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Short communication

5-Fluorouracil toxicity-attributable IVS14 + 1G > A mutation of the dihydropyrimidine dehydrogenase gene in Polish colorectal cancer patients

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Abstract:
DPYD gene encodes dihydropyrimidine dehydrogenase which is the initial and rate-limiting enzyme in the metabolism of 5-fluorouracil (5-FU). The aim of our study was PCR-RFLP based-genetic testing for the most common 5-FU toxicity-attributable IVS14 + 1G > A *DPYD* mutation (*DPYD*2A*) in 252 Polish colorectal cancer (CRC) patients treated with this adjuvant chemotherapeutic regimen after surgery. The *DPYD*2A* allele was identified only in one patient: a male who was one of 4 CRC patients suffering from grades 3–4 myelotoxicity upon 5-FU chemotherapy. We conclude that IVS14 + 1G > A *DPYD* (*DPYD*2A*) variant occurs in the Polish population and is responsible for a significant proportion of life-threatening toxicity of 5-FU.

Key words:
dihydropyrimidine dehydrogenase, 5-fluorouracil toxicity, gene mutation

Abbreviations: CRC – colorectal cancer, DPD – dihydropyrimidine dehydrogenase, *DPYD*2A* – IVS14 + 1G > A *DPYD* mutation, 5-FU – 5-fluorouracil

Introduction

5-Fluorouracil (5-FU) is one of the most commonly used chemotherapeutic drugs for the treatment of several common human malignancies, including breast cancer and colorectal cancer. 5-FU exerts cytotoxic effects *via* its metabolites. The 5-FU metabolite, 5-5-fluoro-deoxyuridine monophosphate inhibits thymidylate synthase thus impairing DNA synthesis. Additional cytotoxic mechanisms of 5-FU metabolites involve their incorporation into RNA and DNA. The amount of 5-FU available for anabolic reactions and subsequent cytotoxicity is determined by its catabolism [16, 20].

Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5-FU. It has been reported that more than 80% of the administered 5-FU is catabolized by DPD, so the activity of this enzyme may be of paramount importance to predict the efficacy and toxicity of this drug. It has been demonstrated that DPD-deficient cancer patients presented severe toxicity after administration of 5-FU, including diarrhea, neutropenia, and neurotoxicity. Although total DPD deficiency is rare, as many as 3% to 5% of the population may have low enzyme activity and thus may be at increased risk of severe toxicity if treated with 5-FU [13, 16, 20].

To date, more than 30 mutations in the *DPYD* gene encoding dihydropyrimidine dehydrogenase have been identified, some of which result in decreased DPD activity. The most common *DPYD* mutation resulting in severe DPD deficiency is a G to A transition in the GT 5'-splice recognition site of intron 14 (IVS14 + 1G > A). Recent studies have reported that approximately one third of the patients who had experienced severe 5-FU toxicity (WHO grade 3–4) were heterozygous for this exon-skipping mutation [5, 13, 16, 20, 22, 23, 25, 29]. In addition, random screening for the IVS14 + 1G > A *DPYD* variant (*DPYD*2A* allele) in Caucasians showed an allele frequency of approximately 0.75% [1, 5–7, 13, 14, 17, 18, 20, 25, 27].

The aim of our study was to evaluate whether the IVS14 + 1G > A *DPYD* mutation is present in the Polish population.

Materials and Methods

The study was approved by the local ethics committee, and written informed consent was obtained from each patient. The study group consisted of two hundred fifty two consecutive CRC patients (146 men and 106 women, mean age 62 years) treated within the period 1998–2005 in the Department of Oncological Surgery, Pomeranian Medical University, Szczecin, Poland. Chemotherapy toxicity was evaluated according to the common toxicity criteria of the National Cancer Institute version 2.0 [12]. Four of the 252 subjects (1.6%) suffered from grades 3–4 neutropenia upon 5-FU chemotherapy. Genomic DNA was extracted from blood leukocytes using standard techniques and genotyping was performed by means PCR-RFLP method developed by van Kuilenburg et al. [25]. The *DPYD* exon 14 and its flanking 5' donor intronic region was amplified by PCR using the following primer pair: 5'-ATCAGGACATTGTGACATATGTTTC-3' as a sense primer, and 5'-CTTGTTTTAGATGTTAAATCACACATA-3' as an anti-sense primer. The amplification was carried out in a total volume of 20 μl containing: 40 ng of template DNA, 4 pM of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.08% Nonidet P40, 1.5 mM $MgCl_2$, 200 μM each dNTP (dATP, dCTP, dGTP and dTTP), and 0.5 U *Taq* DNA polymerase (MBI Fermentas, Lithuania). The PCR was performed in Mastercycler gradient thermocycler (Eppendorf, Germany) under the following conditions: initial denaturation at 94°C for 5 min, and then 36 cycles: denaturation at 94°C for 20 s, annealing at 54°C for 40 s, and extension at 72°C for 40 s. The final 72°C incubation was extended by 8 min. For RFLP assay, a 15 μl aliquot of PCR product was incubated overnight at 37°C with 3 U endonuclease *NdeI*. The restriction fragments were separated by electrophoresis on 3% agarose gel, stained with ethidium bromide. The wild-type allele (IVS14 + 1G) was identified by cleaving the 198 bp PCR product into 181 bp and 17 bp fragments. In contrast, the mutant allele (IVS14 + 1A) after digestion with *NdeI* produced three fragments: 154 bp, 27 bp and 17 bp. Results were recorded with photographs of gels in UV light. All samples were independently genotyped on blind basis in duplicate (A.B.-K. and W.P.).

Results and Discussion

One of 252 CRC patients was identified as the heterozygote for IVS14 + 1G > A *DPYD* mutation. The mutation was found in the male who was one of four CRC patients suffering from grades 3–4 myelotoxicity upon 5-FU chemotherapy. The remaining 251 subjects were homozygous for the wild-type allele.

For decades, 5-FU has been a staple of therapy for various solid tumors [11]. In 1985 Tuchman et al. and 3 years later Diasio et al. established that dihydropyrimidine dehydrogenase (DPD) is an important enzyme in the catabolism of 5-FU and that a heritable defect in enzyme function could lead to abnormal 5-FU metabolism and severe toxicity [4, 11, 19].

Tab 1. The prevalence of IVS14 + 1G > A *DPYD* mutation in subjects from different countries

Country	Population, (n)	No. of heterozygous individuals	Frequency of the mutation	Frequency of the mutated allele	References
Poland	CRC patients, (252)	1	0.44%	0.22%	This study
Portugal	CRC patients, (73)	1	1.37%	0.68%	[16]
Germany	CRC patients, (267)	3	1.12%	0.56%	[13]
Scotland	CRC patients, (72)	1	1.39%	0.69%	[15]
Scotland	CRC patients, (37)	1	2.70%	1.35%	[3]
Turkey	CRC patients, (200)	3	1.50%	0.75%	[2]
Japan	CRC patients, (104)	0	0.00%	0.00%	[29]
The Netherlands	Healthy adults, (1357)	24	1.77%	0.88%	[25]
Finland	Healthy adults, (90)	2	2.22%	1.11%	[28]
Japan	Healthy adults, (50)	0	0.00%	0.00%	[28]
USA (Afro-Americans)	Healthy adults, (105)	0	0.00%	0.00%	[28]
Taiwan	Healthy adults, (300)	0	0.00%	0.00%	[8]
Turkey	Healthy adults, (250)	0	0.00%	0.00%	[18]
Germany	Healthy adults, (157)	0	0.00%	0.00%	[17]
Egypt	Healthy adults, (247)	0	0.00%	0.00%	[7]
USA (Caucasians)	Healthy adults, (95)	0	0.00%	0.00%	[1]
USA (Afro-Americans)	Healthy adults, (95)	0	0.00%	0.00%	[1]

n – sample size

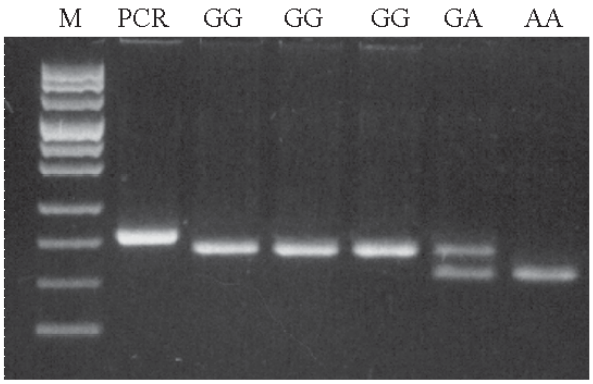

Fig. 1. Restriction fragment length polymorphism (RFLP) analysis of IVS14 + 1G > A mutation in the *DYPD* gene. Lanes: M – molecular weight marker pUC Mix Marker 8 (MBI Fermentas), PCR – undigested PCR product, GG – GG homozygote, GA – GA heterozygote, AA – AA homozygote

The human DPD gene (*DPYD*) is present as a single copy gene on the chromosome 1p22 and consists of 23 exons. A physical map indicates that *DPYD* is at least 950 kilobase (kb) in length with 3 kb of coding sequence and an average intron size of about 43 kb [20, 27]. The vast majority of different *DPYD* mutations and polymorphisms have been detected in patients with a complete DPD deficiency accompanied by a wide variety of clinical presentations [20, 21, 26]. A number of studies have demonstrated the presence of a *DPYD* mutated allele in patients suffering from severe toxicity after the administration of 5-FU [3, 9, 10, 13, 16, 20, 22, 23, 25]. In this group of patients, 11 mutations have been identified, including IVS14 + 1G > A splice-site transition (*DPYD*2A* allele). This mutation results in a 165-bp deletion (corresponding to exon 14) in the *DPYD* mRNA. The resulting protein product is truncated by 55 amino acids, and its catalytic activity is virtually absent [13, 16, 20]. Analysis of the prevalence of the various mutations among cancer patients suffering from severe 5-FU-associated toxicity showed that IVS14 + 1G > A *DPYD* mutation was the most common one [20]. Van Kuilenburg et al. reported that the exon 14-skipping (IVS14 + 1G > A) *DPYD* variant was almost completely confined to the group of patients with a decreased DPD activity, and 44% of these patients were

carriers of this mutation. However, one patients with a low-normal DPD activity proved to be heterozygous for the IVS14 + 1G > A *DPYD* mutation [24].

Several reports have been published on the *DPYD*2A* prevalence in subjects of different ethnic backgrounds (Tab. 1). The sample size in almost all of these reports was too small to yield reliable and consistent data, thus leading to conflicting conclusions [13]. However, some studies including our report suggest that IVS14 + 1G > A *DPYD* mutation is present only in Caucasians [1–3, 8, 13, 15, 16, 25, 28].

To the best of our knowledge, we report, for the first time, occurrence of the exon 14-skipping (IVS14 + 1G > A) *DPYD* mutation in a Polish population (*DPYD*2A* frequency of approximately 0.2%). In addition, we confirm, on the basis of a sample of four colorectal cancer patients who had experienced WHO grade 3–4 myelotoxicity upon 5-FU treatment, that the IVS14 + 1G > A *DPYD* mutation is responsible for a significant proportion of life-threatening toxicity of 5-FU.

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