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The relationship of polymorphisms in *ABCC2* and *SLCO1B3* with docetaxel pharmacokinetics and neutropenia: CALGB 60805 (Alliance)

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Abstract

Docetaxel-related neutropenia was associated with polymorphisms in the drug transporters *ABCC2* and *SLCO1B3* in Japanese cancer patients. We hypothesized that this association is because of reduced docetaxel clearance, associated with polymorphisms in those genes. We studied 64 US cancer patients who received a single cycle of 75 mg/m^2 of docetaxel monotherapy. We found that the *ABCC2* polymorphism at rs-12762549 trended to show a relationship with reduced docetaxel clearance ($P = 0.048$), but not with neutropenia. There was no significant association of the *SLCO1B3* polymorphisms with docetaxel clearance or neutropenia. We conclude that the relationship between docetaxel-associated neutropenia and polymorphisms in drug transporters identified in Japanese patients was not confirmed in this cohort of US cancer patients.

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Conflicts of interest M.J.R. declares that he has acted as a consultant to Sanofi-Aventis (not relating to docetaxel). For the remaining authors there are no conflicts of interest.

Keywords

ABCC2 (pharmacogenetics); docetaxel; neutropenia; pharmacokinetics; *SLCO1B3*

Docetaxel, a semisynthetic taxane, is a cytotoxic agent used widely for the treatment of breast, non-small-cell lung and prostate cancer [1,2]. Previous pharmacokinetic studies have evaluated both the erythromycin breath test and genetic variation in drug-metabolizing enzymes and transporters as determinants of the variability in docetaxel clearance [3], but the major determinants of this variability are incompletely defined. The differential expression and function of polymorphic drug-metabolizing enzymes and/or transporters at the sites of drug elimination could play a major role in this variability. CYP3A4 and CYP3A5 are the primary enzymes involved in hepatic oxidation of docetaxel to its major metabolite, C-13-hydroxydocetaxel [3,4]. Hepatocellular uptake of taxanes is regulated, at least in part, by the solute carrier *OATP1B3* (*SLCO1B3*; OATP8) [5], whereas the ATP-binding cassette (ABC) transporters P-glycoprotein (*ABCB1*) and MRP2 (*ABCC2*; cMOAT) [6] are involved in the secretion of taxanes from the liver into the bile [7]. Neutropenia is the dose-limiting toxicity of docetaxel [2,3]. To identify the genetic factors determining the risk of docetaxel-induced neutropenia, Kiyotani *et al*. [8] carried out a retrospective case–control study of 140 Japanese cancer patients who received docetaxel monotherapy, with 84 cases of grade 3/4 neutropenia and 56 controls (no neutropenia). The researchers identified a strong association of grade 3/4 neutropenia with the SNPs rs-12762549 in *ABCC2* and rs-11045585 in *SCLO1B3*. The presence of polymorphisms in both genes yielded a seven-fold (95% confidence interval 2.95–16.59) increase in the odds ratio for developing docetaxel-associated neutropenia. As docetaxel-associated neutropenia has been related to drug exposure (AUC) [3], we hypothesized that rs-12762549 in *ABCC2* and rs-11045585 in *SLCO1B3* are associated with reduced docetaxel clearance and neutropenia.

We studied this hypothesis retrospectively in a subgroup of patients who originally participated in CALGB 9871 (Alliance) [9] and who consented to have DNA collected for studies of genes involved in docetaxel disposition and pharmacodynamics. The protocol was approved by local Institutional Review Boards. Details of the study design, patient eligibility criteria, docetaxel regimen, neutropenia monitoring, docetaxel concentration measurement, and population pharmacokinetic modeling using NONMEM have been described previously [9,10]. All patients in this retrospective study received a single intravenous dose of 75 mg/m^2 docetaxel. The original study found no difference in docetaxel clearance with ethnicity; thus, this patient population was considered a single subgroup.

Blood samples (4.5 ml) for genotyping were collected in citrated tubes and DNA was isolated using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instructions. Genotyping of *ABCC2* and *SLCO1B3* polymorphisms was carried out at the University of California San Francisco. The genotyping of de-identified DNA samples was approved by the University of California San Francisco Institutional Review Board. TaqMan SNP genotyping was carried out for rs-12762549 (*ABCC2*) and rs-11045585 (*SLCO1B3*). TaqMan SNP genotyping 40 × primer/probe assays were purchased from Applied Biosystems (Foster City, California, USA; ABI assay ID numbers C_11214917_10 for rs-12762549 and C_31106434_10 for rs-11045585). Three additional polymorphisms selected for strong linkage disequilibrium to the target SNPs in Asians were also genotyped. Primers and probes were purchased from Applied Biosystems [ABI assay ID numbers C_31980850 for rs-11190298 (*ABCC2*) and C_25766123 for rs-16923270 (*SLCO1B3*)] and were custom designed by Applied Biosystems for *SLCO1B3* rs-4149155

Forward primer: 5′-TTGTAGGAAGAACAGAGTATATAGGCATA-3′.

Reverse primer: 5′-CAGATGTATTTGATCTACTCTTCTCTCCCTAT-3′.

VIC-labeled reporter 1: 5′-CAGAGGGAAGAAAGAGT-3′.

FAM-labeled reporter 2: 5′-ACAGAGGGAATAAAGAGT-3′.

The 5 μl reactions contained 5 ng of genomic DNA, 1 × TaqMan Universal Master Mix and 1 × primer/probe mix (900 nmol/l final concentration of primers and 200 μmol/l final concentration of FAM-labeled and VIC-labeled probes). PCR and post-reads were performed on a 7900 Real-Time PCR system (Applied Biosystems, Foster City, California. USA). Amplification conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Negative controls and random sample duplicates were included on each plate for quality control purposes. All SNPs were tested for deviation from Hardy–Weinberg equilibrium using the χ^2-test. Haplotypes were statistically inferred using the program PHASE.

The primary objective of this retrospective study was to investigate the association of the *ABCC2* polymorphism (rs-12762549) and the *SLCO1B3* polymorphism (rs-11045585) with docetaxel clearance. The specific hypotheses, on the basis of the results of Kiyotani *et al.* [8], were that carriers of rs-12762549 or rs-11045585 have reduced docetaxel clearance and an increased risk of neutropenia, compared with their reference genotypes. Each hypothesis was tested using the Wilcoxon rank-sum test at the one-sided marginal level of 0.025. A secondary objective was to investigate the association between drug transporter genotypes and neutropenia. To this end, the Wilcoxon test for absolute neutrophil count nadir and Fisher's test for neutropenic event (toxicity grade) were used. All secondary and exploratory analyses were carried out using a two-sided marginal level of 0.05 and not adjusted for multiple testing.

Ninety-nine patients were originally enrolled in CALGB 9871; of these, 64 patients had DNA samples available for pharmacogenetic analysis with concomitant docetaxel pharmacokinetic and neutropenia data. The demographics of this patient subgroup are shown in Table 1. Patients with rs-12762549 showed some evidence of reduced docetaxel clearance compared with those patients with the reference genotype (left panel in Fig. 1a, $P = 0.048$), although this did not achieve the predefined level of statistical significance ($P < 0.025$). There was no association between rs-11045585 and docetaxel clearance (right panel Fig. 1a, $P = 0.799$). There was no association between the absolute neutrophil count nadir and either the *ABCC2* genotype ($P = 0.86$) or the *SLCO1B3* genotype ($P = 0.92$), Fig. 1b. Additional analyses indicated no relationship between these drug transporter genotypes and the occurrence of grade 3/4 neutropenia. Three additional polymorphisms (rs-4149155, rs-16923270, rs-11190298) were also tested for their association with docetaxel clearance and neutropenia, on the basis of the observation that the two tag SNPs identified in the Kiyotani *et al.*'s [8] study had different linkage disequilibrium patterns in Caucasians and African-Americans compared with the Asian population. However, in our study population, no association was found between any of these SNPs and docetaxel clearance or neutropenia (data not shown). Post-hoc power analyses for the *SLCO1B3* polymorphism to define an odds ratio of 6 for ≥ grade 3 neutropenia yielded a power of 0.84 (one sided $\alpha = 0.025$); for the *ABCC2* polymorphism, the power was 0.33 (one sided $\alpha = 0.025$) to define an odds ratio of 3 for ≥ grade 3 neutropenia.

This study investigated whether the polymorphisms in *ABCC2* (rs-12762549) and in *SLCO1B3* (rs-11045585) [11,12] that were previously associated with docetaxel-induced neutropenia in Japanese cancer patients [8] were similarly associated with docetaxel

clearance and hematopoietic toxicity in US Caucasian and African-American patients enrolled in CALGB 9871. We observed decreased docetaxel clearance in association with the *ABCC2* polymorphism, but this did not result in greater neutropenia. Similarly, in CALGB 9871 [9], we did not establish a pharmacokinetic–pharmacodynamic relationship for docetaxel-associated neutropenia. Given the findings of Kiyotani *et al*. [8], this is weak evidence in support of a relationship between the *ABCC2* rs-12762549 polymorphism and docetaxel clearance. Furthermore, our study did not identify an association of *SLCO1B3* polymorphisms with docetaxel clearance or hematologic toxicity. To further elucidate whether our findings may have been confounded by the derivation of the docetaxel clearance using population pharmacokinetics models, we added the transporter genotypes to the Bruno docetaxel NONMEM model [10], but this did not yield additional docetaxel pharmacokinetic–drug transporter genotype associations (data not shown). Confounding results of the association of drug transporter polymorphisms with docetaxel pharmacokinetics highlight the importance of replication of pharmacogenetic results. Baker *et al*. [13] studied 92 White US and European cancer patients receiving docetaxel therapy (at different doses, either as monotherapy or in combination therapy) and found no relationship between docetaxel clearance and *ABCC2*, *SLCO1B3*, or *ABCB1* genotypes. The relationships between transporter genotype and neutropenia were not explored in this study because of the variation in the docetaxel dose used and the use of combination chemotherapy. In a population of Asian nasopharyngeal carcinoma patients treated with weekly docetaxel monotherapy ($n = 54$), an influence of functional polymorphisms in *SLCO1B3* and *ABCB1* with interindividual variability in docetaxel clearance was reported [14]. Although there are conflicting reports on the association of the *ABCB1* genotype with docetaxel clearance [13–17] and toxicity [18], the prevailing evidence does not support a significant role for *ABCB1* (P-glycoprotein) polymorphisms in determining docetaxel clearance.

There are a number of possible explanations why the previous Japanese data [8] were not replicated in this present study. Selection bias cannot be ruled out in any retrospective study with small cohorts; furthermore, the doses of docetaxel administered to Japanese patients were not defined. Differences in the linkage disequilibrium patterns for *ABCC2* and *SLCO1B3* were considered in the current study and tested by the inclusion of three additional SNPs. There was no evidence that this was the reason for nonreplication of the previous findings in our US population. Interestingly, the minor allele frequencies in the study patients for rs-11045585 and rs-1276259 were 0.148 and 0.460, respectively, similar to the minor allele frequencies in HapMap CEU Environmental differences between study populations; variations in treatment paradigms and concomitant medications may all affect the phenotypes studied and therefore any genetic associations related to drug exposures. It is not possible to identify which one (or combination) of these factors may have influenced the current findings. It is possible that ongoing genome-wide association studies of docetaxel will provide additional insights into the variable toxicity of this agent.

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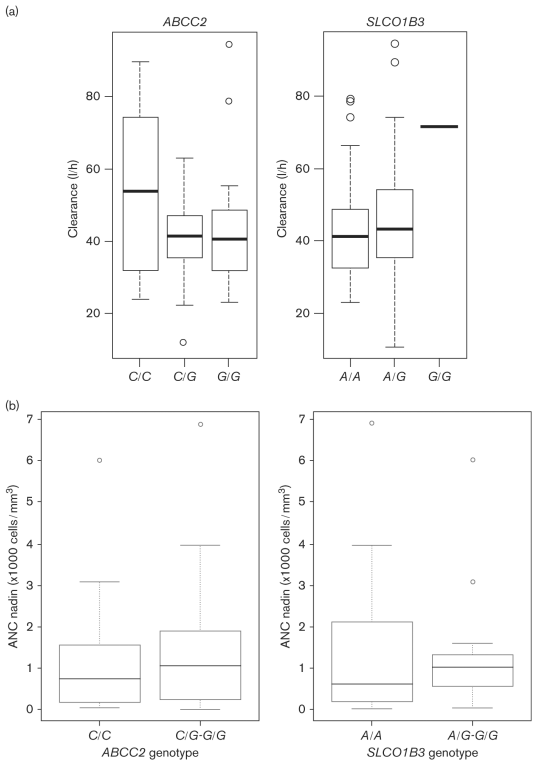

Fig. 1.

(a) Box and whisker plots of docetaxel clearance in patients who received a single intravenous dose of 75 mg/m^2 of docetaxel and *ABCC2* (left panel) and *SLCO1B3* (right panel) genotype. The horizontal lines represent the median values of docetaxel clearance for each genotype. For *ABCC2* ?? vs CG/GG rs-12762549, the Wilcoxon–Mann–Whitney rank-sum test ($P = 0.048$). For the *SLCO1B3* AA vs AG/GG rs-11045585, the Wilcoxon–Mann–Whitney rank-sum test ($P = 0.799$). (b) Box and whisker plots of the nadir absolute neutrophil count (ANC) and *ABCC2* rs-12762549 (left panel) and *SLCO1B3* rs-11045585 (right panel) genotype. The Wilcoxon–Mann–Whitney rank-sum test ($P = 0.861$ and 0.922) for the *ABCC2 CC* vs. *CG/GG* and *SLCO1B3 AA* vs. *AG/GG* genotypes, respectively.

Table 1

Patient demographics for CALGB 60805 (Alliance) (a subgroup of patients from CALGB 9871: Alliance)

	African-American	Caucasian	Total
Number of patients enrolled	20	44	64
Median age (years)	59.5 (range 42–70)	62.5 (range 42–79)	62 (range 42–79)
Sex			
Male	13 (65%)	33 (75%)	46 (72%)
Female	7 (35%)	11 (25%)	18 (28%)
Performance status			
0	2 (10%)	7 (16%)	9 (14%)
1	15 (75%)	24 (55%)	39 (61%)
2	3 (15%)	12 (27%)	15 (23%)
Unknown	0 (0%)	1 (2%)	1 (2%)
Tumor types			
Lung	12 (60%)	30 (68%)	42 (66%)
Breast	0 (0%)	1 (2%)	1 (2%)
GI and pancreas	3 (15%)	3 (7%)	6 (9%)
Head and neck	1 (5%)	1 (2%)	2 (3%)
Prostate	1 (5%)	1 (2%)	2 (3%)
Other	3 (15%)	8 (18%)	11 (17%)
Docetaxel dose −75 mg/m^2	20 (100%)	44 (100%)	64 (100%)
Median baseline WBC×10^3/μl (range)	8.9 (2.7–18.7)	6.7 (2.5–12.8)	7.0 (2.5–18.7)