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*Pharmacogenomics J.* 2016 February ; 16(1): 54–59. doi:10.1038/tpj.2015.23.**Clinical validity of new genetic biomarkers of irinotecan neutropenia: an independent replication study****DJ Crona<sup>1</sup>, J Ramirez<sup>2</sup>, W Qiao<sup>3</sup>, A-J de Graan<sup>4</sup>, MJ Ratain<sup>2</sup>, RHN van Schaik<sup>5</sup>, RHJ Mathijssen<sup>4</sup>, GL Rosner<sup>6</sup>, and F Innocenti<sup>1,2,7</sup>**

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**Abstract**

The overall goal of this study was to provide evidence for the clinical validity of nine genetic variants in five genes previously associated with irinotecan neutropenia and pharmacokinetics. Variants associated with absolute neutrophil count (ANC) nadir and/ or irinotecan pharmacokinetics in a discovery cohort of cancer patients were genotyped in an independent replication cohort of 108 cancer patients. Patients received single-agent irinotecan every 3 weeks. For ANC nadir, we replicated *UGT1A1*\*28, *UGT1A1*\*93 and *SLCO1B1*\*1*b* in univariate analyses. For irinotecan area under the concentration–time curve ( $AUC_{0-24}$ ), we replicated *ABCC2* -24*C>T*; however, *ABCC2* -24*C>T* only predicted a small fraction of the variance. For SN-38  $AUC_{0-24}$  and the glucuronidation ratio, we replicated *UGT1A1*\*28 and *UGT1A1*\*93. In addition to *UGT1A1*\*28, this study independently validated *UGT1A1*\*93 and *SLCO1B1*\*1*b* as new predictors of irinotecan neutropenia. Further demonstration of their clinical utility will optimize irinotecan therapy in cancer patients.

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**CONFLICT OF INTEREST**

Dr Federico Innocenti and Dr Mark J Ratain disclose that they receive royalties from *UGT1A1* genotyping. Dr Gary L Rosner discloses that he owns stock in Pfizer. The remaining authors state no conflict of interest.

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## INTRODUCTION

Irinotecan is an anticancer agent commonly used for the treatment of metastatic colorectal cancer and other solid tumors. Irinotecan is a potent inhibitor of topoisomerase I, and is initially hydrolyzed to its active metabolite, SN-38, which is then subsequently inactivated through UGT1A1-mediated glucuronidation. A significant proportion of patients treated with irinotecan develop toxicities, including severe neutropenia. Neutropenia is a common, serious, dose-dependent and dose-limiting toxicity of irinotecan.<sup>1</sup>

A common, germline genetic variation in *UGT1A1* predisposes patients to an increased risk of irinotecan-induced toxicities.<sup>2,3</sup> The number of TA repeats in the *UGT1A1* promoter is inversely proportional to the transcriptional efficiency of the gene,<sup>4</sup> mRNA expression<sup>5</sup> and protein levels.<sup>6</sup> Patients with the *UGT1A1*\*28 variant have seven TA repeats (compared with six repeats in patients with *UGT1A1*\*1), have decreased SN-38 glucuronidation<sup>7</sup> and experience increased systemic exposure to SN-38, which results in a higher risk of severe neutropenia.<sup>1</sup> As a result, an FDA-approved *UGT1A1*\*28 genotyping test has been made commercially available,<sup>8</sup> and the irinotecan label has been revised to include *UGT1A1*\*28 as a predisposing factor for severe neutropenia.<sup>9</sup>

Irinotecan-induced neutropenia is a complex, polygenic phenotype. There is significant interindividual variation in systemic exposure to both irinotecan and SN-38 that cannot be explained solely by *UGT1A1*\*28. Several additional genetic variants contribute to both variability in irinotecan pharmacokinetics and the risk of severe neutropenia.<sup>10-16</sup> The FDA-approved *UGT1A1*\*28 genetic test has only moderate predictive power for severe toxicity due to its low positive predictive value,<sup>8</sup> and therefore the genetic test has not been incorporated into routine clinical practice. The discovery of additional variants associated with neutropenia is needed to improve the utilization of irinotecan genetic testing.

Pharmacogenetic studies have identified a vast set of genetic variants as predictors of chemotherapy efficacy and toxicity. The majority of these proposed variants have failed to produce similar results across different studies, which has limited the clinical utility of pharmacogenetics.<sup>17,18</sup> Therefore, prospective replication of pharmacogenetic findings in independent and external cohorts of patients is essential to hasten the implementation of pharmacogenetics into routine clinical practice.

In a previous study of cancer patients treated with single-agent irinotecan, novel gene variants that were associated with irinotecan disposition and toxicity were identified.<sup>16</sup> In addition to *UGT1A1*\*28, other variants, mostly in drug transporter genes, were associated with neutropenia and irinotecan pharmacokinetics. Therefore, we conducted a replication study to test the clinical validity of these variants in an external cohort of cancer patients treated with single-agent irinotecan.

## MATERIALS AND METHODS

### Study design

The overall goal of the study was to replicate genetic associations for irinotecan neutropenia and pharmacokinetics previously identified in a discovery cohort.<sup>16</sup> The primary objective was to validate the associations between four genetic variants and absolute neutrophil count (ANC) nadir by testing them in an external replication cohort. The secondary objective was to validate the effects of eight genetic variants previously associated with pharmacokinetic parameters in the discovery cohort by analyzing them in the replication cohort. Thus, a total of nine common variants in five genes (ANC nadir and the pharmacokinetic phenotypes shared two variants) were genotyped in the replication cohort and tested for associations. Variants for replication testing were selected based on significant genotype–phenotype associations ( $P < 0.05$ ) observed in the discovery cohort. All patients in the replication cohort were White, and therefore only the previously genotyped White patients comprised the discovery cohort ( $n = 67$ ).<sup>16</sup>

### Patient characteristics

In the discovery cohort, advanced solid tumor patients were treated at the University of Chicago (Chicago, IL, USA) with a 90-min infusion of single-agent irinotecan every 3 weeks at  $300 \text{ mg m}^{-2}$  ( $n = 18$ ) or  $350 \text{ mg m}^{-2}$  ( $n = 49$ ). Eligibility criteria included adequate hematopoietic function (white blood cell count  $> 3500$  per  $\mu\text{l}$ , ANC  $> 1500$  per  $\mu\text{l}$ , platelets  $> 100\,000$  per  $\mu\text{l}$ ), normal renal and hepatic function (creatinine  $< 1.5 \text{ mg dl}^{-1}$ , total bilirubin  $< 1.25 \times$  upper limit of normal (ULN), and AST/ALT  $< 5 \times$  ULN), and adequate performance status (Karnofsky score  $\geq 70\%$ ). Plasma pharmacokinetic parameters of irinotecan and metabolites were measured during and after the first cycle infusion of irinotecan. Forty-two genetic variants in twelve candidate genes of the irinotecan pathway were previously genotyped and tested for association with irinotecan pharmacokinetics and ANC nadir, measured during cycle 1.

In the replication cohort, 108 White advanced solid tumor patients were treated at the Erasmus University Medical Center, Erasmus MC Cancer Institute (Rotterdam, The Netherlands).<sup>19–21</sup> Patients received a 90-min infusion of single-agent irinotecan every 3 weeks at 600 mg (flat dose,  $n = 58$ ),  $350 \text{ mg m}^{-2}$  ( $n = 31$ ), or 380–1060 mg (flat dose calculated according to an algorithm,<sup>19</sup>  $n = 19$ ). Eligibility criteria included adequate hematopoietic function (ANC  $> 2000$  per  $\mu\text{l}$ , platelets  $> 100\,000$  per  $\mu\text{l}$ ) and normal renal and hepatic function (creatinine clearance  $> 60 \text{ ml min}^{-1}$ , total bilirubin  $< 1.25 \times$  ULN and AST/ALT  $< 3 \times$  ULN). Plasma pharmacokinetics of irinotecan and metabolites were measured during and after the first cycle infusion.

All patients in the discovery and replication cohorts provided written informed consent and the local institutional review boards approved the clinical protocols. Patient characteristics from the discovery and replication cohorts are provided in Table 1.

### Patient phenotyping: pharmacokinetic parameters and ANC nadir

In both cohorts, pharmacokinetic parameters included: irinotecan area under the concentration–time curve to the last time of sampling ( $AUC_{0-24}$ ),  $AUC_{0-24}$  of the active SN-38 metabolite,  $AUC_{0-24}$  of the inactive SN-38 glucuronide (SN-38G) and the ratio of SN-38G  $AUC_{0-24}$  to SN-38  $AUC_{0-24}$  (glucuronidation ratio).

For the discovery cohort, samples were collected on day 1 of cycle 1 at baseline before irinotecan infusion, during the infusion (30, 60 and 90 min), and after the infusion (10, 20, 30 and 45 min, 1, 1.5, 2, 4, 6, 8, 12 and 24 h). Plasma concentrations of irinotecan and metabolites were measured, as previously reported.<sup>10</sup> Pharmacokinetic parameters were calculated by non-compartmental analysis (WinNonlin, Pharsight, Cary, NC, USA).

For the replication cohort, samples were collected on day 1 of cycle 1 at baseline before infusion, during the infusion (30 and 90 min) and after the infusion (10, 20 and 30 min, and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h). Plasma concentrations of irinotecan and metabolites were measured, as previously reported.<sup>20,22,23</sup> Pharmacokinetic parameters were calculated by non-compartmental analysis (PK Solutions v2.0, Summit Research Services, Montrose, CO, USA).

In both cohorts, complete blood counts were taken at baseline, weekly throughout cycle 1, and then before the start of cycle 2 to obtain the measurements of the ANC nadir.

### Genotype data

Nine common variants, previously associated with irinotecan pharmacokinetics and ANC nadir in the discovery cohort, were genotyped in the replication cohort: *ABCB1 IVS9 44 A>G*, *ABCC1 1684 T>C*, *ABCC1 IVS11 -48C>T*, *ABCC2 3972C>T*, *ABCC2 -24C>T*, *SLCO1B1\*1b*, *SLCO1B1\*5*, *UGT1A1\*28* and *UGT1A1\*93*. DNA isolated from peripheral blood was used for genotyping. All genotyping assays were performed on an Applied Biosystems TaqMan 7500 (Life Technologies, Grand Island, NY, USA). *UGT1A1\*93* was genotyped by restriction fragment length polymorphism PCR, using 5'-ACCTCTAGTTACATAACCTGAA-3' as the forward primer sequence and 5'-ATAAACCCGACCTCACCAC-3' as the reverse primer sequence. *UGT1A1\*28* genotyping methods for the replication cohort have been previously described.<sup>20</sup> All other variants were genotyped using TaqMan SNP genotyping assays (Life Technologies) as per the manufacturer's instructions. Positive controls of known genotypes were used in the assays.

### Statistics

Data for all phenotypes for both cohorts were log<sub>10</sub> transformed. Hardy–Weinberg Equilibrium was evaluated for all nine variants genotyped in both the discovery and replication cohorts (Supplementary Table 1). In the discovery cohort, associations between genetic variants and clinical phenotypes were analyzed using linear regression, and were adjusted for sex, age and irinotecan dose (300 or 350 mg m<sup>-2</sup>). All ANC nadir analyses were also adjusted for baseline ANC.

In the replication cohort, we prospectively tested associations between the nine gene variants described above and phenotypes of ANC nadir and irinotecan pharmacokinetics. The same statistical methodologies employed for the discovery cohort were applied: linear regression adjusted for sex, age and irinotecan dose (350 mg m<sup>-2</sup>, 600 mg flat dose or dose by an algorithm<sup>19</sup>), with baseline ANC used to adjust the ANC nadir analysis. Flat doses were converted to mg m<sup>-2</sup> according to the body-surface area of each patient. The same mode of inheritance (dominant, recessive or additive) used in the discovery cohort was also used in the replication cohort.

No general consensus exists to provide standardized criteria for replication cohort analyses. We considered a given variant's association to be replicated based on direct comparison of the observed estimates of effect in the discovery and replication cohorts: an association's estimate of effect in the replication cohort had to be in the same direction as in the discovery cohort (an increased or decreased estimate of phenotype change in both cohorts), and lie within the 95% confidence interval (CI) of the discovery cohort's estimate. Two-sided *P*-values are reported for reference. Since comparisons between discovery and replication cohort estimates of effect were pre-specified and rely on 95% CIs from the discovery cohort, not on hypothesis testing in the replication cohort, issues related to multiplicity are not present. Therefore, no correction for multiple comparisons was performed.

## RESULTS

This study sought to replicate, in an independent, external cohort of White cancer patients from the Netherlands, nine variants from five genes that had previously associated with ANC nadir or irinotecan pharmacokinetics.<sup>16</sup> Baseline clinical patient characteristics and pharmacokinetic data (Table 1), as well as allele and genotype frequencies (Supplementary Table 1), were comparable between the two cohorts. Below we report the replication results of each variant for neutropenia and irinotecan pharmacokinetics (Table 2).

### Replication of variants previously associated with ANC nadir

For ANC nadir, four variants that previously associated with ANC nadir in the discovery cohort were tested in the replication cohort. In the discovery cohort, *UGT1A1\*28* (additive model), *UGT1A1\*93* (recessive model) and *ABCC1 IVS11 -48C>4 T* (recessive model) were associated with decreased ANC nadir; *SLCO1B1\*1b* (dominant model) was associated with increased ANC nadir. In the replication cohort, we considered *UGT1A1\*28*, *UGT1A1\*93* and *SLCO1B1\*1b* replicated, since the direction of the estimate of the effect for each variant was consistent between both cohorts (decreased ANC nadir for *UGT1A1\*28* and *UGT1A1\*93*, as well as increased ANC nadir for *SLCO1B1\*1b*) and each was within the 95% CIs for its respective discovery cohort estimate. *ABCC1 IVS11 -48C>T* failed to replicate (Table 2).

### Replication of variants associated with the pharmacokinetic parameters of irinotecan

For irinotecan AUC<sub>0-24</sub>, two variants that were previously associated with irinotecan AUC<sub>0-24</sub> in the discovery cohort were tested in the replication cohort. In the discovery cohort, *ABCC2 -24C>T* and *SLCO1B1\*5* (both dominant model) were associated with

increased irinotecan AUC<sub>0-24</sub>. In the replication cohort, we considered *ABCC2 -24C>T* replicated since the direction of the estimate of the effect was consistent between both cohorts (increased AUC<sub>0-24</sub> for both variants), and was within the 95% CIs for the discovery cohort estimate. *SLCO1B1\*5* failed to replicate (Table 2).

For SN-38 AUC<sub>0-24</sub>, three variants that were previously associated with SN-38 AUC<sub>0-24</sub> in the discovery cohort were tested in the replication cohort. In the discovery cohort, *UGT1A1\*28* and *UGT1A1\*93* (both additive model) were associated with increased SN-38 AUC<sub>0-24</sub>, while *ABCB1 IVS9> -44A>G* (dominant model) was associated with decreased SN-38 AUC<sub>0-24</sub>. In the replication cohort, we considered *UGT1A1\*28* and *UGT1A1\*93* replicated since the direction of the estimate of the effect for each variant was consistent between both cohorts (increased AUC<sub>0-24</sub> for both variants), and each was within the 95% CIs for its respective discovery cohort estimate. *ABCB1 IVS9 -44A>G* failed to replicate (Table 2).

For SN-38G AUC<sub>0-24</sub>, although *ABCC2 3972C>T* (recessive model) was associated with increased SN-38G AUC<sub>0-24</sub> in the discovery cohort, it failed to replicate when tested in the replication cohort (Table 2).

For the glucuronidation ratio, three variants that associated with the glucuronidation ratio in the discovery cohort were tested in the replication cohort. In the discovery cohort, *UGT1A1\*28* (additive model), *UGT1A1\*93* (additive model) and *ABCC1 1684T>C* (dominant model) were associated with a decreased glucuronidation ratio. In the replication cohort, we considered *UGT1A1\*28* and *UGT1A1\*93*, replicated, since the direction of the estimate of the effect for each variant was consistent between both cohorts (decreased glucuronidation ratio for all variants), and each was within the 95% CIs for its respective discovery cohort estimate. Although the association between *ABCC1 1684 T>C* (dominant model) and glucuronidation ratio also satisfies our criteria for replication, we are less convinced of the association, given the 84% reduction in the magnitude of the estimate as compared with that of the discovery cohort (Table 2).

## DISCUSSION

In this replication study, we validated the clinical effects of new germline genetic variants for neutropenia and irinotecan pharmacokinetics using an independent, external cohort of White cancer patients treated with single-agent irinotecan.

The most important result of this study was the clinical validation of *SLCO1B1\*1b*. To our knowledge, this provides the first replicated data implicating *SLCO1B1\*1b* as a protective marker against irinotecan-induced neutropenia. *SLCO1B1* encodes for organic anion transporter family member 1B1 (OATP1B1), and mediates hepatic uptake of both endogenous<sup>24,25</sup> and xenobiotic compounds.<sup>26</sup> OATP1B1 is a hepatic uptake transporter of SN-38,<sup>27,28</sup> but not irinotecan.<sup>28</sup> In this study, we have replicated results from the discovery cohort, and have shown that the variant *\*1b* allele was associated with a higher ANC nadir compared with the reference sequence *\*1a* allele (Figure 1a). Since *SLCO1B1\*1b* is a non-synonymous variant (asparagine to aspartate amino-acid change), and *SLCO1B1* is primarily

expressed in the liver,<sup>29</sup> we postulate this variant might associate with reduced neutropenia by altering systemic SN-38 exposure. The effect of *SLCO1B1\*1b* on SN-38 AUC<sub>0-24</sub> was  $-0.083 \pm 0.076$  (mean  $\pm$  s.e.) in the White patients of the discovery cohort ( $n = 67$ ;  $P = 0.278$ ), and because the  $P$ -value was  $>0.05$ , this association was not selected for analysis in the replication cohort. However, an exploratory univariate analysis (adjusted for dose (mg m<sup>-2</sup>), age and sex) revealed that *SLCO1B1\*1b* was associated with decreased SN-38 AUC<sub>0-24</sub> in the replication cohort ( $n = 84$ ;  $-0.128 \pm 0.055$ ,  $P = 0.023$ ). These results support the hypothesis that the protective effect of *SLCO1B1\*1b* against neutropenia could be due to increased hepatic uptake of SN-38, resulting in increased SN-38 elimination from the plasma after irinotecan infusion.

While the pharmacokinetic data are supportive of the protective effect of *SLCO1B1\*1b* against neutropenia, the functional effect of this variant is less clear. Using RNA expression data from human livers,<sup>30</sup> *SLCO1B1\*1b* (as well as variants in linkage disequilibrium  $r^2 = 0.8$ ) did not associate with changes in the mRNA expression of *SLCO1B1* (results not shown). In oocyte studies, the uptake of SN-38 was higher for *SLCO1B1\*1b* than *SLCO1B1\*1a* (the reference sequence allele), but the observed difference was not statistically significant (see Figure 6a of Nozawa *et al.*<sup>28</sup>). Our results provide evidence that *SLCO1B1\*1b* results in a gain of function, which leads to increased hepatic uptake of SN-38 from the plasma. Although this seems the most plausible hypothesis, other mechanisms related to the widespread functions of this transporter on several endogenous constituents cannot be ruled out.

Another important conclusion of this study is that *UGT1A1\*93* confers an increased risk of irinotecan-induced neutropenia. We replicated results from the discovery cohort, and have shown that the *\*93* variant was associated with a lower ANC nadir compared with the reference sequence *\*1* allele (Figure 1b). *UGT1A1\*93* is a  $-3156G>A$  change discovered during a resequencing study of the region 5' to the *UGT1A* exon 1.<sup>31</sup> According to an analysis of more than 150 human livers where genome-wide genotyping data were available, *UGT1A1\*93* is a major determinant of decreased levels of the *UGT1A1* protein (Pearson's  $r = -0.46$ ,  $P = 3.5 \times 10^{-9}$ ),<sup>30,32</sup> and additional preliminary data corroborate these findings.<sup>33</sup> Because *UGT1A1\*93* is in partial linkage disequilibrium with *UGT1A1\*28* among White patients ( $r^2 = 0.68$ ),<sup>34</sup> our results suggest that *UGT1A1\*93*, based on its greater estimate of effect for ANC nadir, may be a more robust marker for neutropenia than *UGT1A1\*28* (Table 2). While the *UGT1A1\*93* variant has not yet been included in the FDA-revised irinotecan label, we envision that recommendations supporting *UGT1A1\*93* genotyping could eventually replace *UGT1A1\*28* in the irinotecan drug label.

The association between *ABCC2 -24C>T* and increased irinotecan AUC<sub>0-24</sub> was also replicated (Figure 1c). *ABCC2* encodes for the multidrug resistant protein-2 and contributes to the biliary clearance of irinotecan, SN-38 and SN-38G.<sup>35,36</sup> The  $-24C>T$  variant has been associated with a nearly 20% reduction in promoter activity.<sup>37</sup> This observation is consistent with our results, where the variant T allele was associated with increased irinotecan AUC<sub>0-24</sub>, likely due to decreased biliary clearance. However, the estimate of effect size was relatively small (Table 2), and additional studies should be conducted to elucidate the extent of its clinical relevance.

Established criteria for conducting pharmacogenetic replication studies do not currently exist, but we provide a general framework for conducting such studies. Pharmacogenetic replication studies are beset with numerous challenges, including dosing and population heterogeneity between the discovery and replication cohorts. In our study, we attempted to control for population heterogeneity by comparing patients in the replication cohort to only the White patients from the original discovery cohort.<sup>16</sup> Dosing heterogeneity between the two cohorts may have affected our ability to replicate some variants, but it did not confound all associations, as evidenced by the detection of associations serving as ‘positive controls’, such as *UGT1A1*\*28 versus SN-38 AUC<sub>0-24</sub> and *UGT1A1*\*28 versus glucuronidation ratio (but not irinotecan AUC<sub>0-24</sub>). Moreover, we are confident that dosing heterogeneity did not significantly confound our replication results because irinotecan has been shown to demonstrate dose linear pharmacokinetics over a wide range of doses.<sup>38</sup> Regarding our statistical approach, the assessment of replicated associations is not based on hypothesis testing, and therefore using *P*-values as our main criteria for replication would have been inappropriate. Moreover, given the influence of sample size on *P*-values, utilization of *P*-values as the main criteria for replication could have resulted in false negative results. We also cannot exclude the possibility that between-cohort differences limited our ability to detect phenotypic differences and replicate several variants.

This replication study allowed us to demonstrate the clinical validity of associations between *UGT1A1*\*93 and *SLCO1B1*\*1b and neutropenia. The effects of these two variants on neutropenia should be confirmed in studies where irinotecan is given in combination with other anticancer agents that have neutropenic effects (for example, with 5-fluorouracil). Additionally, the effects of these replicated variants can currently be applied only to White patients. Efforts should be made to validate these variants in patients from other races who receive irinotecan. Further validation of their clinical utility will aid in the implementation of routine irinotecan pharmacogenetic testing and optimization of personalized treatments for cancer patients.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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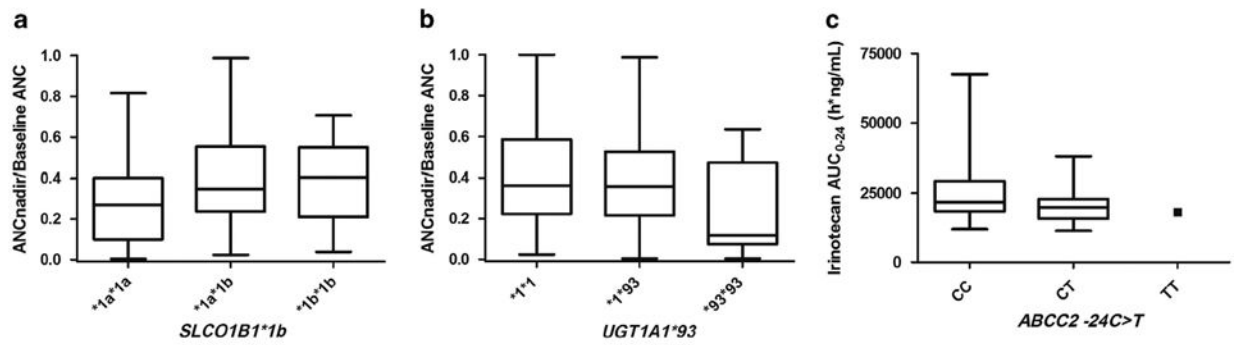
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**Figure 1.**

Associations between *SLCO1B1*\*1*b* and absolute neutrophil count (ANC) nadir (a), *UGT1A1*\*93 and ANC nadir (b) and *ABCC2* -24C>T and log<sub>10</sub> irinotecan area under the concentration–time curve (AUC<sub>0-24</sub>) (c) in the replication cohort. For the purpose of illustrating the replicated genetic associations, the data are not adjusted for the same factors used in the univariate analyses, and the differences among genotypes might not be the same as the ones reported in Table 2. ANC nadir is normalized to the baseline pretreatment ANC. Data are expressed as medians, 25th and 75th percentiles, minimums and maximums.

**Table 1**

Baseline patient characteristics and pharmacokinetic data from the discovery and the replication cohorts

	Discovery cohort (n = 67)		Replication cohort (n = 108)	
	Median	Range	Median	Range
<i>Dose</i>				
300 mg m <sup>-2</sup>	18 (26.9%)		—	
350 mg m <sup>-2</sup>	49 (73.1%)		31 (29.7%)	
Flat dose (600 mg)	—		58 (53.7%)	
Dose by algorithm (380–1060 mg)	—		19 (17.1%)	
<i>Sex</i>				
Male	42 (62.7%)		60 (55.6%)	
Female	25 (37.3%)		48 (44.4%)	
	Median	Range	Median	Range
Age (years)	57	34–85	58	26–75
BSA (m <sup>2</sup> )	1.87	1.46–2.55	1.88	1.36–2.50
Baseline ANC (cells per $\mu$ l)	5.27	2.18–14.36	5.12	1.30–13.60
ANC Nadir (cells per $\mu$ l)	2.21	0.05–7.83	1.79	0.03–7.13
<b>Pharmacokinetic parameters</b>				
	Mean	Range	Mean	Range
Irinotecan AUC <sub>0-24</sub> (h ng ml <sup>-1</sup> )	23251	8857–65305	22776	11422–67560
SN-38 AUC <sub>0-24</sub> (h ng ml <sup>-1</sup> )	385	38–1957	364	79–1776
SN-38G AUC <sub>0-24</sub> (h ng ml <sup>-1</sup> )	1824	360–8214	2238	396–6912
SN-38G AUC <sub>0-24</sub> to SN-38 AUC <sub>0-24</sub> ratio (glucuronidation ratio)	5.85	0.78–37.57	7.32	1–24.07

Abbreviations: ANC, absolute neutrophil count; AUC, area under the concentration–time curve. Flat dosing and dosing by algorithm<sup>19</sup> were used only in the replication cohort. The distribution of the algorithm-derived doses includes: 380 mg (*n* = 1), 500 mg (*n* = 1), 520 mg (*n* = 2), 540 mg (*n* = 1), 560 mg (*n* = 1), 620 mg (*n* = 2), 640 mg (*n* = 1), 660 mg (*n* = 1), 680 mg (*n* = 1), 720 mg (*n* = 2), 740 mg (*n* = 3), 780 mg (*n* = 1), 900 mg (*n* = 1) and 1060 mg (*n* = 1).

Table 2

Univariate analyses of the associations between genetic variants and phenotypes

Variant	Reference	Discovery cohort (n = 67)			Replication cohort (n = 74-103)		
		Estimate ± s.e.	95% CI	P-value	n	Estimate ± s.e.	P-value
<i>Log<sub>10</sub> ANC Nadir</i>							
<i>ABCC1</i> IVS11-48 (C>T)	CC/CT	-0.489±0.201	(-0.095, -0.883)	0.018	74	0.053±0.117	0.652
<i>UGT1A1</i> *28 (TA6>TA7)	*1*1=1 *28*28=2 *28*28=3	-0.257±0.061	(-0.137, -0.377)	<0.001	75	<b>-0.139±0.081</b>	0.090
<i>UGT1A1</i> *93 (G>A)	GG/AG	-0.607±0.129	(-0.354, -0.860)	<0.001	103	<b>-0.417±0.170</b>	0.016
<i>SLCO1B1</i> *1b (A>G)	AA	0.240±0.106	(0.032, 0.448)	0.027	84	<b>0.278±0.107</b>	0.012
<i>Log<sub>10</sub> irinotecan AUC<sub>0,24</sub></i>							
<i>ABCC2</i> -24 (C>T)	CC	0.090±0.035	(0.021, 0.159)	0.012	94	<b>0.067±0.030</b>	0.040
<i>SLCO1B1</i> *5 (T>C)	TT	0.084±0.036	(0.013, 0.155)	0.023	83	-0.010±0.035	0.769
<i>Log<sub>10</sub> SN-38 AUC<sub>0,24</sub></i>							
<i>UGT1A1</i> *28 (TA6>TA7)	*1*1=1 *1*28=2 *28*28=3	0.140±0.046	(0.050, 0.190)	0.004	75	<b>0.189±0.043</b>	<0.001
<i>UGT1A1</i> *93 (G>A)	GG=1 AG=2 AA=3	0.130±0.047	(0.038, 0.222)	0.007	103	<b>0.171±0.033</b>	<0.001
<i>ABCB1</i> IVS9-44 (A>G)	AA	-0.180±0.070	(-0.043, -0.317)	0.013	77	0.021±0.054	0.703
<i>Log<sub>10</sub> SN-38G AUC<sub>0,24</sub></i>							
<i>ABCC2</i> 3972 (C>T)	CC/CT	0.250±0.100	(0.054, 0.446)	0.019	105	-0.104±0.086	0.232
<i>Log<sub>10</sub> SN-38G AUC<sub>0,24</sub> / Log<sub>10</sub> SN-38 AUC<sub>0,24</sub></i>							
<i>ABCC1</i> 1684 (T>C)	TT	-0.316±0.153	(-0.016, -0.616)	0.043	95	<b>-0.052±0.059*</b>	0.382
<i>UGT1A1</i> *28 (TA6>TA7)	*1*1=1 *1*28=2 *28*28=3	-0.170±0.048	(-0.076, -0.264)	<0.001	103	<b>-0.243±0.053</b>	<0.001
<i>UGT1A1</i> *93 (G>A)	GG=1 AG=2 AA=3	-0.150±0.051	(-0.050, -0.250)	0.004	75	<b>-0.214±0.042</b>	<0.001

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Abbreviations: ANC, absolute neutrophil count; AUC, area under the concentration–time curve; CI, confidence interval. Data were adjusted for age, sex and dose ( $\text{mg m}^{-2}$ ). ANC nadir was also adjusted for baseline ANC. The genotype reference groups were the same for all discovery and replication cohort analyses, with the exception of *ABCC1 IVS11 -48C>T* and ANC nadir. For *ABCC1 IVS11 -48C>T* and ANC nadir in the replication cohort, the reference genotype was only CC, as there were no TT genotypes. The estimates of effect of replicated variants are denoted in bold. The number of patients genotyped per variant in the replication cohort varied due to insufficient DNA quantity.

\* Although the association between *ABCC1 1684T>C* (dominant model) and glucuronidation ratio satisfies our criteria for replication, we are less convinced of the association, given the 84% reduction in the magnitude of the estimate as compared with that of the discovery cohort.