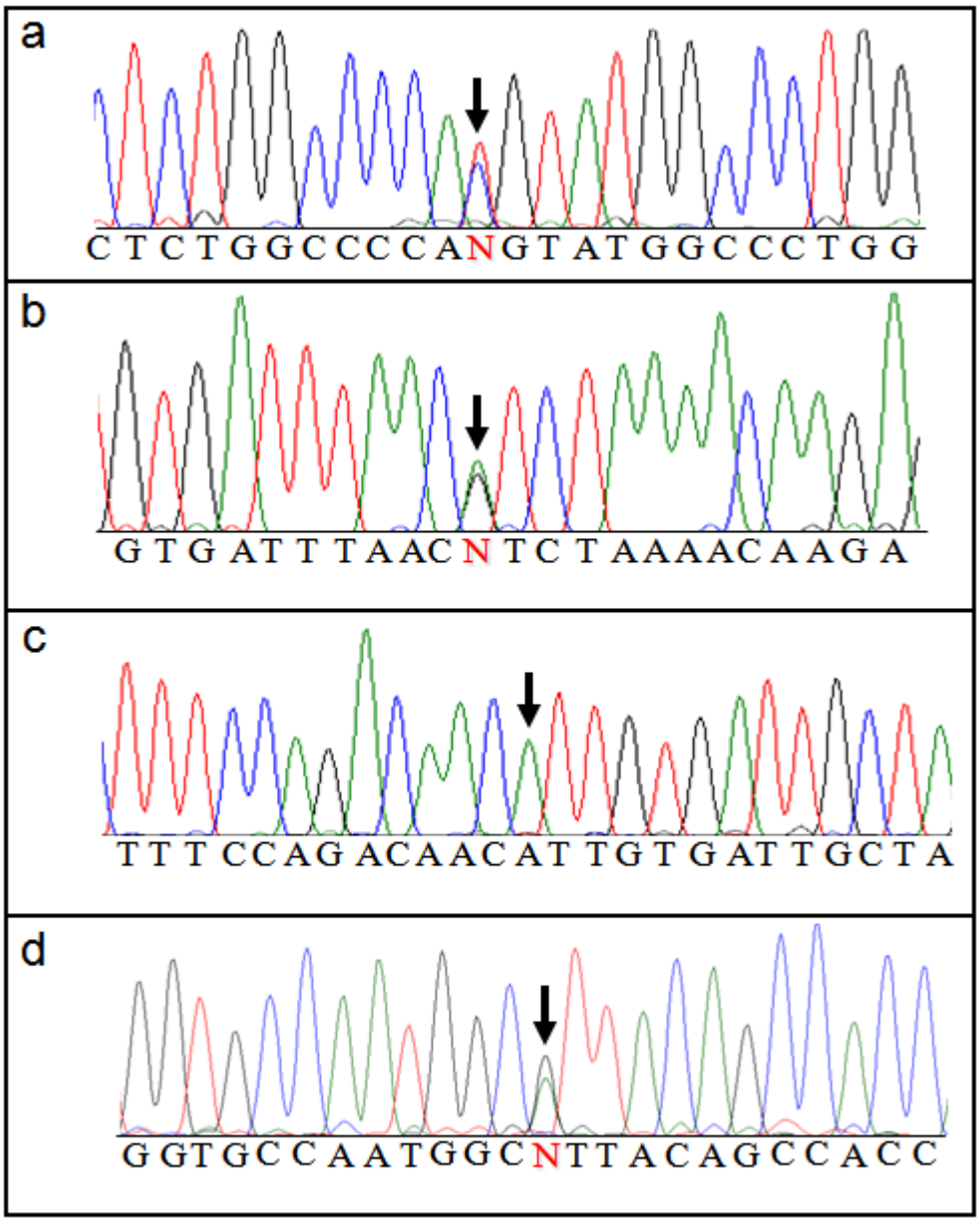

Fig. 1. Electropherogram showing two novel mutations in the DPYD gene. (a) One patients was heterozygous for DPYD c.1796T>C (p.M599T), which led to an amino acid shift from methionine to threonine. (b) One patients showed heterozygosity for the splice-site mutation DPYD IVS14+17A>G. (c) The polymorphism V7321 was detected in exon 15 in the patient heterozygous for DPYD IVS14+17A>G, which excludes the possibility of allele dropout. (d) In the patient heterozygous for DPYD IVS14+17A>G, we sequenced mRNA in the flanking exons and found that all exons, including exon 14, were present. This variant did not therefore affect the splicing process.

The PCR program consisted of 33 cycles for 95°C/5 min, 95°C/60 sec, 55°C/60 sec and 72°C/60 sec. The PCR product of 334 bp was cleaved by the restriction enzyme HpyCH4 IV (In vitro AB, Stockholm, Sweden) generating two fragments of 285 and 49 bp each. If the

splice-site mutation IVS14+1G>A is present, the cleaving site for HpyCH4 IV is abolished. The PCR products were then visualized on 2.5% agarose gel.

When the PCR-RFLP analysis was positive for the IVS14+1G>A mutation, or if the quality of the analysis did not reach the standard quality for clinical testing, the finding was confirmed by sequencing using ABI Prism BigDye Terminator Cycle sequencing v3.1 kit (Applied Biosystems, Stockholm, Sweden) and an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequencing was performed from both directions**,** giving a forward and a reverse sequence. Total RNA was isolated from blood in Tempus^{TM} tubes and RNA preparation was performed with RNeasy® Mini Kit (Qiagen, Maryland, USA), according to the manufacturer's protocol. cDNA was synthesized using SuperScript™II RNase H^{-} Reverse Transcriptase (Invitrogen™, Sweden), and sequencing was performed with the following primers: 5'CCAGCACATCAATGATTCGAAGAG-3'and 3'AGACCACTTTCAGCAGAGTCAATTCC-5';and 5'ATCGGTGAATGATGGAAAGCAAGC-3'and 3'AGCCAAAATGGGAAATCCAGGCAG-5'

3. RESULTS

3.1 Population frequency of DPYD IVS14+1G>A

Analysis of the splice-site mutation IVS14+1G>A of the DPYD gene was performed in 400 cancer patients that were eligible for 5-FU treatment. Of these, 14 patients were found to be heterozygous for the splice-site mutation DPYD IVS14+1G>A, which corresponds to a frequency of 3.5%. The population frequency in 242 controls was 2.5% (6/242).

3.2 New gene variants in the DPYD gene

Case 1: In the first patient, we detected an unexpected cleavage pattern and the DPYD gene was therefore sequenced. The patient was found to be heterozygous for DPYD c.1796T>C (p.M599T). This mutation was detected because it introduces a new cleavage site for the restriction enzyme HpyCH4 IV. This mutation gives rise to an amino acid shift from methionine, which is a hydrophobic amino acid with a non-polar side chain, to threonine that is hydrophilic with a polar side chain. The mutation was not found in 662 patients and controls, which give an expected gene frequency of 0.15%.

Case 2: The restriction cleavage in this patient was inconclusive and sequence analysis was therefore performed. The analysis revealed heterozygosity for DPYD IVS14+17A>G. To investigate whether this splice-site mutation had any effect on the splicing process, we sequenced the mRNA from the flanking exons and found that all exons, including exon 14, were present in the cDNA. A previously described heterozygous polymorphism V7321 was detected in exon 15 [10], which excludes the possibility of allele dropout. In conclusion, this splice-site mutation did not appear to affect the splicing process and may therefore be regarded as a polymorphism.

4. DISCUSSION

The enzyme DPD plays a critical role in the catabolism of the anti-cancer agent 5-FU. Non-functional alleles of the gene encoding for DPD may result in life-threatening events in cancer patients receiving systemic 5-FU treatment.

Since January 2004, at the Department of Oncology at Sahlgrenska University Hospital, Göteborg, Sweden, we have been implementing routine clinical genetic testing for the splice-site mutation DPYD IVS14+1G>A for patients prior to receiving continuous infusion of 5-FU (750-1000mg/m^2) to identify patients running an increased risk of severe 5-FU-induced toxicity. This mutation alone is estimated to be present in about 25 percent of patients suffering from severe toxicity [7]. The population frequency was 2.5% in 242 controls and slightly higher, 3.5%, in 400 cancer patients. This suggests that there may be a percentage of patients that are subjected to genetic analysis because of the occurrence of toxicity and the analysis is therefore not presymptomatic in all patients. Given the relatively high frequency of this splice-site mutation in the general population, mandatory genetic screening prior to 5-FU treatment may be considered for patients receiving intense 5-FU treatment schedules. Moreover, a simplified cost-benefit analysis has shown that the cost of the genetic screening is advantageous.

We report two cases of 5-FU-associated toxicity, where one patient had the c.1796T>C variant allele which led to a deleterious amino acid change from methionine to threonine. This variant was uncommon, 0.15%. However, it was recognized by the RFLP designed to identify the DPYD IVS14+1G>A and both mutations may therefore be identified by this single test.

In the second case, we identified a splice-site mutation that did not appear to affect the splicing process. This mutation can therefore be regarded as a polymorphism and does not explain the toxicity from which the patient suffered.

A huge number of patients are treated for different tumor types with high or conventional dosages of fluoropyrimidines, in adjuvant or metastatic therapy.

Further advances in predicting and preventing the occurrence of 5-FU-associated toxicities are therefore essential. However, there is as yet no consensus on the DPYD mutations that should be screened for prior to 5-FU treatment. Many DPYD allele variants have been discovered, but there has been a lack of consistency between the genetic variant and the degree of toxicity; many such individuals have toxicity profiles similar to those of the general population [9]. Although screening for the splice-site mutation IVS14+1G>A alone may have incomplete predictive ability in identifying patients at risk of 5-FU toxicity, we believe that testing for this mutation alone is sufficient. Given the relatively frequent presence in the population and the simplicity of the genetic test, many cases of severe or even lethal toxicity may be avoidable.

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