



Published in final edited form as:

Pharmacogenomics J. 2018 January ; 18(1): 35–42. doi:10.1038/tpj.2016.75.

ABC Transporter Polymorphisms are Associated with Irinotecan Pharmacokinetics and Neutropenia

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Abstract

Neutropenia is a common dose-limiting toxicity associated with irinotecan treatment. While *UGT1A1* variants have been associated with neutropenia, a fraction of neutropenia risk remains unaccounted for. To identify additional genetic markers contributing to variability in irinotecan pharmacokinetics and neutropenia, a regression analysis was performed in 78 irinotecan-treated patients to comprehensively analyze three hepatic efflux transporter genes (*ABCB1*, *ABCC1* and *ABCG2*). rs6498588 (*ABCC1*) and rs12720066 (*ABCB1*) were associated with increased SN-38 exposure, and rs17501331 (*ABCC1*) and rs12720066 were associated with lower absolute neutrophil count nadir. rs6498588 and a variant in high linkage disequilibrium are located in transcriptionally active regions or are predicted to alter transcription factor binding sites. While enhancer activity was not evident *in vitro* for genomic regions containing these SNPs, rs6498588 was significantly associated with *ABCC1* expression in human liver. These results suggest that genetic variation in *ABCC1* and *ABCB1* may contribute to irinotecan-induced neutropenia by altering expression of transporters involved in irinotecan metabolite disposition.

Introduction

Irinotecan is an antineoplastic prodrug approved for first-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the colon or rectum and as a single agent for second-line therapy of fluorouracil refractory metastatic colorectal

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Conflict of Interest

Drs. Innocenti and Ratain are patent holders on the *UGT1A1* testing for irinotecan neutropenia. Dr. Ratain also receives intermittent royalties on multiple patents related to irinotecan pharmacogenetics and is an inventor on a pending patent application for a genomic prescribing system. The remaining authors declare no conflicts of interest.

Supplementary information is available at *The Pharmacogenomics Journal's* website.

cancer. Common dose-limiting toxicities are diarrhea and neutropenia¹, with up to 34% of patients experiencing grade 3–4 neutropenia². Interindividual variation in irinotecan toxicity has prompted the search for genetic biomarkers to guide safer irinotecan treatment.

Irinotecan is activated to form the more potent topoisomerase I inhibiting active metabolite, SN-38³. Elevated SN-38 plasma concentrations have been associated with neutropenia during irinotecan treatment^{4–7}. SN-38 is subsequently inactivated to SN-38 glucuronide (SN-38G) by members of the uridine diphosphate glucuronosyl transferase family, primarily UGT1A1⁸ (Figure 1).

*UGT1A1**28 has been associated with increased risk of neutropenia during irinotecan treatment^{5,9–12}. The TA repeat polymorphism is located in the proximal promoter region of *UGT1A1* and results in reduced expression relative to the *UGT1A1**1 reference allele. Patients homozygous for *UGT1A1**28 glucuronidate SN-38 less efficiently than patients who have one or more reference alleles¹⁰; subsequently, homozygous patients are especially susceptible to toxicities resulting from increased SN-38 systemic exposure (as measured by area under the plasma concentration-time curve (AUC)). *UGT1A1**93 (rs10929302), a promoter polymorphism in high linkage disequilibrium (LD) with *UGT1A1**28, has also been proposed as a predictor of neutropenia, with the variant being strongly associated with increased hematologic toxicity¹³, increased SN-38 exposure, and lower absolute neutrophil count (ANC) nadir^{14–16}.

While *UGT1A1**28-guided irinotecan dosing does reduce dose-limiting toxicities¹⁷, variability in SN-38 AUC within genotype-normalized doses suggests that *UGT1A1**28 does not account for all the toxicity observed during irinotecan therapy. Other studies have demonstrated more modest effects of *UGT1A1* variants and propose that other metabolizing enzymes and drug transporters may be involved^{18–20}.

Irinotecan toxicity and increased SN-38 exposure have been previously associated with several hepatic transporter polymorphisms^{14,21–23}. The ATP-binding cassette (ABC) family includes proteins that transport irinotecan and its metabolites out of the cell²⁴. *ABCB1* variants have been associated with increased exposure to irinotecan and SN-38²¹, reduced irinotecan and SN-38 clearance^{21,25}, and other toxic events^{15,26}. *ABCC1* variants were previously associated with ANC nadir, SN-38 AUC, and the ratio of SN-38G AUC to SN-38 AUC¹⁴, and a common *ABCG2* haplotype was also associated with neutropenia²⁷. However, these associations have yet to be replicated and validated. Compared to *UGT1A1*, hepatic transporters are relatively unexplored yet could play an important role in the distribution and accumulation of irinotecan and its metabolites.

In the current study, a candidate gene and functional analysis were performed to examine associations of *ABCB1*, *ABCC1*, and *ABCG2* polymorphisms with irinotecan pharmacokinetics and neutropenia. An earlier pharmacogenetic analysis in this cohort included only a small number of polymorphisms in these three genes¹⁴. The present study covers the entire length and flanking regions of these genes for a more comprehensive analysis and tests the hypothesis that ABC transporter variants can contribute to additional variability in neutropenia observed during irinotecan treatment.

Subjects and Methods

Subjects

Human investigations were performed after review and approval by the Biological Sciences Division/University of Chicago Hospitals Institutional Review Board and in accordance with Federalwide Assurance for the protection of human subjects. Informed consent was obtained from all subjects. The study cohort consisted of 85 advanced cancer patients treated with single-agent irinotecan (300 mg/m² or 350 mg/m²) every 3 weeks. Pharmacokinetic and clinical data, including race, sex, age, body surface area (BSA), SN-38 AUC, ANC nadir, and baseline ANC, were collected. This patient population was examined in previous studies^{5,14}, and thus the sample size was not based on *a priori* calculation.

Genotyping

Tag single nucleotide polymorphisms (SNP) were selected from genomic regions covering 25 kb upstream of the transcription start site to 5 kb downstream of the 3'-UTR of each candidate gene (*ABCB1*, *ABCC1*, *ABCG2*). Additional SNPs were chosen to directly interrogate nonsynonymous SNPs in the UCSF Pharmacogenetics of Membrane Transporters database (<http://pharmacogenetics.ucsf.edu/>), SNPs with published clinical associations, and SNPs in LD with clinically associated SNPs. Fifty *ABCB1* SNPs, 93 *ABCC1* SNPs, and 38 *ABCG2* tag SNPs were assayed. Genotyping was performed by the Mayo Clinic Genotyping Shared Resource using the Illumina VeraCode (BeadXpress) platform. Following quality control measures to eliminate suboptimal samples and markers and filtering for minor allele frequency < 0.05 using PLINK v1.07²⁸, 85 individuals and 123 SNPs were retained for analysis (Supplementary Table 1).

Statistical analysis

SNPs were tested for deviation from Hardy-Weinberg equilibrium using an exact test. Genotype-phenotype associations were analyzed using a two-stage regression analysis in R v3.2.2²⁹. Dose-adjusted SN-38 AUC and ANC nadir values were log₁₀-transformed for the analysis. In the univariate analysis, simple regressions were first performed on each variant and clinical covariate separately, and those with a significance level of P < 0.15 were kept for further analysis. Step-wise backward selection of this subset of genotypes using the rms package³⁰ for R led to the final multivariable models, and model fit was assessed by examining R² values. An additive genetic model was first assumed for all genotype associations. To eliminate the driving effect of any sparse homozygous variants, when a particular variant had n = 3 observations in the homozygous genotype group and was significant, these genotypes were grouped with the heterozygous genotypes; the variant was kept in the model if still significant after combining genotypes.

Other statistical analyses were performed using GraphPad Prism v6.0 (GraphPad Software, La Jolla, CA, USA). Comparisons for the risk alleles analysis and luciferase reporter assays were analyzed using a Mann-Whitney test or, if there were more than two groups, a Kruskal-Wallis test followed by Dunn's multiple comparisons test. Variance was assumed to be similar between compared groups, and two-sided P-values < 0.05 were considered statistically significant.

Bioinformatic analysis

Significant SNPs and SNPs in high LD ($r^2 \geq 0.8$) were examined for evidence of changes in function and regulatory activity. HaploReg³¹ and RegulomeDB³² were used to determine whether the SNPs overlap with experimentally predicted functional elements (chromatin state segmentation³³, DNase hypersensitivity peaks, ChIP-seq peaks) from the ENCODE Project³⁴ and the Roadmap Epigenomics Project³⁵. HaploReg³¹ and TRANSFAC Match³⁶ were used to identify and predict changes in transcription factor (TF) binding.

Measurement of enhancer activity in vitro

Primers were designed with the aid of Primer3^{37,38} to target 500 bp genomic regions flanking each significant SNP (Supplementary Table 2). These regions were PCR-amplified using PfuTurbo (Agilent, Santa Clara, CA, USA) from human genomic DNA and cloned into the pGL4.23[*Luc2*/minP] luciferase reporter vector (Promega, Madison, WI, USA). The QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent) was used to create the desired point mutation. Correct sequences were verified by Sanger sequencing. Reporter plasmids containing reference or variant sequences were transfected into Hep G2 (ATCC[®] HB-8065[™], human hepatocellular carcinoma, passage 2–6) cells using Lipofectamine LTX and PLUS reagents (Invitrogen, Carlsbad, CA, USA). The *Renilla* luciferase construct pGL4.73 (Promega) was co-transfected to correct for transfection efficiency. Empty pGL4.23 vector and a known apolipoprotein E liver enhancer³⁹ were also transfected as negative and positive controls, respectively. Cells were cultured in Eagle's Minimum Essential Medium with EBSS, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, 1500 mg/L sodium bicarbonate, and 10% fetal bovine serum. Twenty-four hours after transfection, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) and quantified using the GloMax-96 Microplate Luminometer (Promega). Background-subtracted firefly luciferase activity was normalized with respect to *Renilla* luciferase activity and expressed as a mean value relative to empty pGL4.23 vector.

Code availability

The R code for the univariate and multivariate regression analyses is available at <https://github.com/kroetzlab/irinotecan-ABC>.

Results

Cohort analysis

The original study cohort consisted of 85 patients, with the majority being Caucasian or African American. Seven subjects (2 Asian, 1 Filipino, 4 Hispanic) were omitted from the initial regression analyses to avoid dilution of association signals by genetic heterogeneity. Characteristics of patients included in the primary analysis are listed in Supplementary Table 3. The analyzed cohort had a median age of 57 and slightly more males (58%). The majority (76%) received a 350 mg/m² dose of irinotecan, while 24% received a 300 mg/m² dose. The outcomes examined in this study were SN-38 AUC (adjusted for irinotecan dose) and ANC nadir. Distributions of these phenotypes in the analyzed cohort are shown in Supplementary

Figure 1. None of the clinical covariates (race, sex, age, BSA, baseline ANC) were significantly associated with SN-38 AUC or ANC nadir.

Association of ABC SNPs with SN-38 exposure

Eight SNPs met the $P < 0.15$ threshold in univariate regression analyses for SN-38 exposure. Of these, rs12720066, rs6498588, rs35621, and rs2373586 had P -values < 0.05 (Table 1). The genotype frequencies for these SNPs in Caucasians and African Americans are shown in Supplementary Table 4. All eight ABC SNPs were taken into the multivariate analysis along with the *UGT1A1**93 genotype (rs10929302). The final log SN-38 AUC model included *UGT1A1**93, an intronic *ABCB1* variant (rs12720066), and an *ABCC1* variant (rs6498588) in the 5'-flanking region (Table 1). The model explained 34% ($R^2 = 0.345$) of the variation in SN-38 exposure, with all three SNPs contributing similar variances to the model (rs12720066: $\beta = -0.337$; rs6498588: $\beta = 0.325$; rs10929302: $\beta = 0.390$). The *UGT1A1* and *ABCC1* SNPs were associated with increased exposure of patients to SN-38, while the *ABCB1* SNP was associated with decreased exposure (Figure 2).

Inclusion of only Caucasian genotypes in the association analyses yielded similar results as obtained with the combined population (Supplementary Table 5). The multivariable model for log SN-38 AUC also contained *UGT1A1**93, rs12720066, and rs6498588, with similar effect sizes as those for the model including African Americans. Inclusion of the seven Asian, Filipino, and Hispanic subjects into the final model (Supplementary Table 6) resulted in similar coefficients and P -values.

Association of ABC SNPs with neutropenia

In univariate linear regression analyses of ABC SNPs with ANC nadir, ten SNPs met the $P < 0.15$ threshold and three (rs17501331, rs12720066, and rs3743527) had P -values < 0.05 (Table 2). Genotype frequencies of these SNPs are listed in Supplementary Table 4. The final model following multivariable regression and inclusion of *UGT1A1**93 included an intronic SNP in *ABCB1* (rs12720066) and an intronic SNP in *ABCC1* (rs17501331) (Table 2). This model accounted for 39% ($R^2 = 0.390$) of the variation in ANC nadir, with *UGT1A1**93 explaining more variance in the outcome ($\beta = -0.479$) than the other two SNPs (rs17501331: $\beta = -0.295$; rs12720066: $\beta = 0.286$). The *ABCB1* SNP was associated with increased ANC nadir and the *UGT1A1* and *ABCC1* SNPs were associated with reduced nadirs (Figure 3). As for the SN-38 AUC analysis, the final multivariable model for log ANC nadir for Caucasians only contained the same SNPs with similar effect sizes as those for the model including African Americans (Supplementary Table 7). Inclusion of the seven Asian, Filipino, and Hispanic subjects into the final model (Supplementary Table 8) also resulted in similar coefficients and P -values.

Contribution of multiple risk alleles

To examine the combined effects of risk alleles, composite genotypes were constructed for each outcome. Patients carrying more risk alleles for rs12720066, rs6498588, and *UGT1A1**93 had significantly higher SN-38 AUC ($P < 0.0001$), while patients carrying more risk alleles for rs12720066, rs17501331, and *UGT1A1**93 had significantly lower ANC values ($P = 0.0006$) (Supplementary Figure 2). Almost all (91%) patients with grade

3–4 neutropenia (< 1000 cells/ μ l) carried at least four risk alleles among all three ABC SNPs and *UGT1A1**93, compared to 53% of patients with no or less severe neutropenia ($P = 0.001$). Of the patients with grade 3–4 neutropenia, 91% are homozygous for rs12720066 and 46% are homozygous for *UGT1A1**93, while of patients with no or less severe neutropenia, 73% are homozygous for rs12720066 and 7% are homozygous for *UGT1A1**93. Dose-adjusted SN-38 AUC was also confirmed to be higher in patients with grade 3–4 neutropenia (Supplementary Figure 3; $P = 0.0001$).

Bioinformatic analysis

To investigate the putative function of the SNPs in the final models, overlapping regulatory elements and protein binding sites were examined. The intronic *ABCC1* SNP rs17501331 is in a predicted region of transcriptional transition in Hep G2 cells and is predicted to alter two regulatory motifs, DMRT1 and SOX6. rs6498588, located 11 kb upstream of *ABCC1*, is predicted to alter three regulatory motifs (GR, Mef2, YY1). rs4148330, a SNP in high LD ($r^2 = 0.8$) with rs6498588, resides 1.7 kb upstream of *ABCC1* in a region with H3K4 methylation in Hep G2 cells and DNase hypersensitivity in Hep G2 cells and hepatocytes, and is predicted to alter E2F and NRSF TF binding. rs12720066 and other SNPs in high LD with rs6498588 were also predicted to alter regulatory motifs but were not associated with an enrichment in enhancer or DNase activity in liver or liver-derived cells.

Enhancer assays

Genomic regions containing the *ABCB1* and *ABCC1* SNPs in the final models for SN-38 AUC and ANC nadir plus additional SNPs in high LD with these variants (Supplementary Table 2) were tested *in vitro* for enhancer activity and the effect of genetic variation on this activity. None of the tested genomic regions exhibited significant enhancer activity and the introduction of the indicated variants did not change the transcriptional activity (Figure 4).

Discussion

This study provides a comprehensive analysis of the genetic variants spanning three hepatic ABC transporter genes of the irinotecan pharmacokinetic pathway. Three SNPs (rs12720066, rs6498588, rs17501331) were significantly associated with changes in SN-38 AUC or ANC nadir. These variants could potentially predict neutropenia during irinotecan treatment and provide additional insight into the molecular mechanism of the toxicity.

*UGT1A1**93 (rs10929302) was confirmed as a strong predictor of irinotecan-induced neutropenia, reflected by associations with higher SN-38 AUC and lower ANC nadirs. The effect sizes of *UGT1A1**93 for associations with SN-38 AUC or ANC nadir were similar or slightly more significant than those of *UGT1A1**28. *UGT1A1**93 was previously associated with increased hematologic toxicity, increased SN-38 exposure, and lower ANC nadir^{13–15}. A recent study confirmed *UGT1A1**93 as a more robust marker for neutropenia than *UGT1A1**28¹⁶.

The three ABC transporter SNPs in the final multivariable models are in noncoding regions, either intronic or upstream of their respective genes, and are not in high linkage disequilibrium with any coding SNPs. This was expected, considering the low number of

high-frequency coding SNPs in these genes. Previous studies of the effects of nonsynonymous SNPs on the expression and activity of these ABC transporters have yielded mostly negative or inconclusive results^{40–42}. Of the SNPs with $P < 0.15$ in the univariate analyses, only one (rs1128503) is in a coding region and one intronic SNP (rs2373586) is in high LD with a common missense SNP, rs2032582. rs1128503 and rs2032582 are both in *ABCB1* and, together with rs1045642, make up the *ABCB1**2 haplotype. This haplotype has been previously associated with early toxicity during irinotecan treatment²⁶. However, these SNPs dropped out of significance during the selection of the multivariable models. These coding SNPs have been frequently studied together and individually for association with transporter activity and changes in exposure and response to numerous drugs, but with discordant results⁴⁰.

In contrast to coding SNPs, due to lower selective pressures and more modest effects, variants in the regulatory regions of membrane transporter genes are more abundant and exist at higher allele frequencies^{43,44}. Functional studies of variants in the proximal promoter^{44–47}, untranslated^{48,49} and intronic^{50,51} regions of ABC and SLCO transporter genes have demonstrated changes in mRNA or protein expression levels and interindividual variation in pharmacokinetics^{52,53}. In light of this, it is likely that noncoding genetic variation has a more widespread impact on transporter function. Our results highlight the importance of including noncoding SNPs in pharmacogenetic studies and the need to further characterize regulatory regions in pharmacogenes.

The significant variants identified in this study may contribute to irinotecan-induced neutropenia by altering expression of *ABCB1* or *ABCC1*. *ABCB1* encodes P-glycoprotein, which is involved in irinotecan and SN-38 biliary excretion^{54,55}. An increase in *ABCB1* expression could result in increased biliary secretion of SN-38 and a corresponding reduction in plasma SN-38 levels, which are inversely correlated with ANC nadir. *ABCC1* encodes MRP1, a hepatic basolateral efflux transporter of SN-38⁵⁶. Increased *ABCC1* expression is hypothesized to result in increased systemic levels of SN-38, which is consistent with the decreased ANC nadir associated with this variant. It should be noted that *ABCC1*/MRP1 expression in the liver is relatively low⁵⁷, so increased expression may have a significant impact on basolateral efflux of SN-38 back into the blood.

Bioinformatic analyses predicted 5′-flanking *ABCC1* SNP rs6498588 to alter TF binding and rs4148330 (in LD with rs6498588) to have both enhancer activity in hepatic cells and effects on TF binding. A preliminary eQTL meta-analysis of human liver samples found rs6498588 to be significantly associated (meta-adjusted $P = 0.05$) with increased *ABCC1* expression⁵⁸. These data are consistent with the association of rs6498588 with increased SN-38 AUC, as higher *ABCC1* expression would result in increased transport of SN-38 into the blood. However, our *in vitro* enhancer assays did not confirm these predictions for either rs6498588 or rs4148330. Enhancer assays conducted in additional hepatic cell lines or with different-sized genomic regions are warranted.

A combined allele analysis showed that a higher number of risk alleles from our models is correlated with irinotecan-induced toxicity. A significantly greater proportion of patients with grade 3–4 neutropenia carried at least four risk alleles for both phenotypes when

compared to the group with no or less severe neutropenia. rs12720066 and *UGT1A1**93 appear to be driving this effect, with the directions of their effects consistent with the toxicity in both pharmacokinetic and pharmacodynamic outcomes. While rs6498588 was only in the final SN-38 AUC model, a SNP in high LD (rs8050881) had a weaker univariate association with ANC nadir; the variant alleles of these SNPs are also associated with the toxicity in both outcomes. However, the contribution of rs17501331 is less clear; this variant was only associated with ANC nadir and not at all with SN-38 AUC, suggesting a mechanism that may affect neutrophil count independent of SN-38 exposure. Given the complexity of the many metabolizing enzymes and transporters acting on SN-38, further eQTL and functional characterization studies are needed to validate the effect of these SNPs before proposing a combined PK/PD model.

The addition of rs6498588 to our previously published model¹⁴ of log SN-38 AUC containing rs35605 (*ABCC1*), rs10276036 (*ABCB1*) and *UGT1A1**93 improved the percent variation of SN-38 AUC explained by genetic determinants (adjusted R² increased from 0.221 to 0.284, P = 0.009, ANOVA), with all four variants in the model remaining significant. When rs12720066 and rs17501331 were added to our previous model of log ANC nadir¹⁴ containing rs3765129 (*ABCC1*), rs2306283 (*SLCO1B1*), and *UGT1A1**93, only rs17501331 had marginal significance (P = 0.05) and a smaller effect than the other variants in the initial model. The previously identified SNPs, especially *UGT1A1**93, may explain more of the genetic variation in ANC nadir. Again, a better mechanistic understanding of this toxicity is needed before proposing a predictive multivariable model for irinotecan-induced neutropenia.

There are no known clinical associations of the variants identified in the current association studies. However, there is still enough compelling functional evidence to suggest that genetic variants in *ABCB1* and *ABCC1* are relevant to irinotecan toxicity. MRP1-overexpressing KB-3-1 cells showed increased SN-38 resistance compared to normal cells⁵⁶, affirming that expression of the transporter does affect SN-38 transport. In *ABCB1* knockout mice, irinotecan and SN-38 plasma levels were increased relative to the wildtype mice, consistent with a role for P-gp in biliary secretion of this drug/metabolite pair⁵⁵. Cell lines overexpressing BCRP (encoded by *ABCG2*) are also resistant to irinotecan⁵⁹ and SN-38⁶⁰, and *ABCG2* 421C>A reduced gene expression and conferred irinotecan resistance in cancer cell lines⁶¹. In the current study, there was no association with any of the tested *ABCG2* variants and SN-38 exposure or ANC nadir.

Other genes in the irinotecan pharmacokinetic pathway including *ABCC2*, *SLCO1B1*, *CES1*, *CES2*, *CYP3A4* and additional variants in *UGT1A1* and other UGT genes may also contribute to SN-38 exposure and neutropenia and warrant further study⁶²⁻⁶⁴. In contrast, genetic variation in genes responsible for irinotecan pharmacodynamics do not seem to play a role⁶⁵. More comprehensive genotyping of pharmacokinetic genes has the potential to identify additional genetic variants more strongly associated with irinotecan exposure and toxicity. Discovery of additional genetic and non-genetic determinants of irinotecan-induced neutropenia will improve the predictive power of a clinical pharmacogenetic algorithm over using a single *UGT1A1**28 test for drug selection and dosing. Replication and prospective

studies in large independent cohorts as well as in other ethnic groups are necessary to validate and expand these findings.

A current gap in the field of pharmacogenetics is understanding the clinical value of variants. Our study used a pharmacokinetic/pharmacodynamic phenotyping approach to discover new variants associated with neutropenia during irinotecan treatment. Further investigation of these variants and others in ABC transporter genes may not only potentially contribute to the improvement of individualized treatment with irinotecan, but may also have broader implications for other substrates of ABC transporters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH/NIGMS U01GM061390 (Pharmacogenetics of Membrane Transporters), NIH/NIGMS U01GM0061393 (PAAR-Pharmacogenomics of Anticancer Agents Research Group), NIH/NIGMS T32 GM007175, NIH/NIDDK R21DK081157, and NIH/NCI K07CA140390-01.

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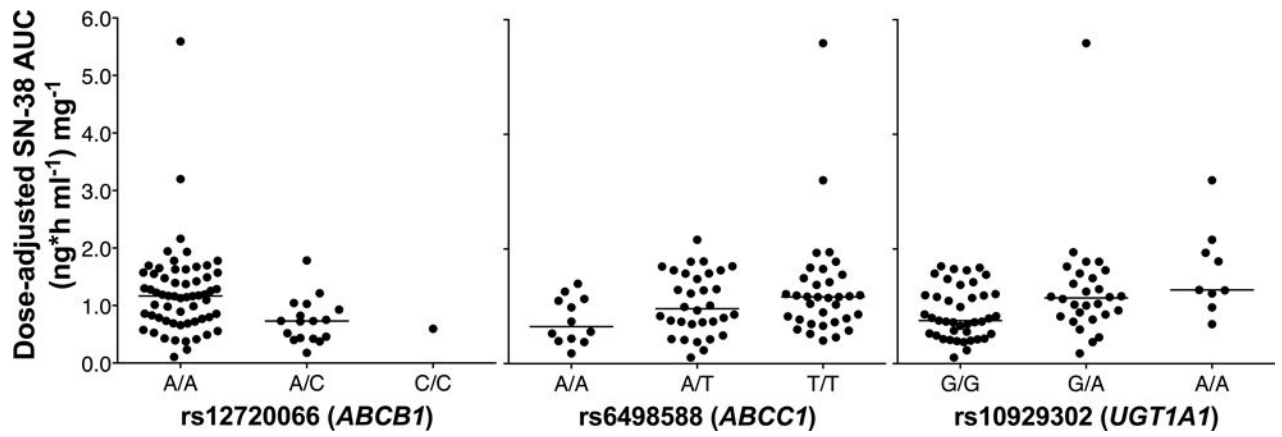


Figure 2. Association of ABC and UGT variants with SN-38 AUC

The relationship between genotype and SN-38 AUC (adjusted for irinotecan dose) is shown for all analyzed patients. Horizontal bars represent the median values for each genotype group.

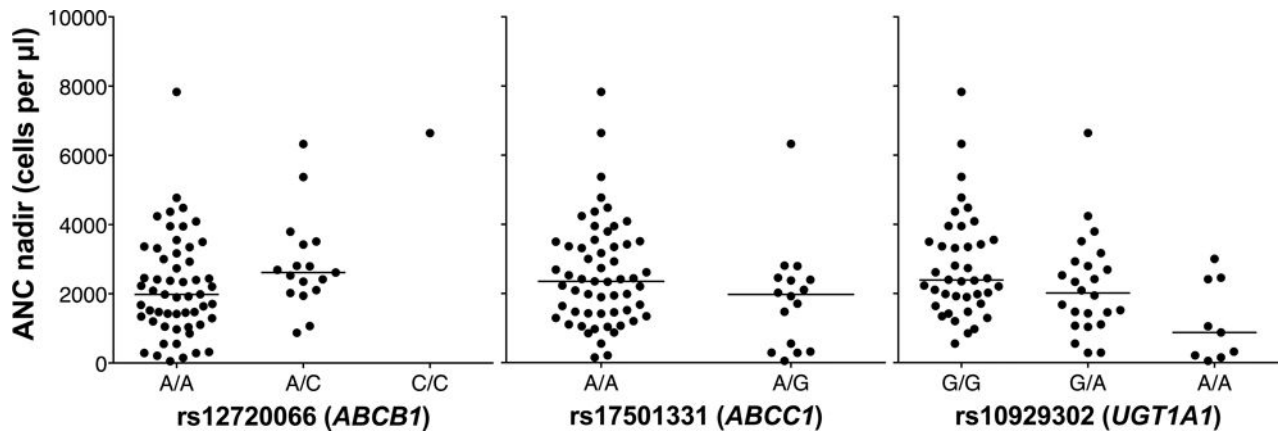


Figure 3. Association of ABC and UGT variants with ANC nadir

The relationship between genotype and absolute neutrophil count nadir is shown for all analyzed patients. Horizontal bars represent the median values for each genotype group.

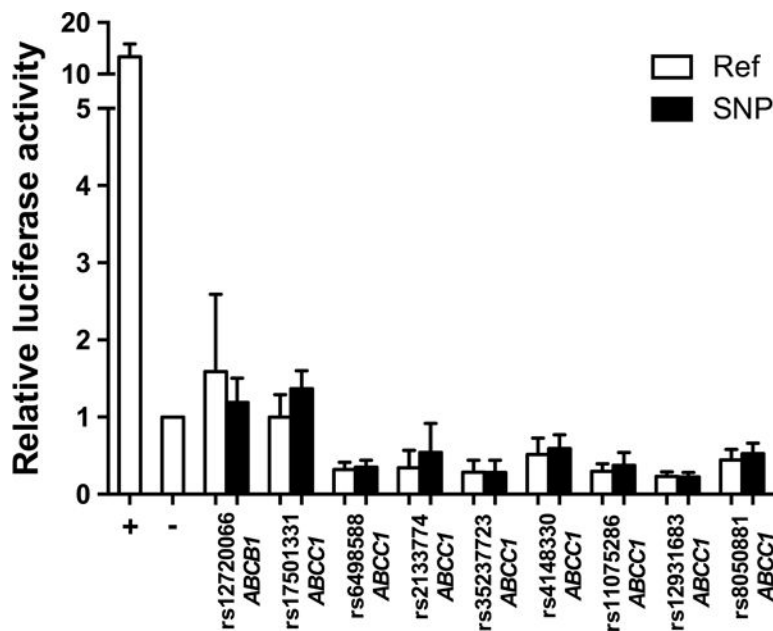


Figure 4. Analysis of genomic regions and genetic variants for enhancer activity

Enhancer activity was measured in Hep G2 cells using a luciferase reporter gene assay. The values plotted represent mean \pm SD of the relative luciferase activity in cells transfected with the indicated genomic region without (Ref) and with (SNP) the variant compared to empty pGL4.23 construct (-). A known liver enhancer of the *APOE* gene was used as the positive (+) control. Data are representative of at least three independent experiments of six biological replicates each.

Table 1Univariate and multivariate analysis results for log SN-38 AUC¹

rsid	Gene	Function	Ref>Alt	Univariate			Multivariate ³		
				Estimate	SE	P	Estimate	SE	P
rs12720066 ²	<i>ABCB1</i>	intrinsic	A>C	-0.204	0.070	0.005	-0.217	0.061	6.24×10^{-4}
rs6498588	<i>ABCC1</i>	5'-gene	A>T	0.111	0.042	0.010	0.123	0.036	9.50×10^{-4}
rs35621 ²	<i>ABCC1</i>	intrinsic	C>T	0.191	0.083	0.025			
rs2373586	<i>ABCB1</i>	intrinsic	A>C	0.086	0.040	0.033			
rs8187843	<i>ABCC1</i>	intrinsic	G>A	-0.160	0.084	0.061			
rs3743527 ²	<i>ABCC1</i>	3'-UTR	C>T	0.113	0.062	0.071			
rs215095 ²	<i>ABCC1</i>	intrinsic	G>A	0.118	0.073	0.109			
rs212082	<i>ABCC1</i>	intrinsic	A>G	-0.080	0.054	0.142			
rs10929302	<i>UGT1A1</i>	promoter	G>A	0.145	0.042	0.001	0.154	0.037	9.00×10^{-5}

¹ SN-38 AUC expressed in units of ng*hr/mL; adjusted for irinotecan dose² Homozygous alternate genotypes grouped with heterozygous genotypes³ R² = 0.345

Table 2

Univariate and multivariate analysis results for log₁₀ ANC nadir^{1/}

rsid	Gene	Function	Ref>Alt	Univariate			Multivariate ³		
				Estimate	SE	P	Estimate	SE	P
rs17501331	<i>ABCC1</i>	intronic	A>G	-0.255	0.106	0.019	-0.281	0.088	0.002
rs12720066 ²	<i>ABCB1</i>	intronic	A>C	0.227	0.102	0.030	0.261	0.084	0.003
rs3743527 ²	<i>ABCC1</i>	3'-UTR	C>T	-0.194	0.089	0.033			
rs212090	<i>ABCC1</i>	3'-UTR	T>A	0.102	0.059	0.089			
rs1128503	<i>ABCB1</i>	synonymous	A>G	-0.097	0.056	0.090			
rs8050881	<i>ABCC1</i>	5'-gene	A>G	-0.098	0.061	0.110			
rs2725264	<i>ABCG2</i>	intronic	C>T	-0.127	0.080	0.118			
rs35626	<i>ABCC1</i>	intronic	G>T	-0.107	0.069	0.123			
rs1967120	<i>ABCC1</i>	intronic	G>A	-0.108	0.070	0.124			
rs152023	<i>ABCC1</i>	intronic	C>T	-0.093	0.063	0.144			
rs10929302	<i>UGT1A1</i>	promoter	G>A	-0.262	0.057	1.98×10^{-5}	-0.268	0.052	1.99×10^{-6}

^{1/}ANC nadir expressed as cells/ μ L^{2/}Homozygous alternate genotypes grouped with heterozygous genotypes^{3/}R² = 0.390