EPIDEMIOLOGY

CYP2C8*3 predicts benefit/risk profile in breast cancer patients receiving neoadjuvant paclitaxel

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Abstract Paclitaxel is one of the most frequently used chemotherapeutic agents for the treatment of breast cancer patients. Using a candidate gene approach, we hypothesized that polymorphisms in genes relevant to the metabolism and transport of paclitaxel are associated with treatment efficacy and toxicity. Patient and tumor characteristics and treatment outcomes were collected prospectively for breast cancer patients treated with paclitaxel-containing regimens in the neoadjuvant setting. Treatment response was measured before and after each phase of treatment by clinical tumor measurement and categorized according to RECIST criteria, while toxicity data were collected from physician notes. The primary endpoint was achievement of clinical complete response (cCR) and secondary endpoints included clinical response rate (complete response + partial response) and grade 3+ peripheral neuropathy. The genotypes and haplotypes assessed were CYP1B1*3, CYP2C8*3, CYP3A4*1B/CYP3A5*3C, and ABCB1*2. A total of 111 patients were included in this study. Overall, cCR was 30.1 % to the paclitaxel component. CYP2C8*3 carriers (23/111, 20.7 %) had higher rates of cCR (55 % vs. 23 %; OR = 3.92 [95 % CI: 1.46–10.48], corrected $p = 0.046$). In the secondary toxicity analysis, we observed a trend toward greater risk of severe neuropathy (22 % vs. 8 %; OR = 3.13 [95 % CI: 0.89–11.01], uncorrected $p = 0.075$) in subjects carrying the CYP2C8*3 variant. Other polymorphisms interrogated were not significantly associated with response or toxicity. Patients carrying CYP2C8*3 are more likely to achieve clinical complete response from neoadjuvant paclitaxel

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treatment, but may also be at increased risk of experiencing severe peripheral neurotoxicity.

Introduction

Interpatient variability in toxicity and response are important problems in the use of cancer chemotherapy. For example, paclitaxel, one of the most commonly used therapies for breast cancer and other cancers, has interpatient variability of 19–26 % in (unbound) drug clearance [1], causes grade 3 or higher neuropathy and neutropenia in 5–8 % and 2–4 % of patients, respectively [2] and a response rate as first line, single-agent treatment in metastatic breast cancer of 20–30 % [3]. Studying host factors responsible for the variability in chemotherapeutic outcomes and developing strategies to individualize therapy to maximize response and minimize toxicity are active areas of research. Pharmacogenomics, study of the interplay of genetics and drug therapy outcomes, is one promising approach to achieving individualized therapy [4]. Genetic variation can influence therapy by a number of mechanisms. Variants in genes relevant to drug disposition or metabolism can modulate the patient's exposure to the drug, whereas variation in genes that are involved in drug action can influence the patient's sensitivity. For example, germline genetic polymorphisms have been discovered that increase the likelihood of a patient experiencing severe toxicity to irinotecan [5, 6] or modulate the optimal dose of a patient's warfarin therapy [7].

A number of putative pharmacogenetic markers for paclitaxel outcomes, in breast cancer and in other solid tumors, have been evaluated [8–17]. Most of these studies focused on known mutations in biologically relevant candidate genes, such as CYP2C8, CYP3A4, and ABCB1, which code for the enzymes involved in paclitaxel metabolism and the transporters that influence paclitaxel disposition. More recent genome-wide association studies used an unbiased approach to examine the entire genome to address this question, and have reported intriguing candidate SNPs in genes not previously investigated [14, 15]. The clinical phenotypes most often studied were severe toxicities, such as neuropathy, or measures of survival. Some of these retrospective pharmacogenetic studies suggest that genetic variability may be associated with clinical outcome to paclitaxel therapy, and others do not. However, the toxicity endpoints are often confounded by prior or combination therapy, whereas the survival endpoint is confounded by a multitude of factors including ER status or tumor subtype [18] and stage at diagnosis [19]. To our knowledge, no published pharmacogenomic study has exclusively utilized a neoadjuvantly treated population, in which toxicity and tumor response to paclitaxel therapy can be assessed in the absence of these confounding factors.

In this study, we genotyped a cohort of patients treated with neoadjuvant paclitaxel for polymorphisms that have previously demonstrated significant associations with efficacy or toxicity. These subjects are uniquely informative because tumor response and toxicity data were collected exclusively for the taxane treatment phase. We hypothesized that polymorphisms in genes relevant to paclitaxel metabolism (CYP2C8 & CYP3A4/3A5), transport (ABCB1), or mechanism (CYP1B1) would influence the likelihood that a patient would respond to, or experience severe toxicity from, paclitaxel therapy.

Materials and methods

Patients and treatments

Relevant candidate SNPs were evaluated in a cohort of patients treated between 2005 and 2009 and derived from the University of North Carolina Lineberger Comprehensive Cancer Center (UNC LCCC) Breast Cancer Neoadjuvant Database, which includes prospective annotation of clinical data, treatment details, toxicity, and outcome. Eligible women received neoadjuvant paclitaxel-containing regimens and enrolled in both the UNC neoadjuvant database and a concurrent IRB approved clinical trial that collected genomic DNA from all newly diagnosed patients. All patients received paclitaxel (T) treatment guided by standard neoadjuvant protocols that had a defined and conventional treatment dose, schedule, and duration. In most cases, patients received neoadjuvant doxorubicin and cyclophosphamide (AC) either before or after the paclitaxel; however, the tumors were measured before and after each phase of therapy so that clinical response to the anthracycline component and the taxane component could be separately identified. Some patients with HER2 overexpressing tumors received trastuzumab concurrent with the paclitaxel. Tumor size was clinically measured by the patient's medical oncologist, and percent change in tumor size was calculated from these measurements. Clinical response was defined as complete response (cCR; 100 % reduction in tumor size), partial response (cPR; 30–99 % reduction), stable disease (cSD; 29 % reduction–20 % enlargement of tumor), or progressive disease (cPD; >20 % enlargement) according to RECIST criteria [20]. Patients who achieved complete response to AC before the start of taxane therapy were

excluded from the efficacy analysis because they were not evaluable for response to taxane treatment. Pathological response, which could not be evaluated between treatment regimens, was not used as an endpoint in this study because of the inability to separate the confounding effects of other chemotherapy treatments. Toxicities were evaluated during paclitaxel treatment, recorded prospectively, and coded by NCI CTC AE V4.0 based on the physician's description [21]. Patients who were treated at outside institutions did not have toxicity data and were excluded from that part of the analysis. All patients signed informed consent to participate and agreed to allow DNA to be collected for additional pharmacogenetic studies. The study protocol was approved by the UNC Institutional Review Board.

SNP genotyping

A 30 mL blood sample was collected from each patient at the time of study enrollment. DNA used for genotyping was extracted by the UNC Biospecimen Processing Facility and plated at 5 ng/uL. Target gene region amplification was carried out by PCR in a 20 μL reaction including 2 μL genomic DNA and polymorphisms were genotyped on a Pyromark Pyrosequencer Q96 MD as previously described [22, 23]. The polymorphisms genotyped were as follows: CYP1B1*3 (rs1056836, 4326C>G), CYP2C8*3 (rs11572080, 416G>A and rs10509681, 1196A>G), CYP3A4*1B (rs2740574, -392A>G), CYP3A5*3C (rs776746, 6986A>G), and ABCB1*2 (rs1045642, 3435C>T, rs2032582, 2677G>T/A, and rs1128503, 1236C>T). Genotyping was carried out blinded to clinical data with negative controls included in each, run and at least 5 % of samples were repeated for quality control to ensure accuracy of assay results. Any assay with call rate or concordance with repeated samples <95 % was excluded from analysis. Additional details for gene variants including PCR and pyrosequencing primers are included in Supplementary Table 1.

CYP3A4*1B/CYP3A5*3C and ABCB1 1236C > T, 2677G > T/A, and 3435C > T were included in haplotype analyses. Haplotypes were inferred using PHASE Version 2 [24, 25] for polymorphisms with LD > 0.7. Any subject with missing genotype information at any locus of the haplotype was considered to have an unknown haplotype and excluded from analysis. CYP3A4/3A5 haplotypes were grouped according to Baker et al. (*1: CYP3A4*1A/CYP3A5*3C, *2: CYP3A4*1B/CYP3A5*1A, *3: CYP3A4*1A/CYP3A5*1A, and *4: CYP3A4*1B/CYP3A5*3C) [26]. After ABCB1 haplotype inference (wild-type: C-G-C, Variant: T–T(A)-T, mixed: other), each patient was assigned a diplotype number (1–5) in order of increasing genetic variation as described in Sissung et al. [27].

Statistical analysis

Genotype calls were assessed for concordance with Hardy–Weinberg Equilibrium (HWE) using a Pearson's chi-square test with df = 1. Assays with HWE p value < 0.05 in the cohort were then tested in the Caucasian subcohort as population admixture violates key assumptions of HWE [28]. Each genotype or haplotype was individually tested for an association with efficacy or toxicity using logistic regression modeling. In the haplotype analysis, the "variant" group was defined by grouping diplotypes. For CYP3A4/3A5, any individual carrying the *2 haplotype (CYP3A4*1B/CYP3A5*1A) was considered a variant carrier. For ABCB1, any individual with diplotype 4 or 5 was considered a variant carrier and was compared with non-carrier diplotypes 1–3. For the genotype analyses, variant carriers were compared with homozygotic wild-type individuals (dominant genetic model). The primary efficacy endpoint was clinical complete response (cCR) to taxane therapy (yes vs. no). The primary toxicity endpoint was any grade 3 or higher adverse event during taxane therapy (yes vs. no). Secondary endpoints of efficacy and toxicity were clinical response rate (cRR; cCR + cPR = cRR) and grade 3 or higher neuropathy during paclitaxel therapy, respectively. Following univariate testing, additional covariates for efficacy (estrogen receptor (ER) status, tumor grade, concurrent trastuzumab treatment, whether paclitaxel treatment was preceded by other chemotherapy phase) were included in a multivariable model to adjust for their prognostic importance. Backward selection was used to eliminate covariates that did not significantly contribute to the model using AIC as a selection criterion. Self-reported race was used as stratification factor for significant associations to account for racial heterogeneity in the cohort. To correct the primary efficacy analysis for multiple comparisons, the p values were multiplied by 7, the number of independent statistical associations performed (a Bonferroni correction for multiple comparisons) so that the p value to be compared with the standard significance threshold of $\alpha = 0.05$ is valid. P values of all secondary and sub-analyses are uncorrected as these are exploratory in nature and should be interpreted as such. All statistical analyses were performed in R Statistical Software, version 2.13.0 (R Development Core Team, Vienna, Austria).

Results

Patient population

A total of 111 patients neoadjuvantly treated with paclitaxel-containing regimens were eligible for analysis. After excluding subjects missing efficacy or toxicity data, 103

subjects were included in the efficacy and 109 subjects in the toxicity analysis. Demographic data including patient, treatment, and tumor characteristics for the whole population are presented in Table 1.

Allele frequencies

The two highly linked SNPs in CYP2C8*3 (rs11572080, 416G>A and rs10509681, 1196A>G) were completely concordant in this population. CYP3A4 and CYP3A5 were out of HWE before accounting for race, as expected given the large difference in allele frequencies among Caucasian and African-American individuals [29], but no significant deviations were seen in stratified samples. Allele frequencies in Caucasian subjects for all variants were consistent with those reported in The International HapMap Project [30] or the NCBI EntrezSNP database [31] and replicated samples were 100 % concordant with the original genotype calls; thus, no assays were excluded from analysis (Supplementary Table 2). As expected, significant LD was seen between CYP3A4*1B and CYP3A5*3C ($r = 0.93$) and the three polymorphisms in ABCB1 ($r > 0.7$), which were then grouped into haplotypes as planned (Supplementary Table 3).

Response by genotype

Clinical complete response to paclitaxel for the efficacy cohort was 30.1 % and the mean change in tumor size was a 49 % reduction (Table 2). Response by genotype is presented in Table 3, demonstrating significance only for CYP2C8; the odds ratio for an individual carrying CYP2C8*3 to achieve clinical complete response was 3.92 with a 95 % confidence interval of 1.46–10.48 (corrected $p = 0.046$) (Fig. 1). Of the 22 subjects carrying the CYP2C8*3 variant, 12 achieved clinical complete response (55 %) as compared with only 19 out of the 81 wild-type subjects (23 %). To ascertain whether this association was independent of other prognostic factors, a multivariable model that included tumor grade, ER status, concomitant trastuzumab, and whether paclitaxel was preceded by another phase of chemotherapy was tested. After backward elimination of covariates that were not significant, the final model included tumor grade and whether paclitaxel was preceded by another phase of chemotherapy. After controlling for these prognostic factors, the association of CYP2C8*3 status remained significant in the final model (uncorrected $p = 0.003$, corrected $p = 0.022$), while the other covariates were not significantly associated with achievement of clinical complete response (Table 4). Next, the association of CYP2C8*3 and clinical complete response was stratified by race to ensure that racial

Table 1 Patient characteristics ($N = 111$)

Demographic data of patients	
Self-reported race	
Caucasian	79 (71 %)
African-American	27 (24 %)
Other	5 (5 %)
Age (years)	
Median	50 (11.2)
Range	27–78
Menopausal status	
Pre-menopausal	57 (51 %)
Post-menopausal	54 (49 %)
Grade at diagnosis	
1	7 (6 %)
2	29 (26 %)
3	60 (54 %)
Unknown	15 (14 %)
Receptor status	
ER+	56 (50 %)
ER or PR+	63 (57 %)
HER2+	31 (28 %)
Stage at diagnosis	
IIA–IIB	42 (38 %)
IIIA–IIIC	59 (53 %)
IV	10 (9 %)
Taxane regimen[a]	
T	16 (14 %)
TC	2 (2 %)
TCH	1 (1 %)
TH	1 (1 %)
AC-T	73 (66 %)
AC-TH	18 (16 %)
Taxane schedule and dose	
Weekly (80–90 mg/m^2)	36 (32 %)
Q2 Weeks (175 mg/m^2)	52 (47 %)
Q3 Weeks (175 mg/m^2)	22 (20 %)
Q2.5 Weeks (175 mg/m^2)	1 (1 %)
Early paclitaxel discontinuation	
Toxicity	11 (10 %)
Disease progression	1 (1 %)
Total weeks of Taxane	
Median	9.0 (2.9)
Range	1–23

Counts and percentages (in parentheses) are presented for categorical data. Medians and standard deviations (in parentheses) are presented for quantitative data

A doxorubicin (Adriamycin), *C* cyclophosphamide (Cytoxan), *T* paclitaxel (Taxol), *H* trastuzumab (Herceptin)

[a] Regimen includes all drugs taken before or during paclitaxel treatment but not after. "–" indicates these are sequential treatments

Table 2 Response to paclitaxel therapy in efficacy cohort ($n = 103$)

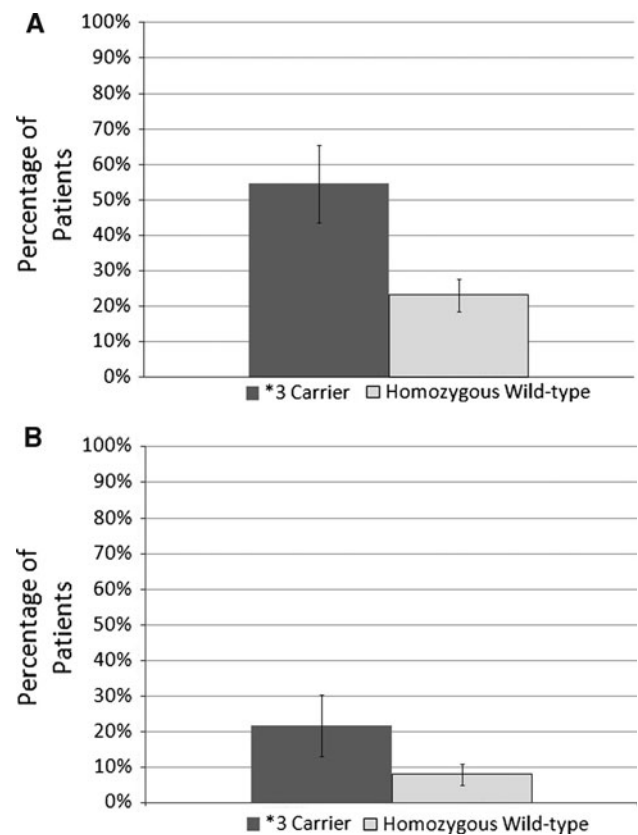| Therapy response	Data
Percentage change in tumor size	
Mean	−49 %
Median	−43 %
Maximum decrease	100 %
Maximum increase	29 %
Clinical response	
Clinical complete response	31 (30.1 %)
Clinical partial response	34 (33.0 %)
Clinical stable disease	36 (35.0 %)
Clinical progressive disease	2 (1.9 %)
Excluded (unevaluable)	8

Counts and percentages (in parentheses) are presented for categorical data

Table 3 Clinical complete response to treatment in efficacy cohort ($n = 103$) by genotype or haplotype, comparing variant carriers with wild-type homozygous patients

Gene variant	Odds ratio (95 % CI)	Uncorrected p value	Bonferroni corrected p value
CYP1B1*3	0.53 (0.21–1.33)	0.1761	1.0000
CYP2C8*3	3.92 (1.46–10.48)	0.0066	0.0459
CYP3A4*1B	0.88 (0.32–2.37)	0.7929	1.0000
CYP3A4/3A5 haplotype	1.16 (0.42–3.21)	0.7798	1.0000
ABCB1 3435	1.23 (0.50–3.01)	0.6548	1.0000
ABCB1 2677	1.98 (0.83–4.73)	0.1245	0.8716
ABCB1 haplotype	0.49 (0.15–1.61)	0.2389	1.0000

Fig. 1 Percentage of patients carrying CYP2C8*3 vs. CYP2C8*1 wild-type homozygotes achieving clinical complete response (**a**: *left*) or experiencing severe peripheral neuropathy (**b**: *right*). Patients carrying CYP2C8*3 were more likely to achieve clinical complete response (OR = 3.92, 95 % CI: 1.46–10.48, corrected p = 0.046). There was a trend toward greater risk of severe neuropathy in patients carrying the *3 variant, though it did not achieve statistical significance (OR = 3.13, 95 % CI: 0.89–11.01, uncorrected p = 0.075)

Table 4 Final multivariable model of clinical complete response in efficacy cohort ($n = 103$) including CYP2C8*3 and relevant covariates selected by backward elimination

	Odds ratio	95 % Confidence interval	Uncorrected p value
CYP2C8	5.11	1.73–15.12	0.003
Tumor grade	1.08	0.50–2.35	0.840
Preceded by another phase of chemotherapy	0.59	0.19–1.79	0.350

heterogeneity was not falsely inflating our results. In the self-reported Caucasian subjects, the magnitude of effect was marginally greater and the significance similar to that seen in the entire efficacy cohort (OR = 5.31, 95 % CI: 1.59–17.67, corrected p = 0.049). Only two non-Caucasians carried the *3 variant in our cohort so the association in non-Caucasians was not analyzed.

To evaluate the robustness of our finding, a secondary efficacy analysis was carried out with clinical response rate (cRR = cCR + cPR). The clinical response rate in the cohort was 63 % (65/103). In the CYP2C8*3 carriers, the response rate was 82 % (18/22) versus 58 % (47/81) in the CYP2C8 wild-type subjects. In the univariate logistic regression model, this association showed a strong trend in the same direction with an odds ratio of 3.16 (95 % CI: 0.98-10.19, uncorrected p = 0.054), supporting our primary findings.

To examine whether, as hypothesized, this finding was specific to paclitaxel, we then tested the association between CYP2C8*3 status and clinical complete response

Table 5 Severe toxicities (≥ grade 3) during paclitaxel treatment in toxicity cohort (n = 109)

Toxicities	Data
Any toxicity	
Any	34 (31 %)
None	75 (69 %)
Excluded	2
Specific toxicities	
Neuropathy	12 (11 %)
Neutropenia	10 (9 %)
Myalgia	9 (8 %)
Hypersensitivity	7 (6 %)
Fatigue	4 (4 %)
Gastrointestinal	3 (3 %)
Anemia	2 (2 %)
Other	4 (4 %)

Counts and percentages (in parentheses) are presented

to the doxorubicin–cyclophosphamide (AC) phase of therapy in patients who received the combination. Out of 100 subjects who received the AC combination who had evaluable response, the rate of clinical complete response was not significantly different between *3 carriers (2/22 = 9.0 %) and wild-type homozygotes (8/78 = 10.3 %) (OR = 1.14, 95 % CI: 0.62–15.96, uncorrected $p = 0.872$).

Toxicity by genotype

Of the 109 subjects included in the toxicity analysis, 34 experienced at least one grade 3 or higher toxicity (31.2 %) (Table 5); however, none of the genetic markers were associated with this cumulative endpoint (data not shown). Analysis of the secondary toxicity endpoint, grade 3 or higher peripheral neuropathy, revealed a trend toward increased neuropathy in subjects carrying the CYP2C8*3 variant (5/23 = 22 %) versus wild-type individuals (7/86 = 8 %) (OR = 3.13, 95 % CI: 0.89–11.01, $p = 0.075$) (Fig. 1, Supplementary Table 4).

Discussion

This study investigated pharmacogenetic predictors of breast cancer treatment outcomes following neoadjuvant paclitaxel. By measuring the tumor before and after each phase of sequential chemotherapy, and collecting toxicity during each phase separately, we were able to isolate the taxane-specific outcomes from the sequential therapy. We employed a candidate polymorphism replicate strategy based on reported associations with clinical outcomes in previous pharmacogenetic studies in taxane-treated cancer patients. These candidate genes covered the major metabolic pathways of paclitaxel, CYP3A4/3A5 and CYP2C8,

the efflux transporter ABCB1, and CYP1B1 that has been shown to influence taxane treatment efficacy.

Our results indicate that patients carrying the CYP2C8*3 polymorphism are more likely to achieve clinical complete response than patients homozygous for the wild-type isozyme. This finding is supported by the strong trend in the same direction for clinical response rate. The association with tumor response remained significant after adjustment for covariates and stratification by self-reported race. There was no association between CYP2C8 genotype and clinical complete response to the AC phase of sequential therapy, dismissing the possibility that patients carrying CYP2C8*3 had more chemosensitive tumors. Thus, there seems to be a true pharmacogenetic association between the CYP2C8*3 polymorphism and clinical response to neoadjuvant paclitaxel therapy.

Clinical response, instead of the more accepted pathological response, which has prognostic implications for future survival [32–34], was selected because of the collection of tumor size data between phases of sequential therapy, which enables us to isolate the response to the paclitaxel phase of treatment from that of the other administered therapy. Although clinical measurement is not a component of RECIST classification, that methodology was designed with radiographic measurements in the metastatic setting in mind. Conversely, pathologic response, a more conventional endpoint for neoadjuvant studies, cannot differentiate among drugs given preoperatively so would have introduced considerable noise from the inclusion of anthracycline and antimetabolite effect in the efficacy estimates. Furthermore, the ability to use response to the AC component of therapy as an internal control for the specificity of the findings for paclitaxel would have been lost. There is a documented relationship between clinical measurement and pathologic response [35] supporting the use of easily obtained serial clinical measurements in the palpable lesions relevant in this setting. The reported relationship between clinical and pathological response indicates that our finding may have an important influence on survival; however, it is essential that these findings are confirmed with pathological or radiographic tumor measurements before and after taxane therapy in independent patient cohorts.

Two previous groups have reported that patients carrying CYP2C8*3 are at a higher risk of paclitaxel-induced peripheral neuropathy [10, 11], but this finding was not observed in other studies [16, 36, 37]. All of these studies primarily included patients who were on combination therapy or who had been previously treated with chemotherapy. Our results, in previously untreated patients not receiving combination therapy with other neurotoxins, are consistent with those of Leskela et al. [11] and Green et al. [10] and suggest that there may be a true association

between CYP2C8*3 and risk of peripheral neuropathy. The difference between our study and that of Leskela et al. [11] is the event rate. Their study included grade 2+ neurotoxicity, a substantially more common phenotype than our grade 3+ endpoint, but one that does not require a change in therapy, unlike higher grades of neuropathy as were measured in this study. Additionally, they used a cumulative dose-to-event analysis, which is consistent with the cumulative nature of neurotoxicity. This was not feasible in our study given the relatively small number of patients (12) who experienced grade 3 or higher neuropathy. In fact, it is important to note that a general limitation of the current study is the modest sample size.

The paclitaxel parent compound, not its metabolites, is thought to be responsible for the drug's efficacy and toxicity [38]. Paclitaxel clinical outcomes are related to the amount of time the total drug concentration remains above a threshold level [39, 40] and the cumulative exposure may determine the extent of neuropathy development [10, 41]. The CYP2C8*3 variant has diminished in vitro metabolic activity for paclitaxel [42–45] and carriers of this variant have decreased clearance of free paclitaxel, and a corresponding increase in drug exposure [46]. These findings provide a rational mechanism for the increased paclitaxel treatment response and toxicity seen in CYP2C8*3 carriers in this study.

It is not possible to distinguish the influence of each of the two non-synonymous polymorphisms in CYP2C8*3 in this population because of the complete concordance, and it will be difficult to do this in any clinical study based on their high linkage disequilibrium. However, in vitro data suggest that the causative SNP is the K399R variant (rs10509681) that, unlike the R139K variant, has diminished paclitaxel metabolic activity when each is tested in isolation [42, 47]. If this were true, then it would be important for future researchers to focus their analyses specifically on the K399R variant that is sometimes present in patients without the R139K variant [48].

These patients were treated according to standard neoadjuvant protocols, which specify an appropriate treatment dose, schedule, and duration. Recent data demonstrate that the 3-weekly regimen received by 20 % of these patients is inferior to the weekly or every 2-week regimen [49]. In follow-up analyses, multivariable models that included treatment schedule were analyzed to see if schedule had a significant influence on the achievement of clinical complete response, which it did not (uncorrected $p = 0.100$, data not shown). The predefined treatment duration also ensures that the assessment of response and neuropathy is not confounded by dramatic differences in cumulative paclitaxel received. Only 12 patients discontinued paclitaxel before receiving the full course of therapy, 11 for toxicity, and 1 because of disease progression during treatment. Indeed,

carriers of the CYP2C8*3 variant received a similar number of cycles (median = 4) and weeks of therapy (mean = 9.99 vs. 9.60) compared with wild-type patients so it is unlikely that differences in response or neurotoxicity are attributable to differences in cumulative paclitaxel administered.

Numerous groups have investigated polymorphisms in other genes relevant to paclitaxel exposure or mechanism. The polymorphisms in CYP3A4 and CYP3A5 have been independently interrogated and typically do not show associations with outcome [13, 36, 50], though associations have been reported [51]. However, recent data suggest that looking at the CYP3A4*1B/3A5*1A variants as a high metabolic activity haplotype may be a superior strategy [26]. We were unable to identify a statistically significant association with paclitaxel treatment outcomes for either the CYP3A4*1B variant alone or the two variants in combination.

The ABCB1 variants have also been the focus of a number of retrospective pharmacogenetic studies, with inconsistent results. Variants at the 3435 position have been associated with shorter overall survival and worse progression free survival in paclitaxel-treated cancer patients [8, 9]. Variants at the 3435 and 2677 position have been implicated in higher risk of paclitaxel-induced neutropenia [17] and docetaxel treatment outcomes [27]. In our study, the variants of ABCB1 tested individually or in haplotypes did not have a statistically significant effect on paclitaxel treatment outcome.

CYP1B1 is not involved in taxane metabolism [52], yet an association between taxane efficacy and the CYP1B1*3 variant has been repeatedly demonstrated [13, 53–55]. In vitro studies by Sissung et al. [27] reveal that CYP1B1*3 enhances estrogen metabolism to compounds that antagonize the mechanism of action of docetaxel and paclitaxel, and covalently bind docetaxel, providing two plausible mechanisms for the decreased efficacy seen in patients with this genotype [53]. We found no evidence of a link between CYP1B1 genotype and paclitaxel efficacy.

In conclusion, we report evidence that CYP2C8*3 carriers are more likely to achieve complete clinical response to neoadjuvant paclitaxel. This association was independent of other important clinical covariates. The odds of an individual who carried the *3 variant achieving clinical complete response were nearly four times higher than those for an individual carrying two wild-type alleles. Additionally, our results support the previously reported possibility that individuals carrying this variant are at increased risk of experiencing paclitaxel related neuropathy. Our data suggest a potential biomarker for identifying patients before treatment who are more likely to benefit from therapy, but may be at an increased risk of experiencing certain adverse events. The results of this small study warrant further investigation of this association in larger neoadjuvantly treated patient cohorts, and if confirmed,

may prompt studies of dose individualization based on host genotype.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All experiments comply with current laws of the United States of America.

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