Cancer Chemother Pharmacol (2016) 78:875–880
DOI 10.1007/s00280-016-3137-0

SHORT COMMUNICATION

Patients homozygous for *DPYD* c.1129-5923C>G/haplotype B3 have partial DPD deficiency and require a dose reduction when treated with fluoropyrimidines

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Received: 26 June 2016 / Accepted: 12 August 2016 / Published online: 20 August 2016

Abstract
Purpose Dihydropyrimidine dehydrogenase (DPD) is a critical determinant of 5-fluorouracil pharmacology, and reduced activity of DPD as a result of deleterious polymorphisms in the gene encoding DPD (*DPYD*) can result in severe treatment-related toxicity. Dosing recommendations to individualize treatment have been provided for three *DPYD* variants (*DPYD**2A, c.2846A>T, and c.1679T>G). A fourth variant, c.1129-5923C>G/HapB3, has been shown to increase the risk of fluoropyrimidine-associated toxicity, but little is known about the functional effects of this variant.
Methods By performing a large retrospective screen for *DPYD* variants, we identified three patients who were homozygous for c.1129-5923C>G/HapB3. We describe their clinical course of treatment and analyzed DPD activity and *DPYD* gene expression, to provide insight into the phenotypic effects of c.1129-5923C>G/HapB3.
Results DPD activity could be measured in two patients and was 4.1 and 5.4 nmol/mg/h (DPD activity 41 and 55 % compared to controls, respectively). The fluoropyrimidine dose had to be reduced during treatment in both patients. In line with partial DPD deficiency in both patients, sequence analysis of DPD cDNA demonstrated a normal-sized (wild type) cDNA fragment of 486 bp, as well as a larger-sized (mutant) 530-bp fragment containing an aberrant 44-bp insertion in intron 10. Patient three tolerated treatment well, but DPD activity measurement was not possible as the patient had deceased at the time of performing the study.
Conclusions The presented functional and clinical data indicate that the c.1129-5923C>G variant is both functionally and clinically relevant, and support an upfront dose reduction of the fluoropyrimidine starting dose in patients carrying c.1129-5923C>G homozygously.

Keywords Fluoropyrimidines · *DPYD* · Dihydropyrimidine dehydrogenase · Toxicity · Capecitabine · 5-Fluorouracil

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Introduction

The fluoropyrimidine anticancer drugs 5-fluorouracil (5-FU), capecitabine, and tegafur are used by approximately two million patients per year worldwide for colorectal, gastric, and breast cancer [1–5]. Of these patients, 10–30 % experience severe, sometimes lethal,

fluoropyrimidine-associated toxicity, creating a substantial clinical problem [6]. A well-known cause of intolerance to fluoropyrimidines is deficiency of the main 5-FU metabolic enzyme, dihydropyrimidine dehydrogenase (DPD), which occurs in 3–8 % of all patients [7].

DPD deficiency results from deleterious polymorphisms in the gene encoding DPD (*DPYD*) in the majority of cases, although other mechanisms (including posttranscriptional regulation, e.g., by microRNAs) might affect DPD activity as well [8]. Three *DPYD* variants are established predictors of fluoropyrimidine-associated toxicity—i.e., *DPYD**2A, c.2846A>T, and c.1679T>G (*DPYD**13) [9]. For heterozygous carriers of these variants, a 50 % dose reduction is recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC), based on the fact that a dysfunctional *DPYD* allele results in ~50 % reduction of DPD enzyme activity and a 1.5–2-fold increase in 5-FU exposure when patients are treated with full-dose fluoropyrimidines [9–11]. Additional *DPYD* variants have previously been associated with fluoropyrimidine-related toxicity, including c.1236G>A [12, 13]. Importantly, it was recently shown that upfront *DPYD* screening and dose reduction in variant allele carriers improved safety and was feasible in routine clinical practice, underscoring the clinical utility of *DPYD* screening [14].

It was recently shown in a meta-analysis that c.1129-5923C>G, an intronic polymorphism occurring in intron 10 of *DPYD*, significantly increases the risk of severe fluoropyrimidine-associated toxicity [15]. However, a dosing recommendation has thus far not been proposed by the CPIC, and the effect of c.1129-5923C>G/HapB3 on DPD enzyme activity in patients is not well described. c.1129-5923C>G is located in intron 10 and occurs in a haplotype termed haplotype B3 (HapB3), with an allele frequency of ~0.02 in Caucasians [16, 17]. c.1129-5923C>G creates a cryptic splice donor site, which leads to insertion of an aberrant 44-bp fragment into mature *DPYD* mRNA, with a premature stop codon as a result [18]. The fact that the phenotypic effects of c.1129-5923C>G/HapB3 on DPD enzyme activity in patients are not well described, hampers formulation of a rational dosing recommendation. Previously, we showed that four patients carrying c.1129-5923C>G/HapB3 were suffering from partial DPD deficiency [18]. However, DPD activity was measured in these patients because they had experienced severe (grade $\geq$ 3) treatment-related toxicity, and the observations might therefore have been biased toward lower values for DPD activity (since the patients were selected for DPD activity measurement based on their toxicity phenotype) [18]. Because little or no other data are available on the phenotypic consequences of c.1129-5923C>G, it remains uncertain to what extent c.1129-5923C>G reduces DPD activity.

The full phenotypic consequences of *DPYD* variants become evident in patients with homozygous genotypes, and this provides an opportunity to study the variant's effect on enzyme activity. However, the frequency of the homozygous genotype of c.1129-5923C>G/HapB3 is very low and is anticipated to be only ~0.04 % based on the low allele frequency of 2 % [17]. Using a large retrospective screen for *DPYD* variants, we identified three patients who were homozygous for c.1129-5923C>G/HapB3 and treated with fluoropyrimidines. We describe the clinical course of treatment of these patients and analyzed DPD activity and *DPYD* gene expression, to provide insight into the phenotypic effects of c.1129-5923C>G.

Patients and methods

The patients were identified as homozygous carriers of c.1129-5923C>G/HapB3 during two pharmacogenetic analyses, which were performed as a secondary endpoint of two clinical studies (NCT00838370 and NCT01359397 [14, 19]). The studies were approved by the Medical Ethics Committees of the Netherlands Cancer Institute and the local study sites. Patients provided written informed consent for the respective studies and for the additional analyses described here.

Toxicity was monitored and recorded during treatment according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTC-AE) v3.0. Genomic DNA for determination of *DPYD* genotypes was collected prior to treatment, and c.1129-5923C>G was genotyped as described previously [18]. The presence of haplotype B3 was confirmed by genotyping of the haplotype B3 tagging variants c.959-51T>G, c.1129-5923C>G, and c.1236G>A [16]. Other known deleterious *DPYD* variants (*DPYD**2A, c.2846A>T, and c.1679T>G) were genotyped according to previously described methods and were not found to be present in the described patients [12]. *DPYD* genotype status was unknown at the time of treatment. In two patients, DPD enzyme activity in peripheral blood mononuclear cells (PMBCs) was determined, as described previously [18]. In order to determine the functional consequences of c.1129-5923C>G on mRNA expression, sequence analysis of cDNA of intron 10 was performed as reported previously [20]. The latter two assays were performed >4 weeks after the last treatment with fluoropyrimidines, to avoid possible interference between 5-FU treatment and DPD activity measurement.

Results

Clinical course of treatment of patients homozygous for c.1129-5923C>G/HapB3

The clinical characteristics of the patients are summarized in Table 1.

Table 1 Characteristics of the patients carrying homozygous variant genotypes for c.1129-5923C>G/HapB3

	Patient 1	Patient 2	Patient 3
Sex	F	M	M
Age	47	67	69
Body surface area (m^2)	1.76	1.86	1.88
Race	Caucasian	Caucasian	Caucasian
Primary tumor	CRC	GEJ	CRC
WHO performance status	0	0	0
Estimated glomerular filtration rate	>80 mL/min	62 mL/min	57 mL/min
Hematology/blood chemistry prior to treatment	Normal	Normal	Leukocytes increased
DPD activity (nmol/mg/h) (% of normal)[a]	4.1 (41 %)	5.4 (55 %)	NA[b]
Treatment	CAP + RT	DOC + B	CAP + RT
Capecitabine dose (mg/m^2 b.i.d.)	825	850	825
Capecitabine schedule	Days 1–33 (continuous)	Days 1–14 (Q3W)	Days 1–26 (continuous)
Toxicity during treatment (maximum grade)	LEU 2 NEU 2 HFS 1 DIA 1 FAT 1	MAL 2 FAT 2 FEV 1 NEU 2	No toxicity
Dose adaption required during treatment	A 40 % dose reduction was required on day 15, after which treatment could be finished	Treatment 1: had to be discontinued on day 11 of cycle 1 Treatment 2: an a priori 36 % reduced dose was not tolerated	No dose reduction was required to finish treatment

CAP + RT capecitabine combined with radiotherapy, *CRC* colorectal cancer, *DIA* diarrhea, *DOC + B* docetaxel, oxaliplatin, and capecitabine plus bevacizumab, *DPD* dihydropyrimidine dehydrogenase, *GEJ* adenocarcinoma of the gastroesophageal junction, *FAT* fatigue, *FEV* fever, *HFS* hand–foot syndrome, *LEU* leukocytes, *MAL* malaise, *NA* not available (not measured), *NEU* neutropenia; *Q3W* every 3 weeks, *WHO* World Health Organization

[a] Normal range 5.9–14.0 nmol/mg/h (median 9.9 nmol/mg/h)

[b] Patient 3 had deceased at the time of this study, and a DPD activity measurement could therefore not be performed

The first patient was a female, aged 47, treated with neoadjuvant chemoradiotherapy for locally advanced rectal cancer ($T_3N_2M_0$). She received capecitabine 825 mg/m^2 b.i.d. (2 × 1500 mg) for 33 days, combined with 25 fractions of radiotherapy (2 Gy each) on weekdays, to a total dose of 50 Gy. On day 9 of treatment she developed leukocytopenia grade 2 (2.3×10^9/L), neutropenia grade 2 (1.3×10^9/L), hand-foot syndrome (grade 1), diarrhea (grade 1), and fatigue (grade 1). These symptoms intensified, until it was decided on day 15 to reduce the dose of capecitabine by 40 % (capecitabine in the weekend was omitted, and on weekdays the evening dose was reduced to 1000 mg). After dose reduction, treatment was well tolerated. On day 23, the dose of capecitabine was increased slightly (by 10 %). Five days later she again developed leukocytopenia (2.5×10^9/L, grade 2) and neutropenia (1.5 x 10^9/L, grade 1). Despite these symptoms, treatment could be finished at reduced dose. The patient subsequently received surgery and is currently disease-free, four years after treatment.

Patient 2 was a male, aged 67, diagnosed with a metastasized adenocarcinoma of the distal esophagus ($T_3N_1M_1$). He received capecitabine, 850 mg/m^2 (days 1–14), combined with docetaxel (50 mg/m^2), oxaliplatin (100 mg/m^2), and bevacizumab (7.5 mg/kg) on day 1 in three-week cycles. On day 7 of treatment, he experienced fatigue (grade 2). He self-reported the symptoms to be 'intolerable, and related to capecitabine use.' On day 11, the patient was hospitalized with fever (38.7 °C; grade 1, without apparent focus) and neutropenia grade 2 (1.3×10^9/L). He was released after a brief period of hospitalization, at which point he refused further treatment with capecitabine.

Four months later, when his disease had further progressed, a second course of treatment with capecitabine was initiated, as monotherapy (for which the standard dose is 1250 mg/m^2). In view of the side effects experienced during the first treatment, the dose of capecitabine was reduced a priori from 1250 to 800 mg/m^2 b.i.d. During the first cycle, the patient again reported fatigue (grade

2), thought to be capecitabine-related. After one cycle the patient decided not to receive any further treatment.

Patient 3 was a male, aged 69, treated for locally recurrent rectal cancer. He received neoadjuvant chemoradiotherapy, with capecitabine 825 mg/m^2 b.i.d. and concomitant radiotherapy on weekdays, in 20 fractions of 1.8 Gy to a total dose of 36 Gy.

The treatment was well tolerated and could be completed without dose reductions or delays. No adverse events were reported during the 4 weeks of treatment, and hematology after treatment was similar to prior to treatment. After neoadjuvant chemoradiotherapy, the patient's tumor was resected. One year after surgery a relapse was diagnosed, and the patient eventually deceased as a result of progressive disease.

Analysis of DPD activity and intron 10 cDNA

As given in Table 1, patients 1 and 2 were both found to have partial DPD deficiency, with 41 and 55 % activity remaining when compared to normal. Patient 3 had deceased at the time of this study, and a DPD activity measurement could therefore not be performed.

In line with the presence of a partial DPD deficiency in the two patients, analysis of the coding sequence of DPD cDNA demonstrated the presence of a normal-sized (wild type) cDNA fragment, of 486 bp, as well as a larger-sized (mutant) 530-bp fragment (Fig. 1). Sequence analysis revealed that the 486-bp fragment was indeed wild type and that the 530-bp fragment contained the aberrant 44-bp insertion, corresponding to nucleotides c.1129-5967_1129-5924 in intron 10 [18].

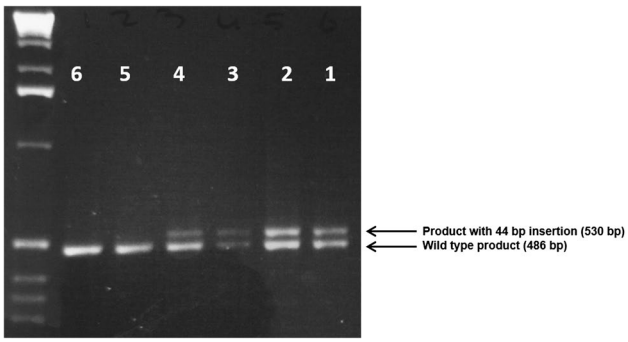

Fig. 1 Fragment analysis of amplified cDNA from intron 10 of *DPYD* in patients with homozygous variant genotypes for c.1129-5923C>G/HapB3. The figure shows the results of the intron 10 cDNA fragment analysis using gel electrophoresis. The 486- and 530-bp fragments correspond to wild-type and mutant cDNA fragments, respectively. *Lanes 1* and *2* contain cDNA of patients 1 and 2, respectively. *Lanes 3* and *4* contain cDNA from two patients reported previously, a patient homozygous for c.1129-5923C>G and a patient heterozygous for c.1129-5923C>G, respectively [18]. *Lanes 5* and *6* contain cDNA of a c.1129-5923C>G wild-type control

Discussion

DPD deficiency as a result of deleterious polymorphisms in *DPYD* is a well-established risk factor for fluoropyrimidine-associated toxicity [9]. The clinical validity of *DPYD* c.1129-5923C>G/HapB3 is only recently being recognized. A meta-analysis summarizing all evidence on the clinical validity of c.1129-5923C>G/HapB3 demonstrated that risk of fluoropyrimidine-associated toxicity was increased 1.6-fold (RR 1.6, 95 %CI 1.29–1.97, $p < 0.0001$) in variant allele carriers [15]. However, little is yet known about the functional effects of this variant, and there is no consensus on the dosing recommendation for patients carrying this variant.

We described three patients homozygous for c.1129-5923C>G/HapB3 who were treated with fluoropyrimidines, and determined the effect of c.1129-5923C>G on DPD enzyme activity in two patients. An approximately 50 % reduction of DPD activity was found in these patients, which was shown to be associated with aberrant mRNA processing, thereby confirming the functional relevance of c.1129-5923C>G, as proposed previously [18]. Given the presence of residual DPD activity in both our patients with homozygous genotypes for whom DPD activity was measured, it is evident that c.1129-5923C>G is not a fully non-functional (i.e., catalytically inactive) variant, such as *DPYD**2A which results in ~0 % DPD activity in homozygous individuals [21, 22]. In fact, the presented data indicate that the magnitude of effect of c.1129-5923C>G on DPD activity may be approximately half that of a fully non-functional variant such as *DPYD**2A, since ~50 % DPD activity remained in the two homozygous individuals. These results are in line with a recent proposal to differentiate between fully non-functional and partially functional *DPYD* variants when reducing the fluoropyrimidine starting dose [23].

In line with the presence of residual DPD activity, the presented clinical data show that the patients homozygous for c.1129-5923C>G/HapB3 were able to tolerate low doses of fluoropyrimidines (note that the starting dose of capecitabine which all three patients received was relatively low compared to the approved dose for monotherapy, i.e., 1250 mg/m^2 b.i.d.). Nevertheless, the administered dose was not tolerated in two out of three patients, since the dose had to be reduced. Since the starting dose of capecitabine was relatively low, it is conceivable that higher doses of capecitabine would have resulted in more pronounced toxicity. In line with this, Amstutz et al. described a patient with a homozygous genotype for c.1129-5923C>G/HapB3 who was treated with full-dose 5-FU plus cisplatin, who experienced fatal toxicity during the first cycle [16].

The available data thus far indicate that full-dose treatment with fluoropyrimidines in patients homozygous for c.1129-5923C>G/HapB3 should be avoided. The degree of dose reduction required to allow safe treatment cannot be determined based on the currently available data, since only a small number of patients with homozygous genotypes of c.1129-5923C>G/HapB3 have been described so far. Considering the presented clinical data and the DPD activity measurements in this study, which showed an approximately 50 % reduction of DPD activity, it seems that a 50 % dose reduction of the fluoropyrimidine dose might be feasible in patients homozygous for c.1129-5923C>G/HapB3. However, additional data are required before a definitive dosing recommendation can be provided. In our view, until more data are available, patients homozygous for c.1129-5923C>G/HapB3 should not be treated with full-dose fluoropyrimidines.

In conclusion, the presented clinical and functional data demonstrate that the c.1129-5923C>G variant is both functionally and clinically relevant. This report confirms the functional relevance of c.1129-5923C>G and adds to the few data available on the effect of c.1129-5923C>G on DPD enzyme activity in patients. The presented data, combined with the functional data reported previously and the available evidence on the clinical validity of c.1129-5923C>G [15, 18], support an upfront dose reduction of the fluoropyrimidine starting dose of approximately 50 % in patients carrying c.1129-5923C>G homozygously, although the exact degree of dose reduction required for patients carrying c.1129-5923C>G should be determined in larger patient populations.

Acknowledgments We gratefully thank the patients for consenting to the performed analyses. We thank Tineke Rienks for her excellent assistance at the Medical Center Leeuwarden.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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