Clinical Pharmacokinetics (2023) 62:1589–1597
https://doi.org/10.1007/s40262-023-01279-7

ORIGINAL RESEARCH ARTICLE

Irinotecan-Induced Toxicity: A Pharmacogenetic Study Beyond *UGT1A1*

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Accepted: 22 June 2023 / Published online: 16 September 2023

Abstract

Background and objective Side effects of irinotecan treatment can be dose limiting and may impair quality of life. In this study, we investigated the correlation between single nucleotide polymorphisms (SNPs) in genes encoding enzymes involved in the irinotecan metabolism and transport, outside *UGT1A1*, and irinotecan-related toxicity. We focused on carboxylesterases, which are involved in formation of the active metabolite SN-38 and on drug transporters.

Methods Patients who provided written informed consent at the Erasmus Medical Center Cancer Institute to the Code Geno study (local protocol: MEC02-1002) or the IRI28-study (NTR-6612) were enrolled in the study and were genotyped for 15 SNPs in the genes *CES1*, *CES2, SLCO1B1*, *ABCB1*, *ABCC2*, and *ABCG2*.

Results From 299 evaluable patients, 86 patients (28.8%) developed severe irinotecan-related toxicity. A significantly higher risk of toxicity was seen in *ABCG2* c.421C>A variant allele carriers ($P = 0.030$, OR 1.88, 95% CI 1.06–3.34). Higher age was associated with all grade diarrhea ($P = 0.041$, OR 1.03, 95% CI 1.00–1.06). In addition, *CES1* c.1165-41C>T and *CES1* n.95346T>C variant allele carriers had a lower risk of all-grade thrombocytopenia ($P = 0.024$, OR 0.42, 95% CI 0.20–0.90 and $P = 0.018$, OR 0.23, 95% CI 0.08–0.79, respectively).

Conclusion Our study indicates that *ABCG2* and *CES1* SNPs might be used as predictive markers for irinotecan-induced toxicity.

Key Points

Even after upfront UGT1A1 genotyping, irinotecan treatment is accompanied by severe adverse events in more than 30% of patients.

We show that single nucleotide polymorphisms in ABCG2 and CES1 are associated with adverse events.

These results may be used to further individualize irinotecan treatment schedules.

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1 Introduction

Irinotecan is an antineoplastic agent that remains a cornerstone treatment for gastrointestinal cancers such as advanced colorectal and pancreatic cancer [1, 2]. Despite its frequent use in clinical practice, the side effects of irinotecan can be dose limiting and may impair quality of life [2]. Most common side effects are diarrhea, febrile neutropenia, anemia, and thrombocytopenia, which occur in around a third of the patients treated with monotherapy irinotecan [3–6]. The incidence is even higher if irinotecan is given in combination with other anticancer agents such as 5-fluorouracil, leucovorin, and oxaliplatin [2].

Irinotecan, a topoisomerase I inhibitor, is a prodrug that is hydrolyzed into the active metabolite SN-38 by carboxylesterase (CES) [7]. Due to its complex metabolic pathway (Fig. 1), only 2–5% of irinotecan will eventually be converted into SN-38, mainly in the liver [7, 8]. A small part of SN-38 is converted in the peripheral blood, and subsequently transported into the liver by the organic anion transporting polypeptide 1B1 (OATP1B1) [9]. In the liver and the intestines, SN-38 is predominantly inactivated

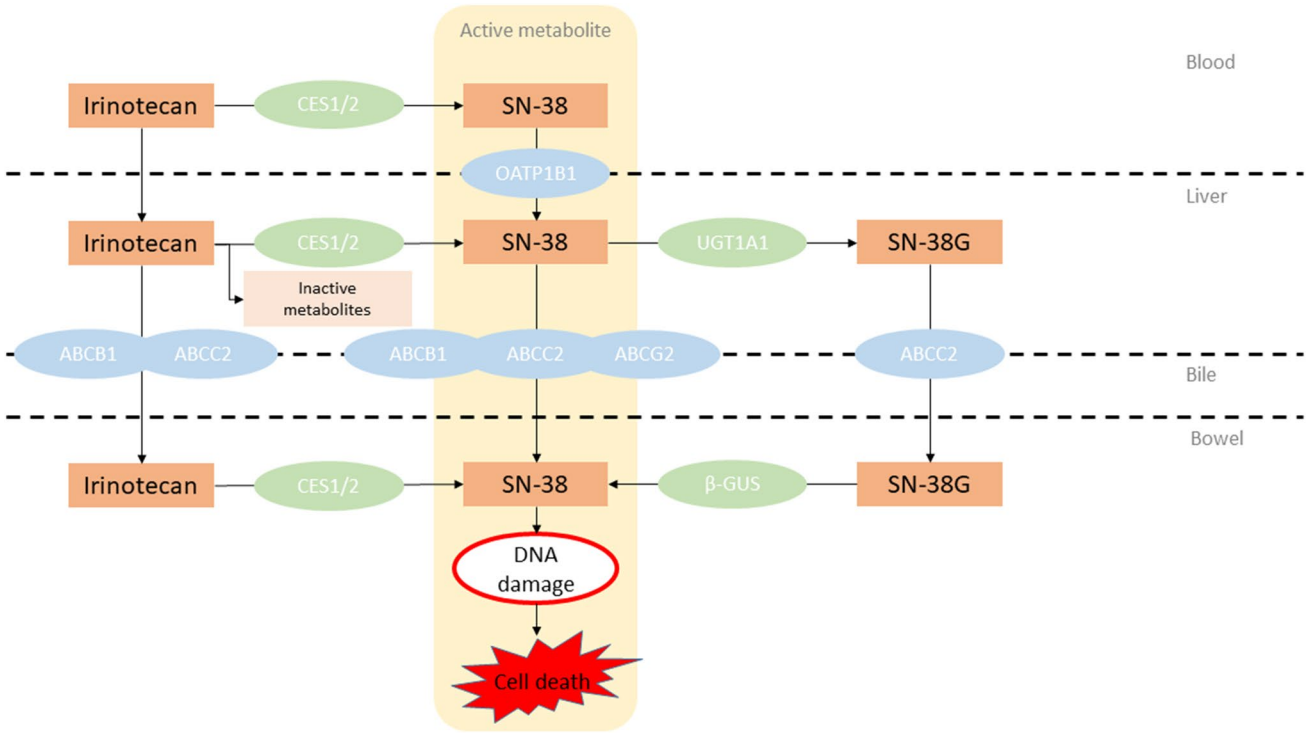

Fig. 1 Irinotecan metabolism. Irinotecan metabolism with the enzymes and membrane transporters of interest. *ABC* ATP-binding cassette, *CES* carboxylesterase, *OATP* organic-anion-transporting polypeptides, *SN-38G* SN-38-glucuronide, *UGT* UDP-glucuronosyltransferase, *ß-GUS* beta-glucuronidase

into SN-38-glucuronide (SN-38-G) by UDP-glucuronosyltransferase 1A1 (UGT1A1), and to a much lesser extent, by cytochrome P450 3A4 (CYP3A4) [10, 11]. Once biliary excreted, SN-38-G can in turn be deconjugated back into SN-38 by bacterial beta-glucuronidase in the intestine [12].

Many studies have provided insights into the risk factors for developing irinotecan-induced toxicity [2]. Much of this research has focused on pharmacogenetic causes of a higher SN-38 exposure [13], predominantly due to reduced UGT1A1 mediated SN-38 metabolism [13]. In particular, the *28 and *93 variants in *UGT1A1* were studied extensively and proven to be clinically relevant in Caucasian patients and potentially suitable for genotype-guided dosing [14–16]. Recently, Hulshof et al. have shown that preemptive genotyping for identification of homozygous carriers of *UGT1A1* *28 and/or *93, followed by a 30% dose reduction, results in significantly lower incidence of febrile neutropenia and irinotecan-related hospital admissions due to adverse events [17].

Furthermore, polymorphisms in membrane transporter genes such as *ABCB1*, *ABCC2*, and *ABCG2* are associated with both irinotecan/SN-38 pharmacokinetics and irinotecan-related adverse events such as diarrhea, febrile neutropenia, anemia, and thrombocytopenia [11, 18, 19], but the results of these findings have not been prospectively validated yet.

Despite all efforts to predict and mitigate toxicity during irinotecan treatment, the incidence of severe adverse events still exceeds 30% in wild type patients for *UGT1A1* polymorphisms [17]. In this study we attempted to further clarify the impact of single nucleotide polymorphisms (SNPs) in genes involved in the irinotecan metabolism or transportation on irinotecan-induced toxicity, predominantly focusing on carboxylesterases, which are involved in SN-38 formation and on drug transporters.

2 Methods

2.1 Study Design and Participants

In this single-center study conducted at Erasmus MC Cancer Institute, we enrolled patients who were treated within this center with irinotecan between July 2001 and June 2021, if they provided written informed consent in the Code Geno study (local protocol: MEC02-1002) or the IRI-28 study (NTR-6612, local protocol: MEC20-007) and if blood samples and clinical data were available. In both studies,

blood samples were collected prospectively for genotyping purposes.

2.2 Single Nucleotide Polymorphism Selection

To identify potentially relevant SNPs in carboxylesterases or transporter genes involved in the metabolism of irinotecan, MEDLINE was searched for *CES1*, *CES2*, *SLCO1B1*, *ABCB1*, *ABCC2*, and *ABCG2*. All SNPs reported to be associated with irinotecan toxicity or pharmacokinetics were included if their estimated minor allele frequency (MAF) was higher than 10% in the Dutch population. To broaden the scope of this research even more, a separate literature search was conducted to identify more carboxylesterase SNPs related to other substrate drugs. Eventually, 15 SNPs in six genes involved in irinotecan metabolism or transport were selected (Table 1) [19–23]. As patients with a genotype homozygous for *UGT1A1* *28 and/or *93 already had an initial 30% dose reduction, which resulted in significantly lower incidence of irinotecan-related toxicity, these patients were excluded from analysis [17].

2.3 Data Collection

The following demographic and clinical data were collected retrospectively; age, sex, body surface area (BSA), Eastern Cooperative Oncology Group Performance Status (ECOG-PS), tumor type, treatment regimen, dose reductions, treatment interruptions, treatment discontinuation, neutropenia, anemia, thrombocytopenia, diarrhea, nausea, and vomiting during irinotecan treatment. Data were collected until 1 month after the last administration of irinotecan. The different side effects were also analyzed as one group and defined as "overall toxicity." Adverse events were graded according to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE) [24]. Only adverse events (AEs) which were possibly, probably, or definitely related to irinotecan treatment were classified as treatment-related AEs. CTCAE grade ≥ 3 was defined as "severe." AE grading was primarily done by the treating physician, or if not registered in the patient file, assessed by the authors (MdW, LvD, EK). Both grade ≥ 1 and grade ≥ 3 adverse events were analyzed.

2.4 DNA Isolation and Genotyping

Ethylenediamine tetraacetic acid (EDTA) whole blood samples were collected for pharmacogenetic analysis. The DNA isolation was performed on the MagCore® SUPER DNA/RNA Extraction Instrument (Artrida, Amersfoort, The Netherlands) with the MagCore® Genomic DNA Whole Blood Kit (for genotyping, type 106). Following DNA isolation, the DNA samples were diluted to a work solution of 10 ng/µL.

Genotyping for the genes *CES1* (rs2244613, rs2244614, rs3217164, rs7187684, rs3785161, rs3815583), *CES2* (rs2241409), *ABCC2* (rs717620, rs3740066), *ABCG2* (rs2231142), *ABCB1* (rs1045642, rs1128503, rs2032582), and *SLCO1B1* (rs4149056, rs2306283) was performed on the TaqMan 7500 software (Applied Biosystems, Life Technologies Europe BV, Bleijswijk, the Netherlands) with

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	c.1165-33C>A	C__11290377_10	80%	17	84	197	0.05
CES1	rs2244614	c.1165-41C>T	C__16195956_10	58%	49	151	97	0.45
CES1	rs3217164	c.690+129delC	C__34030231_10	54%	57	162	80	0.12
CES1	rs7187684	n.95346T>C	C__31071358_20	81%	14	83	202	0.16
CES1	rs3785161	-816A>C	ANPR2J2	23%	174	113	11	0.16
CES1	rs3815583	-75T>G	ANKCKDG	22%	182	100	16	0.64
CES2	rs2241409	1613-108G>A	C___8729601_1	19%	190	86	13	0.42
ABCB1	rs1045642	c.3435C>T	C___7586657_20	52%	73	144	82	0.53
ABCB1	rs1128503	1236C>T	C___7586662_10	43%	97	144	58	0.73
ABCB1	rs2032582	2677G>T	C_11711720C_30	44%	99	138	62	0.28
ABCC2	rs717620	c.-24C>T	C___2814642_10	21%	185	99	14	0.87
ABCC2	rs3740066	3972C>T	C__11214910_20	38%	119	132	48	0.27
ABCG2	rs2231142	c.421C>A	C__15854163_70	14%	218	79	2	0.07
SLCO1B1	rs4149056	c.521T>C; *5	C__30633906_10	42%	93	161	45	0.07
SLCO1B1	rs2306283	c.521T>C; *1B	C___1901697_20	13%	223	73	3	0.26

MAF minor allele frequencies of the study population, *HET* heterozygous variant, *HVAR* homozygous variant, *HWE* Hardy–Weinberg equilibrium, *WT* wild type

a TaqMan GTXpress Mastermix (Applied Biosystems, Life Technologies Europe BV,) in combination with the specific SNP assays (Applied Biosystems, Life Technologies Europe BV). The protocol for the qPCR consists of 40 cycles of 95 °C denaturation lasting 20 s. After the denaturation step, there is an annealing of 92 °C for 3 s and then an extension step of 60 °C for 30 s.

2.5 Statistical Analysis

The distribution of the studied genotypes was tested according to Hardy–Weinberg equilibrium (HWE) using the chi-squared test. SNPs with HWE *P*-value below 0.05 were excluded from analysis. SNPs in the same gene were tested for linkage disequilibrium (LD) by calculating R^2 using LDlink (https://ldlink.nci.nih.gov/). SNPs were considered to be in (partial) LD if $R^2 > 0.8$; in that case, a haplotype of the SNPs was included in HWE analysis. According to the distribution of the genotypes per SNP, the most appropriate genetic model was used; additive, dominant, or recessive.

Adverse events were tested against genotypes, baseline factors (i.e., age, sex, and BSA), irinotecan dosing interval (i.e., every 2 weeks or every 3 weeks) and whether patients were treated with oxaliplatin. Dichotomized data (including the dominant and recessive model) were tested using the Fisher's exact or the chi-squared test. Continuous data were tested using logistic regression analysis. Adverse events associated with genetic polymorphisms and baseline factors with $P < 0.1$ in univariable analysis were entered in multivariable logistic regression analysis (without backward selection). All statistical analyses were performed using SPSS version 28.0.1.0.

3 Results

3.1 Patients

A total of 299 patients were eligible for this analysis. Most patients were treated with irinotecan monotherapy ($n = 168$, 56.2%) or in combination with 5-fluorouracil and oxaliplatin ($n = 115$, 38.5%). Median weekly dose was 225 mg (IQR 110–300 mg) and most patients were treated in a 3-weekly schedule ($n = 172$, 57.5%). Patient characteristics are summarized in Table 2.

3.2 Severe Irinotecan-Related Adverse Events

In total, 86 patients (28.8%) developed severe irinotecan-related adverse events (CTCAE grade ≥ 3) during treatment. Febrile neutropenia was observed in 16 patients (5.4%), severe diarrhea in 44 patients (14.7%), severe anemia in 23 patients (7.7%), and severe thrombocytopenia in 12 patients (4.0%). A complete overview of the irinotecan-related toxicity is presented in Table 2.

3.3 Associations of SNPs with Irinotecan-Related Adverse Events

Table 1 presents MAF of the studied SNPs. Associations of toxicity with the investigated SNPs with $P < 0.1$ in univariable analysis are presented in Table 3. Higher risk of overall severe irinotecan-related toxicity was found in *ABCG2* c.421C>A variant allele carriers ($P = 0.030$, OR 1.88, 95% CI 1.06–3.34) compared with non-carriers of the SNP, also after correction for other potential influencing factors in multivariable analysis. Both carriers of the *ABCB1* 2677TT variant and age were significantly associated with diarrhea in univariable analysis ($P = 0.045$ and $P = 0.014$, respectively). However, in a multivariable model only age remained significant associated ($P = 0.041$, OR 1.03, 95% CI 1.00–1.06). In multivariable analysis, *CES1* c.1165-41C>T variant allele carriers and *CES1* n.95346CC carriers had lower risk on thrombocytopenia, independent of the grade ($P = 0.024$, OR 0.42, 95% CI 0.20–0.90, and $P = 0.018$, OR 0.23, 95% CI 0.08–0.79, respectively).

4 Discussion

In this study, we found that SNPs in *CES1* and *ABCG2* were significantly associated with irinotecan-related toxicity in multivariable analysis: two different SNPs in *CES1* were, independent of each other, associated with all-grade thrombocytopenia, whereas *ABCG2* c.421C>A was associated with grade ≥ 3 overall toxicity. The latter association seems to be explained by significantly more frequent severe anemia and thrombocytopenia in *ABCG2* c.421C>A variant carriers, but these associations could only be tested in univariable analysis due to the relatively low incidence of these adverse events.

The association between this SNP in *ABCG2* and adverse events is supported by in vitro research, where a reduced protein expression and sensitivity to anticancer drugs of the mutant protein was observed [25]. Furthermore, pharmacokinetics of irinotecan and its major metabolites are not significantly different between *ABCG2* c.421C>A variant carriers and wild types [22, 26]. Since *ABCG2* c.421C>A has been repeatedly associated with irinotecan-related toxicity, we hypothesize that the active irinotecan metabolite SN-38 might accumulate intracellularly in organs, causing adverse reactions in carriers of the SNP without affecting systemic pharmacokinetics. Prospective validation of the association with adverse events is needed to evaluate

Table 2 Patient characteristics

Characteristics	$N = 299$ patients
Sex (%)	
Male	176 (58.9)
Female	123 (41.1)
Age (years, median, [IQR])	61 [55–67]
ECOG performance status (%)	
1	208 (76.2)
2	6 (2.2)
3	3 (0.4)
BSA (median, [IQR])	1.92 (1.77–2.06)
Primary tumor type (%)	
Pancreatic	122 (40.8)
Colorectal	111 (37.1)
Gastric	18 (6.0)
ACUP	16 (5.4)
Ovarian	11 (3.7)
Other [A]	21 (7.0)
Irinotecan treatment regimen (%)	
Monotherapy	168 (56.2)
With 5-FU and oxaliplatin	115 (38.5)
With 5-FU	12 (4.0)
With cisplatin	3 (1.0)
With capecitabine	1 (0.3)
Concomitant radiotherapy	2 (0.7)
Irinotecan weekly dose (mg, median, [IQR])	225 (110–300)
Irinotecan dosing interval (%)	
2 weeks	127 (42.5)
3 weeks	172 (57.5)
Irinotecan adjustment due to adverse events (%)	
Dose reduction	79 (26.4)
Discontinuation	4 (1.3)
Of whom already had a reduction during treatment	4 (100)
Febrile neutropenia (%)	16 (5.4)
Diarrhea (%)	
CTCAE grade 1	118 (39.6)
CTCAE grade 2	64 (21.5)
≥ CTCAE grade 3	44 (14.7)
Anemia (%)	
CTCAE grade 1	183 (61.2)
CTCAE grade 2	78 (26.1)
CTCAE grade ≥ 3	23 (7.7)
Thrombocytopenia (%)	
CTCAE grade 1	94 (31.4)
CTCAE grade 2	15 (5.0)
CTCAE grade ≥ 3	12 (4.0)
Nausea (%)	16 (5.4)
Vomiting (%)	17 (5.7)
Irinotecan-related overall toxicity (%)	
CTCAE grade ≥ 3	86 (28.8)

5-FU 5-fluorouracil, *ACUP* adeno carcinoma of unknown primary, *BSA* body surface area, *CTCAE* common terminology criteria for adverse events, *IQR* interquartile range

[A] Cholangiocarcinoma ($n = 8$), non-small cell lung cancer ($n = 5$), duodenum carcinoma ($n = 4$), and sarcoma ($n = 4$)

potential clinical utilization in predicting and mitigating irinotecan-related toxicity.

Our study focused on genetic polymorphisms in carboxylesterase genes, as they are responsible for converting irinotecan into SN-38 [7]. Several associations between CES activity and irinotecan effectiveness were found [27–30], although conflicting studies have been published [31–36]. We recently found that *CES1* polymorphisms are associated with adverse events due to another carboxylesterase substrate, the 5-FU prodrug capecitabine [37]. This was also found by Hamzic et al. and Laizure et al. [20, 23] indicating the potential role of *CES* polymorphisms in the development of irinotecan-related toxicity. In patients treated with irinotecan, however, we found that two *CES1* germline polymorphisms, that is, *CES1* c.1165-41C>T and *CES1* n.95346T>C, had a significant inverse association with "any grade" thrombocytopenia. Interestingly, these SNPs markedly differ from those associated with severe thrombocytopenia (*ABCC2* 3792C>T and *ABCG2* c.421C>A). Although this seems contradictory, low-grade thrombocytopenia might be a sign of cumulative myelosuppression in patients who are treated for a prolonged period, that is, those that tolerate irinotecan well, whereas high-grade thrombocytopenia represents a more acute and severe mechanism of action. The inverse association of *CES1* SNPs with any grade thrombocytopenia might therefore illustrate that these variant carriers form SN-38 at a lower rate than wild type patients for this gene and that they are able to better tolerate irinotecan. Prospective validation of this theory is also warranted, especially in combination with pharmacokinetic research.

In addition to the *ABCG2* SNP, we also investigated other SNPs in genes encoding for other ABC transporters. Irinotecan and SN-38 are both substrates of a spectrum of ABC transporters (Fig. 1), and polymorphisms in their encoding genes could potentially affect the incidence of irinotecan-related toxicity. For *ABCB1* c.3435C>T, for example, an association with toxicity was previously shown [38–42], whereas several other studies could not confirm this association [19, 43–45]. Moreover, this SNP has been associated with lower irinotecan exposure [38, 40], which potentially explains the lack of an association between *ABCB1* c.3435C>T and irinotecan-related toxicity in this study. For *ABCB1* 1236C>T previous studies were also conflicting, reporting either an association with higher exposure and higher incidence of irinotecan-related toxicity [19, 41, 46], with lower risk of toxicity [43], or with no association at all [43, 44].

Furthermore, SNPs in *ABCC2* were significantly associated with severe anemia and thrombocytopenia, but the small number of events for these endpoints stopped us from performing multivariable analyses on these endpoints. Previous studies showed higher irinotecan exposure but a lower risk on grade 3 or 4 neutropenia in *ABCC2*

Table 3 Associations of toxicity with selected single nucleotide polymorphisms

Endpoint	Factor	Comparison	Univariable OR (95% CI)	*P*	Multivariable OR (95% CI)	*P*
Overall toxicity (CTCAE grade ≥ 3)	*CES1* c.1165-33C>A	CC versus CA + AA	0.573 (0.341–0.961)	0.034	0.809 (0.303–2.159)	0.627
	CES1 n.95346T>C	CC versus TC + TT	0.598 (0.355–1.008)	0.053	0.808 (0.303–2.158)	0.671
	ABCB1 1236C>T	TT + CT versus CC	0.642 (0.381–1.084)	0.096	0.780 (0.434–1.403)	0.406
	ABCB1 2677G>T	TT versus GT + GG	0.529 (0.266–1.051)	0.066	0.601 (0.283–1.278)	0.186
	ABCG2 c.421C>A	AA + CA versus CC	1.983 (1.154–3.408)	0.012	**1.883 (1.063–3.335)**	**0.030**
	Treatment schedule	2-weekly versus 3-weekly	2.143 (1.289–3.562)	0.003	0.265 (0.032–2.169)	0.216
Febrile neutropenia	*CES1* n.95346T>C	TC + CC versus TT	1.183 (0.934–1.499)	0.032[A]		
	CES1 -75T>G	GG versus TT + TG	0.246 (0.078–0.776)	0.046[A]		
Diarrhea (All grades)	*CES1* c.1165-41C>T	CT + TT versus CC	1.108 (1.004–1.224)	0.099[A]		0.112
	CES2 1613-108G>A	GA + AA versus GG	1.354 (0.970–1.890)	0.086	0.629 (0.355–1.114)	
	ABCB1 c.3435C>T	CT + TT versus CC	1.135 (0.997–1.292)	0.088	0.631 (0.304–1.311)	0.217
	ABCB1 1236C>T	TT versus CC + CT	1.529 (0.946–2.471)	0.088	0.838 (0.246–2.859)	0.778
	ABCB1 2677G>T	TT versus GG + GT	1.608 (1.021–2.532)	0.045	0.676 (0.202–2.258)	0.525
	Age	*Continuous*	2.818 (2.737–2.901)	0.014	**1.031 (1.001–1.062)**	**0.041**
	Treatment schedule	2-weekly versus 3-weekly	1.675 (0.961–2.919)	0.069	0.600 (0.152–2.363)	0.465
Anemia (All grades)	*ABCC2* 3792C>T	TT vs. CT + CC	0.357 (0.116–1.095)	0.074[A]		
	Treatment schedule	2-weekly versus 3-weekly	11.165 (1.448–86.053)	0.003[A]		
Anemia (CTCAE grade ≥ 3)	*CES1* c.1165-33C>A	CC + CA versus AA	0.236 (0.070–0.793)	0.033[A]		
	ABCB1 1236C>T	TT versus CT + CC	0.175 (0.023–1.323)	0.058[A]		
	ABCC2 3792C>T	TT versus CT + CC	0.826 (0.783–0.872)	0.033[A]		
	ABCG2 c.421C>A	CA + AA versus CC	3.976 (1.668–9.479)	< 0.001		
	Treatment schedule	2-weekly versus 3-weekly	2.746 (1.126–6.693)	0.028[A]		
Thrombocytopenia (All grades)	*CES1* -75T>G	GG versus TG + TT	3.389 (1.147–10.018)	0.033[A]		
	CES1 c.1165-41C>T	CT + TT versus CC	0.374 (0.199–0.701)	0.002	**0.417 (0.195–0.891)**	**0.024**
	CES1 c.1165-33C>A	CC versus CA + AA	0.625 (0.384–1.015)	0.057	2.768 (0.907–8.454)	0.074
	CES1 n.95346T>C	CC versus TC + TT	0.489 (0.299–0.800)	0.004	**0.255 (0.082–0.790)**	**0.018**
	ABCB1 1236C>T	TT versus CT + CC	0.537 (0.289–0.998)	0.047	0.949 (0.276–3.263)	0.934
	ABCB1 2677G>T	TT versus GT + GG	0.575 (0.316–1.045)	0.068	0.495 (0.148–1.661)	0.225
	Sex	Female versus male	1.654 (1.034–2.643)	0.035	1.170 (0.572–2.392)	0.667
	BSA	*Continuous*	0.239 (0.077–0.745)	0.014	0.889 (0.166–4.756)	0.891
	Treatment schedule	2-weekly versus 3-weekly	6.013 (3.621–9.987)	< 0.001	2.411 (0.660–8.809)	0.183
Thrombocytopenia (CTCAE grade ≥ 3)	*CES2* 1613-108G>A	AA versus GA + GG	4.836 (0.944–24.722)	0.096[A]		
	ABCC2 3792C>T	CT + TT versus CC	0.185 (0.050–0.686)	0.008[A]		
	ABCG2 c.421C>A	CA + AA versus CC	3.342 (1.088–10.265)	0.049[A]		
	Treatment schedule	2-weekly versus 3-weekly	8.060 (1.754–37.039)	0.003[A]		
Vomiting (All grades)	*CES1 -75T>G*	TG + GG versus TT	3.430 (0.926–12.708)			

Associations of toxicity with $P \geq 0.1$ with selected single nucleotide polymorphisms

BSA body surface area, *CTCAE* common terminology criteria for adverse events, *OR* odds ratio

[A]Fisher's exact test was used. All other *P*-values for the univariable analyses were calculated using the chi-squared test or logistic regression in case of BSA or age.

c.-24T>C carriers [47], a higher irinotecan and SN-38 exposure and higher risk on irinotecan-related adverse events in *ABCC2* 3972C>T carriers [18, 40, 44, 48], while haplotype analysis of this SNP showed a decreased incidence of diarrhea [49]. The dosing interval of irinotecan was significantly and strongly associated with many of the toxicity endpoints. Although one would expect the lower peak concentrations of irinotecan in a twice-weekly schedule to lead to fewer adverse events than in a thrice-weekly dosing schedule, we found that toxicity was significantly higher with the shorter intervals in univariable analysis. We believe this predominantly reflects the incorporation of oxaliplatin in many of the twice-weekly schedules, as the odds ratios of oxaliplatin-containing regimens versus others were even more extreme than those of the twice-weekly versus thrice-weekly schedules (data not shown). Likewise, fluoropyrimidines might have contributed to the incidence of diarrhea. Another significantly associated clinical parameter was age in the case of diarrhea. Although the median age in this study is relatively low (61 years, IQR 55–67), it is already known that the incidence of severe chemotherapy-related toxicity in elderly patients (65 years or older) is 64% [50]. Geriatric assessments before start with irinotecan treatment can rule out patients not suitable for irinotecan-containing treatment [51], although the value of geriatric treatment in predicting toxicity remains unclear [50]. For severe diarrhea, neither an association with a SNP or baseline factor was found.

A limitation of this study is its retrospective character, which hampered us in studying low-grade adverse events and concomitant medication, querying ambiguities in the older patient files, and adjusting for changes in treatment regimens over time. Our main endpoint, CTCAE grade 3 or higher adverse events, had a lower incidence than we had expected from the literature. The incidence of anemia grade ≥ 3, for example, was too low in variant allele carriers to test the association multivariable. In addition, *ABCG2* c.421C>A carriers have potentially also increased risk on specific adverse events, but the low incidence of specific severe adverse events means that this does not emerge from our analysis. Furthermore, the different treatment regimens might have biased some of the outcomes, but we have countered this by incorporation of the regimens in our multivariable analyses. Additionally, it should be noted that we excluded carriers of *UGT1A1**28 and *93, which allowed us to better study the investigated SNPs, but might have led to different results than in previous studies in an unselected population. The relation between the *UGT1A1* SNPs and the *ABCG2* SNP with toxicity could be investigated in future research.

Overall, pharmacogenetic testing is a low-invasive way to identify patients at high risk for treatment-related toxicity. Previous studies have already shown that patients prone to developing severe adverse events can be identified by pharmacogenetic testing, and that dose reductions in these patients reduce the incidence of treatment-related adverse events in a cost-effective manner [17, 52]. Therefore, it is important to continue searching for novel candidate polymorphisms that could serve as predictive biomarkers, which in general is done in retrospective studies. As for this study, these results need to be validated in larger and preferably prospective studies, as the retrospective and explorative character of these studies and the lack of correction for multiple testing might lead to false positive findings. In general, pharmacogenetic testing could not only improve patient safety during irinotecan treatment, but also reduce treatment-related costs.

5 Conclusion

In this study, we found that *ABCG2* c.421C>A variant allele carriers are at higher risk of severe irinotecan-related overall toxicity. In addition, *CES1* c.1165-41C>T and *CES1* n.95346T>C variant allele carriers had a reduced risk of all-grade thrombocytopenia. Despite the contradictory results from other retrospective cohorts, our study indicates that *ABCG2* and *CES1* SNPs might be used as predictive markers for irinotecan-induced toxicity. Prospective studies addressing both adverse events and pharmacokinetics in patients treated with irinotecan are needed to validate the pharmacogenetic biomarkers found in our study for irinotecan-induced toxicity other than *UGT1A1* *28 and *93.

Acknowledgements We want to thank Prof. H.M.W. Verheul from Erasmus Medical Center Cancer Institute for critically reviewing the manuscript.

Declarations

Conflicts of interest The authors declare no financial or non-financial conflicts of interest.

Funding No funding was received.

Trial registration: Part of the included patients were selected on the basis of their inclusion in the IRI-28 study; trial registration NTR-6612.

Ethics approval of the study: The study was approved by the institutional research ethics committee of the Erasmus Medical Center (local protocol: MEC02-1002 and MEC20-007) and was conducted in accordance with good clinical practice guidelines and the current version of the Declaration of Helsinki.

Author contributions: Conceptualization: MdW, LvD, MM, EO, RvS, RM, and SB. Formal analysis and investigation: MdW, LvD, EK, AvV, MdN, and EO. Writing – original draft preparation: MdW and SB. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Consent to Participate Written informed consent was obtained from every study subject prior to inclusion into the study.

Consent for Publication All subjects signed the informed consent.

Code availability Not applicable.

Availability of Data and Material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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