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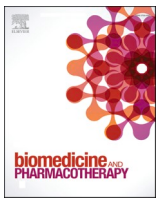

Capecitabine-induced hand-foot syndrome: A pharmacogenetic study beyond DPYD

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ABSTRACT

Aim of the study: Occurrence of hand-foot syndrome (HFS) during capecitabine treatment often results in treatment interruptions (26 %) or treatment discontinuation (17 %), and can severely decrease quality of life. In this study, we investigated whether single nucleotide polymorphisms (SNPs) in genes involved in capecitabine metabolism – other than *DPYD* – are associated with an increased risk for capecitabine-induced HFS.
Methods: Patients treated with capecitabine according to standard of care were enrolled after providing written informed consent for genotyping purposes. Prospectively collected blood samples were used to extract genomic DNA, which was subsequently genotyped for SNPs in *CES1*, *CES2* and *CDA*. SNPs and clinical baseline factors that were univariably associated with HFS with $P \leq 0.10$, were tested in a multivariable model using logistic regression.
Results: Of the 446 patients eligible for analysis, 146 (32.7 %) developed HFS, of whom 77 patients (17.3 %) experienced HFS ≥ grade 2. In the multivariable model, *CES1* 1165–33 C>A (rs2244613, minor allele frequency 19 %) and *CDA* 266 + 242 A>G (rs10916825, minor allele frequency 35 %) variant allele carriers were at higher risk of HFS ≥ grade 2 (OR 1.888; 95 %CI 1.075–3.315; P = 0.027 and OR 1.865; 95 %CI 1.087–3.200; P = 0.024, respectively).
Conclusions: We showed that *CES1* 1165–33 C>A and *CDA* 266 + 242 A>G are significantly associated with HFS grade 2 and grade 3 in patients treated with capecitabine. Prospective studies should assess whether this increased risk can be mitigated in carriers of these SNPs, when pre-emptive genotyping is being followed by dose adjustment or by alternative treatment by a fluoropyrimidine that is not substrate to CES1, such as S1.

1. Introduction

Capecitabine, an oral prodrug of 5-fluorouracil (5-FU), is approved for treatment of solid tumors including colorectal cancer, gastroesophageal cancer, and breast cancer [1]. Most often, it is administered in a 2 weeks on, 1 week off schedule. Hand-foot syndrome (HFS), also known as palmar-plantar erythrodysesthesia, is a major side effect of capecitabine. It has been reported that 53–77 % of patients treated with capecitabine develop HFS, leading to treatment interruption in 26 % of patients and even discontinuation in 17 %.[1–8].

HFS symptoms include palmoplantar numbness, tingling, burning pain and edema which may evolve into desquamation, blistering and ulceration [9]. Symptoms can limit activities of daily living and can seriously impact patients' quality of life [10,11]. In case of HFS grade 2 (skin changes with pain and limiting instrumental ADL) or higher, treatment interruption is recommended in the summary of product characteristics [1,9,12]. Depending on the severity of HFS, dose modifications ranging from dose reductions to permanent discontinuation are advised.[1].

Metabolism of capecitabine into the active agent 5-FU consists of three steps (Fig. 1): carboxylesterase 1 and 2 (CES1/2) convert capecitabine into 5′-DFCR, which is subsequently converted into 5′-DFUR by cytidine deaminase (CDA), and ultimately, 5′DFUR is converted into 5-FU by thymidine phosphorylase (TP). TP is highly expressed in tumor tissue [13]. Over 80 % of 5-FU is catabolized in the liver into inactive metabolites by dihydropyrimidine dehydrogenase (DPD), and around

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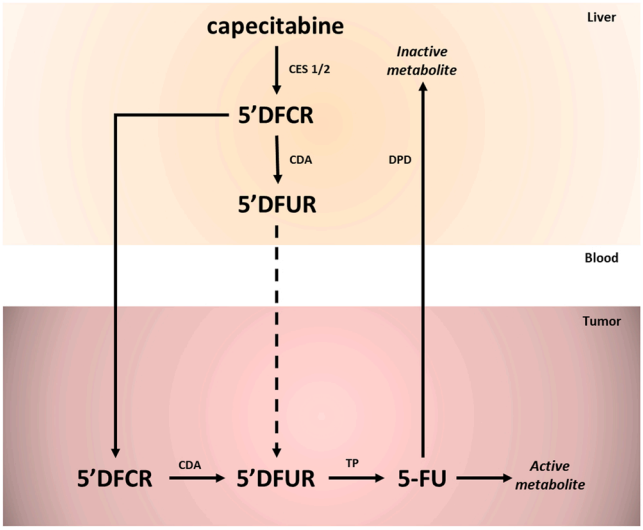

Fig. 1. Capecitabine metabolism Fig. 1 shows the simplistic metabolism of capecitabine. Abbreviations: 5′DFCR: deoxy-5-fluorocytidine; 5′DFUR: 5′-deoxy-5-flurouridine; 5-FU: 5-fluorouracil; CES1/2: carboxylesterase 1 or 2; CDA: cytidine deaminase; TP: thymidine phosphorylase.

20 % is directly excreted through urine [1]. Only 1–5 % of 5-FU is converted into active metabolites which have a cytotoxic effect *via* incorporation into DNA and RNA, and inhibition of thymidylate synthase (TYMS) [14].

Dose modifications based on pre-emptive genotyping of common single nucleotide polymorphisms (SNPs) in *DPYD*, the gene encoding DPD, significantly reduce the incidence of fluoropyrimidine-related toxicity [15,16]. However, it should be noted that the overall incidence of HFS is still high in patients treated with capecitabine, despite upfront *DPYD* testing [2–7]. Potentially, SNPs in other enzymes, involved in capecitabine metabolism, may play a role in the risk on HFS during capecitabine treatment.

In this study, we aimed to assess whether SNPs in *CES1, CES2* and *CDA* can predict the occurrence of HFS, independent of *DPYD* variants, in patients treated with capecitabine.

2. Material and methods

2.1. Study design

In this cohort study, we studied adult patients who were treated with capecitabine according to standard of care at Erasmus MC Cancer Institute between January 2011 and June 2021. Patients were enrolled if they provided written informed consent in the Code-Geno study (local protocol MEC 02–1002) or the M14DPD study (NCT02324452; local protocol MEC 15–358), in which whole-blood samples were collected prospectively for genotyping purposes.

Electronic patient files were retrospectively studied in order to collect the following clinical and demographic data; age, sex, body surface area (BSA), Eastern Cooperative Oncology Group Performance Status (ECOG-PS), tumor type, treatment regimen, dose reductions, treatment interruptions, treatment discontinuation, HFS, diarrhea, nausea, and vomiting during capecitabine treatment according to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE) [12]. Only adverse events (AEs) that were possibly, probably, or definitely related to capecitabine treatment were classified as treatment-related AEs. AE grading was primarily done by the treating physician or – if not registered in the patient file – was assessed by the authors.

2.2. Selection of SNPs

SNPs in genes encoding for the metabolizing enzymes (*i.e.*, CES1, CES2, and CDA) of capecitabine were selected if a previous association with treatment-related AEs was reported in the literature [17–22]. SNPs with a minor allele frequency (MAF) of at least 10% were included.[23].

From the literature, eight potentially relevant SNPs in three genes (*CES1, CES2 and CDA*) encoding enzymes involved in the capecitabine metabolism were selected (Table 1) [17–22]. Patients carrying *DPYD** 2 A, *DPYD** 13, 2846 A>T, 1236 G>A, and/or *DPYD** 7 polymorphisms were excluded from analysis.[16,24].

2.3. DNA isolation and genotyping

DNA was isolated on the MagNaPure Compact Instrument (Roche Diagnostics GmbH, Mannheim, Germany) from 400 μL of the collected whole blood samples using the Nucleic Acid Isolation kit l (Roche Diagnostics GmbH, Mannheim, Germany). Fluorescent-labeled primers and probes were mixed with the TaqMan GTXpress Master Mix (Applied Biosystems, Life Technologies Europe BV, Bleiswijk, the Netherlands) and the obtained 20 ng genomic DNA to perform qPCR. The Taqman qPCR consisted of 40 cycles of denaturation for 20 s on 95 °C, subsequently annealing for 3 s on 92 °C and extension for 30 s on 60 °C. The Taqman 7500 software (Applied Biosystems) was used for allelic discrimination to determine the genotypes by measuring allele-specific fluorescence.

2.4. Statistical analysis

Distribution of SNPs was tested according to Hardy-Weinberg equilibrium (HWE) using the chi-squared test. SNPs in the same gene were tested for linkage disequilibrium (LD) by calculating R^2 using LDlink (https://ldlink.nci.nih.gov/). If $R^2 > 0.8$, only the SNP with the strongest significant association was tested against endpoints.

HFS was categorized in not-limiting activities of daily living (grade 0 or grade 1) and limiting activities of daily living (grade 2 or higher). Genotypes of the SNPs were fitted in the most appropriate of the following models: dominant, recessive or additive. Adverse events were tested against genotypes of the SNPs in the dominant and recessive model and baseline factors (*i.e.*, dichotomized age, sex and ECOG-PS) using the Fisher's exact test or the chi-squared test. For the additive model, logistic regression analysis was used. Genetic polymorphisms and baseline factors associated with a toxicity endpoint with $p < 0.1$ in univariable analysis were entered in multivariable analysis (without backward selection), which was performed using logistic regression analysis. Multivariable associations were internally validated by bootstrapping. One thousand bootstrap samples were generated, with replacement, and the bias-corrected 95% CIs were calculated for ORs. All statistical analyses were performed using SPSS version 28.0.1.0.

3. Results

3.1. Patients

From a total of 573 patients treated with capecitabine, 446 patients were eligible for analysis (Supplementary Fig. S1). The majority of patients was treated with capecitabine in combination with oxaliplatin (n = 170, 38%) or received capecitabine monotherapy with (n = 96, 22%) or without concomitant radiotherapy (n = 80, 18%). Patients were treated with different treatment regimens and different dosing regiments (flat-dosed or dosing based on body surface area)[25]. In total, 217 patients (49%) were treated with flat-dose capecitabine of 3500 mg/day. Patient characteristics are summarized in Table 2.

Table 1
Studied single nucleotide polymorphisms.

Gene	SNP ID	Variant	Assay ID	MAF	No. of WT	No. of HET	No. of HVAR	HWE *P*-value
CES1	rs2244613	1165–33 C>A	C_11290377_10	19%	298	130	18	0.42
CES1	rs2244614	1165–41 C>T	C_16195956_10	58%	82	214	150	0.71
CES1	rs3217164	690 + 129delC	C_34030231_10	51%	111	217	117	0.60
CES2	rs2241409	1613–108 G>A		18%	297	133	15	0.98
CES2	rs11075646	-823 C>G		10%	361	81	4	0.82
CDA	rs2072671	-79A>C * 2	C_25472931_20	35%	197	185	64	0.06
CDA	rs603412	-205 C>G	C___566821_30	42%	155	205	85	0.24
CDA	rs10916825	266 + 242 A>G	C_31573761_10	35%	187	206	53	0.74

Abbreviations: MAF: minor allelic frequencies; WT: wild types; HET: heterozygous variants; HVAR: homozygous variants; HWE: Hardy-Weinberg equilibrium.

Table 2
Patient characteristics.

Characteristics	Total study cohort N = 446 patients	Patients with HFS (all grades) n = 146
Sex (%)	249 (56)	72 (49)
Male	197 (44)	74 (51)
Female		
Age (years, median, [IQR])	62 [54–69]	60 [52–69]
ECOG performance status (%)	302 (68)	1 (1)
1	10 (2)	0
2	1 (<1)	0
3		
BSA (median, [IQR])	1.9 [1.8–2.1]	1.9 [1.7–2.1]
Primary tumor type (%)	295 (66)	95 (65)
Colorectal	80 (18)	18 (12)
Esophagus/Gastric	53 (12)	27 (18)
Breast	8 (2)	2 (1)
Neuro-endocrine[A]	10 (2)	5 (3)
Other[B]		
Metastatic disease (%)	182 (41)	72 (49)
Treatment regimen	80 (18)	40 (27)
Capecitabine monotherapy	96 (22)	20 (14)
Capecitabine + radiotherapy	170 (38)	50 (34)
Capecitabine + oxaliplatin	16 (4)	12 (8)
Capecitabine + bevacizumab	15 (3)	3 (2)
Capecitabine + epirubicin	52 (12)	13 (9)
+ oxaliplatin	7 (2)	2 (1)
Capecitabine + epirubicin	10 (2)	6 (4)
+ cisplatin		
Capecitabine + temozolomide		
Other[C]		
Capecitabine cumulative daily dose	86 (19)	41 (28)
(%)	217 (49)	74 (51)
≥ 4000 mg	143 (32)	31 (21)
3500 mg		
≤ 3000 mg		
Capecitabine adjustment/ discontinuation (%)	126 (28)	59 (40)
Due to adverse events		
Occurrence of hand-foot syndrome	69 (15)	69 (47)
CTCAE grade 1	62 (14)	62 (42)
CTCAE grade 2	15 (3)	15 (10)
CTCAE grade 3		

A Neuro-endocrine tumor: bronchus (n = 5), jejunum (n = 2), pancreas (n = 1), and thymus (n = 1)
B Other tumor types (number of patients in total cohort/number of patients with HFS): appendix (n = 2; n = 2), duodenum (n = 2; n = 1)), goblet cell (n = 1; n = 0), jejunum (n = 2; n = 0), pancreas (n = 1; n = 0), ampulla of Vater (n = 1; n = 1)), and pseudomyxoma peritonei (n = 1; n = 0).
C Other treatment regimen: capecitabine + trastuzumab (n = 4; n = 3), capecitabine + lapatinib (n = 2; n = 1), capecitabine + bevacizumab + paclitaxel (n = 2; n = 1), capecitabine + vinorelbine (n = 1; n = 0), and capecitabine + cisplatin + pembrolizumab (n = 1; n = 0).
Abbreviations: IQR: interquartile range; CTCAE: common terminology criteria for adverse events.

3.2. Hand-foot syndrome

HFS was observed in 146 patients (33 %): grade 1 in 69 patients (16 %), grade 2 in 62 patients (14%) and grade 3 in 15 patients (3%). A complete overview of the occurrence and grades of HFS is shown in Table 2.

3.3. Associations of SNPs with toxicity

Minor allele frequencies of the studied SNPs are provided in Table 1. Higher risk of HFS grade 2 and grade 3 was found in *CES1* 1165–33 C>A (OR 1.9; 95% CI 1.1–3.3; $P = 0.027$) and *CDA* 266 + 242 A>G variant allele carriers (OR 1.9; 95% CI 1.1–3.2; $P = 0.024$), compared with non-carriers of the respective SNPs. However, risk of developing HFS was significantly lower in *CES2* − 823 C>G variant allele carriers (OR 0.4; 95% CI 0.2–0.8; $P = 0.005$). Interestingly, female patients treated with capecitabine were at higher risk of HFS grade 2 and grade 3 (OR 2.1; 95% CI 1.3–3.6; $P = 0.003$), diarrhea (OR 1.5; 95% CI 1.0–2.4; $P = 0.043$), nausea (OR 1.7; 95% CI 1.1–2.5; $P = 0.014$) and vomiting (OR 2.8; 95% CI 1.4–5.4; $P = 0.003$). Haplotype analyses were performed given the strong linkage between *CES1* 1165–33 C>A and *CES1* 1165–41 C>T, but no additional associations in these analyses were found (data not shown).

In multivariable analysis, patients carrying a variant allele of *CES1* 1165–33 C>A were also at significantly higher risk of developing toxicity in all grades (OR 1.6; 95% CI 1.0–2.5; $P = 0.033$) and of developing diarrhea during capecitabine therapy (OR 1.5; 95% CI 1.0–2.4; $P = 0.049$), next to the previously mentioned risk of HFS grade 2 and higher. Carriers of this SNP (either heterozygous or homozygous) more frequently had dose adjustments or treatment discontinuation, but this difference was not significant (61% *vs.* 56%; $P = 0.178$).

All results from multivariable analysis were internally validated by bootstrapping. Results of univariable, multivariable and bootstrap analysis are shown in Table 3.

4. Discussion

In this study, we demonstrated that carriers of *CES1* 1165–33 C>A (rs2244613) and *CDA* 266 + 242 A>G (rs10916825) polymorphisms are at higher risk of developing HFS grade 2 and grade 3 during capecitabine treatment.

CES1 1165–33 C>A has previously been associated with capecitabine-related toxicity, but not with HFS, in 144 Swiss cancer patients.[17] In our study, we validated the presence of the association between this *CES1* SNP with capecitabine-related toxicity and, in contrast to Hamzic et al. [17], we additionally demonstrated *CES1* 1165–33 C>A variant allele carriers are at higher risk of severe HFS during capecitabine treatment.

Carboxylesterase 1, responsible for the conversion of capecitabine to 5-dFCR, is highly expressed in the liver [26]. The reduced CES1 protein function in case of the *CES1* 1165–33 C>A SNP has been illustrated using other CES1 substrates, *e.g.* by measuring significantly lower trough levels of the CES1-formed active metabolite of dabigatran in variant

Table 3
Associations of selected single nucleotide polymorphisms with toxicity.

Endpoint	Factor	Comparison	Univariable OR (95% CI)	*P*	Multivariable OR (95% CI)	Bootstrap 95% CI	*P*
Toxicity[1]	Sex	Female *vs.* Male	1.475 (0.986–2.207)	0.058	1.471 (0.982–2.205)	(0.878–2.250)	0.061
All grades	*CES1* 1165–33 C>A	AA + CA *vs.* CC	1.612 (1.043–2.491)	0.031	**1.608 (1.039–2.488)**	**(1.004–2.643)**	**0.033**
HFS	Sex	Female *vs.* Male	1.479 (0.994–2.201)	0.053	1.385 (0.926–2.073)	(0.922–2.071)	0.113
All grades	*CES2* 823 C>G	GG + CG *vs.* CC	0.412 (0.230–0.739)	0.002	**0.432 (0.240–0.777)**	**(0.214–0.757)**	**0.005**
HFS	Sex	Female *vs.* Male	2.008 (1.220–3.305)	0.006	**2.161 (1.293–3.610)**	**(1.314–3.691)**	**0.003**
≥ grade 2	*CES1* 690 + 129delC	-/- + C/- *vs.* CC	0.576 (0.338–0.980)	0.040	0.758 (0.415–1.384)	(0.424–1.384)	0.367
	CES1 1165–33 C>A	AA + CA *vs.* CC	2.015 (1.222–3.321)	0.005	**1.888 (1.075–3.315)**	**(1.084–3.391)**	**0.027**
	CDA 266 + 242 A>G	GG + AG *vs.* AA	1.747 (1.035–2.951)	0.035	**1.865 (1.087–3.200)**	**(1.099–3.294)**	**0.024**
Diarrhea	Sex	Female *vs.* Male	1.548 (1.018–2.355)	0.040	**1.544 (1.013–2.353)**	**(0.992–2.404)**	**0.043**
All grades	*CES1* 1165–33 C>A	AA + CA *vs.* CC	1.554 (1.008–2.396)	0.045	**1.549 (1.002–2.394)**	**(1.008–2.337)**	**0.049**
Nausea	Sex	Female *vs.* Male	1.702 (1.135–2.553)	0.010	**1.671 (1.109–2.519)**	**(0.125–2.662)**	**0.014**
All grades	Age (years)	≥ 65 *vs.* < 65	0.643 (0.523–0.978)	0.039	0.677 (0.443–1.036)	(0.445–1.041)	0.073
	CES1 690 + 129delC	-/- + C/- *vs.* CC	1.528 (0.936–2.494)	0.089	1.573 (0.958–2.583)	(0.941–2.707)	0.073
Vomiting	Sex	Female *vs.* Male	2.781 (1.421–5.442)	0.002	**2.761 (1.399–5.449)**	**(1.425–6.619)**	**0.003**
All grades	*CDA* -79A>C	CC + AC *vs.* AA	2.112 (1.051–4.242)	0.032	1.812 (0.707–4.643)	(0.795–5.339)	0.216
	CDA − 205 C>G	CG *vs.* GG	2.356 (1.071–5.186)	0.088	1.498 (0.535–4.191)	(0.512–4.807)	0.353
	CES1 690 + 129delC	CC *vs.* GG	1.840 (0.949–3.568)	0.068	0.825 (0.227–2.991)	(0.195 −3.007)	0.120
		-/- *vs.* C/- + CC			1.715 (0.868–3.386)	(0.781 −3.384)	

1 Patients who developed toxicity (*i.e.* hand-foot syndrome, diarrhea, nausea, vomiting, mucositis, neutropenia, hyperbilirubinemia) grade 1 or higher.
Abbreviations: HFS: Hand-foot syndrome.

allele carriers than in wild types [27].

The pathophysiology of HFS is still not known. It is suggested that accumulation of capecitabine and 5-FU metabolites in the skin initiate HFS [28]. This is supported by the fact that higher areas under the curve of 5′-DFCR, 5′-DFUR and 5-FU have been described in patients with HFS [29]. Moreover, Janssen et al. recently showed that the concentration of intracellular FUTP, an active metabolite of 5-FU, was associated with the development and severity of HFS [30]. Our results might add up to this evidence, as slower metabolism of capecitabine prolongs systemic exposure to capecitabine and its metabolites. This might imply that longer exposure to capecitabine and its metabolites, or maybe even more importantly, shorter exposure-free interval until the next treatment cycle, contributes to the development of HFS. Having the relevance of this SNP confirmed, prospective validation of alternative treatment or dosing schedules in carriers is warranted, *e.g.* by starting at a reduced dose, increasing the period off medication between cycles or by choosing an alternative agent that is not metabolized by carboxylesterases, such as S1. Currently, S1 has been reported to be non-inferior to other fluoropyrimidines in colorectal cancer and is recognized as an alternative to 5-FU or capecitabine in case of intolerance to these latter agents.[31] Our results warrant further study whether carriers of *CES1* 1165–33 C>A experience better treatment effects if they are directly treated with S1, rather than after onset of toxicity during treatment with 5-FU or capecitabine.

In contrast, we found the *CES2* − 823 C>G polymorphism (rs11075646) to be associated with a significantly reduced risk of developing HFS. Although this polymorphism has not previously been associated with the incidence of capecitabine-related toxicity, several other *CES2* polymorphisms have been [21,22]. As CES2 is mainly expressed in the gastrointestinal tract, [26] impaired functioning prevents the conversion of capecitabine into its active metabolites, which might lead to intestinal accumulation of capecitabine and reduced systemic availability of its active metabolites. However, replication of these associations is needed to validate the result, preferably combined with additional pharmacokinetic assessments.

In line with previous research, we observed an association between a *CDA* variant and a higher risk of developing capecitabine-related toxicity [20, 21, 32–34]. We found that *CDA* 266 + 242 A>G variant allele carriers are at higher risk than wild types of developing HFS grade 2 and higher. This specific variant allele has previously been associated with overall capecitabine-related toxicity and, particularly, diarrhea grade 2 or higher [17]. This further confirms the potential role for *CDA* polymorphisms in predicting capecitabine-related toxicity. Contrasting prior research on *CDA* − 79A>C carriers, we did not find any association between toxicity and this SNP, whereas previous studies provide conflicting results on the association with adverse events and survival. [18–20, 35] As we performed the largest, but retrospective study, it remains unclear whether the influence of this SNP is valid. Prospective studies could clarify the role of CDA − 79 polymorphism on capecitabine-related adverse events and survival.

Next to the investigated genotypes, we observed that female patients experienced adverse events severely more often. It is known that male and female patients have different pharmacodynamic effects of systemic medical treatment, not in the last place in oncology [36]. Especially for capecitabine, it has been stressed that more research into sex differences is warranted [36–38]. For instance, the subgroup of female patients experienced substantially less benefit from adjuvant capecitabine for biliary tract cancer compared with male patients [39].

Despite the explorative character of this study and the retrospective data collection, the sample size allows us to provide a reliable representation of the occurrence of adverse events, especially HFS during treatment with capecitabine. In addition, the results shown are consistent with previously published studies. It should be noted that we observed grade 3 HFS relatively infrequently (3 %). In this cohort, capecitabine dosing may potentially have been reduced early, to prevent the development of HFS grade 3. As the quality of life in patients developing HFS of all grades is highly affected, early dose reduction or treatment interruptions are the mainstay of HFS management [40]. Preemptive genotyping, which is currently standard of care for *DPYD* in the Netherlands, could be used to identify patients at increased risk for developing adverse events in general, and HFS in particular, during capecitabine treatment.[41] Future studies should assess the best strategy to mitigate adverse events for carriers of *CES1* 1165–33 C>A and *CDA* 266 + 242 A>G, and females. Also, it remains to be studied whether the effects of these intronic SNPs rely on their regulatory effects on gene expression, *e.g. via* alternative splicing, or on their linkage with uncharacterized exonic variants.

Conclusions

We have shown that *CES1* 1165–33 C>A and *CDA* 266 + 242 A>G are potentially important biomarkers for identifying patients at increased risk on HFS grade 2 or higher. Prospective studies should investigate whether preemptive analysis on these SNPs, followed by alternative treatment or adjusted capecitabine dosage, is warranted.

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CRediT authorship contribution statement

Mirjam de With: Conceptualization; Data curation; Investigation; Methodology; Project administration; Visualization; Roles/Writing – original draft; Writing – review & editing. **Leni van Doorn:** Conceptualization; Data curation; Investigation; Methodology; Project administration; Roles/Writing – original draft; Writing – review & editing. **Demi C. Maasland:** Data curation; Formal analysis; Writing – review & editing. **Tessa A.M. Mulder:** Investigation; Methodology; Writing – review & editing. **Esther Oomen-de Hoop:** Conceptualization; Formal analysis; Methodology; Writing – review & editing. **Bianca Mostert:** Writing – review & editing. **Marjolein Y.V. Homs:** Writing – review & editing. **Samira El Bouazzaoui:** Investigation; Methodology; Writing – review & editing. **Ron H.J. Mathijssen:** Conceptualization; Methodology; Supervision; Writing – review & editing. **Ron H.N. van Schaik:** Conceptualization; Methodology; Supervision; Writing – review & editing. **Sander Bins:** Conceptualization; Investigation; Methodology; Supervision; Roles/Writing – original draft; Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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N/A.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114232.

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