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Authors

Baldwin, R Michael

Owzar, Kouros

Zembutsu, Hitoshi

et al.

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A Genome-Wide Association Study Identifies Novel Loci for Paclitaxel-Induced Sensory Peripheral Neuropathy in CALGB 40101

R. Michael Baldwin¹, Kouros Owzar^{5,6}, Hitoshi Zembutsu¹¹, Aparna Chhibber¹, Michiaki Kubo¹¹, Chen Jiang⁶, Dorothy Watson⁶, Rachel J. Eclov¹, Joel Mefford², Howard L. McLeod⁷, Paula N. Friedman⁸, Clifford A. Hudis⁹, Eric P. Winer¹⁰, Eric M. Jorgenson^{3,4}, John S. Witte^{2,4}, Lawrence N. Shulman¹⁰, Yusuke Nakamura¹¹, Mark J. Ratain⁸, and Deanna L. Kroetz^{1,4}

Abstract

Purpose: Sensory peripheral neuropathy is a common and sometimes debilitating toxicity associated with paclitaxel therapy. This study aims to identify genetic risk factors for the development of this toxicity.

Experimental Design: A prospective pharmacogenetic analysis of patients with primary breast cancer, randomized to the paclitaxel arm of CALGB 40101, was used to identify genetic predictors of the onset and severity of sensory peripheral neuropathy. A genome-wide association study in 855 subjects of European ancestry was conducted and findings were replicated in additional European ($n = 154$) and African American ($n = 117$) subjects.

Results: A single nucleotide polymorphism in *FGD4* was associated with the onset of sensory peripheral neuropathy in the discovery cohort [rs10771973; HR, 1.57; 95% confidence interval (CI), 1.30–1.91; $P = 2.6 \times 10^{-6}$] and in a European (HR, 1.72; 95% CI, 1.06–2.80; $P = 0.013$) and African American (HR, 1.93; 95% CI, 1.13–3.28; $P = 6.7 \times 10^{-3}$) replication cohort. There is also evidence that markers in additional genes, including *EPHA5* (rs7349683) and *FZD3* (rs10771973), were associated with the onset or severity of paclitaxel-induced sensory peripheral neuropathy.

Conclusions: A genome-wide association study has identified novel genetic markers of paclitaxel-induced sensory peripheral neuropathy, including a common polymorphism in *FGD4*, a congenital peripheral neuropathy gene. These findings suggest that genetic variation may contribute to variation in development of this toxicity. Validation of these findings may allow for the identification of patients at increased risk of peripheral neuropathy and inform the use of an alternative to paclitaxel and/or the clinical management of this toxicity. *Clin Cancer Res*; 18(18); 5099–109. ©2012 AACR.

Authors' Affiliations: Departments of ¹Bioengineering and Therapeutic Sciences, ²Epidemiology and Biostatistics, ³Neurology, and ⁴Institute for Human Genetics, University of California San Francisco, San Francisco, California; ⁵Department of Biostatistics and Bioinformatics and ⁶CALGB Statistical Center, Duke University, Durham; ⁷Department of Pharmacotherapy and Experimental Therapeutics, University of North Carolina, Chapel Hill, North Carolina; ⁸Department of Medicine, University of Chicago, Chicago, Illinois; ⁹Memorial Sloan-Kettering Cancer Center, New York, New York; ¹⁰Dana-Farber Cancer Institute, Boston, Massachusetts; and ¹¹University of Tokyo and Riken Center for Genomic Medicine, Yokohama, Japan

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R.M. Baldwin and K. Owzar contributed equally to this work.

Corresponding Author: Deanna L. Kroetz, Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, 1550 4th Street RH584E, San Francisco, CA 94158-2911. Phone: 415-476-1159; Fax: 415-514-4361; E-mail: deanna.kroetz@ucsf.edu

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Introduction

Paclitaxel is a useful microtubule-stabilizing agent with efficacy in the treatment of many cancers. It is effective for the treatment of breast cancer in the metastatic, adjuvant, and neoadjuvant settings (1, 2). Sensory peripheral neuropathy remains a significant issue in the clinical use of this agent. More than 50% of patients experience some degree of sensory peripheral neuropathy during their course of paclitaxel treatment, with 5% to 30% experiencing grade 3 or 4 toxicity (3, 4). Paclitaxel-induced sensory peripheral neuropathy is dose-, treatment schedule-, and infusion time-dependent (3). Cumulative dose is a significant predictor of sensory peripheral neuropathy, as is underlying diabetes and concurrent or previous administration of other drugs associated with this toxicity. A recent study suggests that mild to moderate symptoms of sensory peripheral neuropathy can persist for up to 2 years following completion of paclitaxel treatment (5). Long-term neuropathy is

Translational Relevance

Paclitaxel is widely used in the treatment of many cancers, including breast cancer. Treatment with paclitaxel is often limited by the development of peripheral neuropathies that can significantly impact a patient's quality of life. Biomarkers for the prediction of paclitaxel-induced peripheral neuropathy could be used to optimize the use of paclitaxel. A genome-wide genotyping approach in women receiving single-agent paclitaxel as adjuvant therapy for breast cancer identified several novel genetic loci implicated in paclitaxel-induced sensory peripheral neuropathy. In particular, a common genetic variant in *FGD4*, a causal gene for the congenital peripheral neuropathy Charcot-Marie-Tooth disease, was associated with increased onset of neuropathy in both Europeans and African Americans. This variant and others identified in these studies could be validated as genetic predictors of paclitaxel-induced sensory peripheral neuropathy. The genetic variants identified in these studies will also lead to investigations into novel pathways for this common chemotherapy-induced toxicity.

particularly concerning for patients with primary breast cancer, such as those evaluated in the current study, as more than 80% will be long-term survivors whose quality of life will be compromised. Significant sensory peripheral neuropathy during paclitaxel treatment can lead to dose reductions and treatment suspension, possibly resulting in suboptimal disease treatment and the potential for an increased likelihood of relapse. A predictive marker for this dose-limiting toxicity would enable studies to identify whether an individualized assessment of adverse event risk could be useful in the clinical decision making process. It could also provide a possible target for therapeutic interventions.

Substantial interindividual differences in the prevalence, reported and objective severity, and onset of peripheral neuropathy is consistent with an underlying genetic susceptibility to this toxicity. CALGB 40101 is a phase III randomized study comparing cyclophosphamide and doxorubicin versus single-agent paclitaxel as adjuvant therapy for patients with breast cancer at relatively low risk for relapse. In addition, the study compared short versus longer therapy of each regimen as a 2×2 factorial design. A pharmacogenetic companion study (CALGB 60202) was included in this trial to prospectively evaluate germline determinants of interindividual differences in response and toxicity. An initial analysis of treatment outcome in CALGB 40101 has shown no difference in response between the 4 and 6 cycle treatment arms (6); additional analyses of response await complete follow-up data. The goal of this present study was to identify genetic markers predictive of sensory peripheral neuropathies in the paclitaxel treatment arm of CALGB 40101 and to further our understanding of the underlying mechanism of injury and repair. Herein, we report the results of a genome-wide association study

(GWAS) of 1,040 paclitaxel-treated women to identify novel germline susceptibility loci associated with the development of sensory peripheral neuropathies. This represents the largest prospective breast cancer pharmacogenetic study of paclitaxel treatment toxicities to date and provides a paradigm for the identification of genetic markers with potential clinical application in personalized medicine.

Materials and Methods

Participants

All study participants were enrolled in CALGB 40101 and gave their additional consent to participate in the pharmacogenetic companion study (CALGB 60202). CALGB 40101 was open from May 15, 2002 until July 30, 2010. The final total accrual was 3,873 patients. Patients eligible for the treatment protocol were females with histologically confirmed invasive carcinoma of the breast and 0 to 3 axillary nodes positive for cancer. Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, adequate organ function, and absence of congestive heart failure or myocardial infarction in the previous 6 months were required. Enrollment was required within 84 days of breast surgery (either modified radical mastectomy or lumpectomy) and the treatment began within 7 days of registration. Patients with locally advanced, inflammatory, or metastatic breast cancer or involvement of dermal lymphatics were ineligible. Patients were disease-free from any prior malignancies for at least 5 years. Previous trastuzumab, chemotherapy, or hormonal therapy, with the exception of tamoxifen, for the current malignancy was not permitted nor was anthracycline treatment for any previous disease. Patients who received tamoxifen or any other selective estrogen receptor modulators (SERM) for prevention or other indications (e.g., osteoporosis) were eligible. Treatment with tamoxifen, other SERMs, or exogenous hormones (e.g., hormone replacement therapy, oral contraceptives, raloxifene) was discontinued before enrollment. Trastuzumab was recommended for patients with HER2-positive disease. Patients could also enroll in adjuvant studies of bisphosphonates or hormonal therapies (e.g., ovarian suppression concurrent with chemotherapy). All patients provided written informed consent for both the treatment and companion protocols that met state, federal, and institutional guidelines.

Treatment

Patients were randomly assigned with equal probability to 4 or 6 cycles of cyclophosphamide/doxorubicin (AC) or paclitaxel. A full description of the study design is included in a recent publication describing the initial analysis of treatment response (6). The first 570 patients were treated with AC every 3 weeks, or paclitaxel weekly for 12 or 18 weeks. Thereafter, both regimens were administered every 2 weeks for 4 or 6 cycles. Pharmacogenetic samples were collected only from patients enrolled on every 2-week regimens, who received dose-dense paclitaxel for 4 or 6 cycles. Paclitaxel was given for more than 3 hours at 175 mg/m^2 when given every 2 weeks. The 6 cycle treatment arms for

Table 1. Patient demographics

		Randomized ^a	Post Quality Control ^b	Discovery ^c	European Replication	African American Replication
Sample size		1,940	1,023	855	154	117
Age	Mean (SD)	53.4 (9.6)	53.4 (9.6)	53.7 (9.6)	55.2 (9.4)	54.1 (9.2)
Self-reported race and ethnicity	White (Non-Hispanic/Non-Latino)	1,434	788	772	143	1 ^d
	White (Hispanic or Latino)	63	15	1	—	—
	White (Unknown)	115	77	72	11	—
	Black or African American (Non-Hispanic/Non-Latino)	204	85	—	—	101
	Black or African American (Hispanic or Latino)	10	2	—	—	1 ^d
	Black or African American (Unknown)	17	13	1	—	13
	Asian	29	10	—	—	—
	Native Hawaiian or Pacific Islander	2	2	—	—	—
	American Indian or Alaska Native	18	7	2	—	—
	Multiple	2	—	—	—	—
	Unknown (Non-Hispanic/Non-Latino)	9	4	4	—	—
	Unknown (Hispanic or Latino)	24	13	—	—	—
	Unknown (Unknown)	13	7	3	—	1 ^d
Menopausal status	Post	1,176 (61%)	609 (60%)	513 (60%)	99 (64%)	81 (69%)
	Pre	764 (39%)	414 (40%)	342 (40%)	55 (36%)	36 (31%)
ER/PR Status	ER+/PR+	1,059 (55%)	546 (53%)	475 (56%)	108 (70%)	35 (30%)
	ER+/PR—	223 (11%)	119 (12%)	98 (11%)	15 (10%)	18 (15%)
	ER+/PR unknown	3 (<1%)	2 (<1%)	2 (<1%)	1 (1%)	0 (<1%)
	ER-/PR+	24 (1%)	15 (1%)	13 (2%)	1 (1%)	2 (2%)
	ER-/PR—	629 (32%)	341 (33%)	267 (31%)	29 (19%)	62 (53%)
	ER unknown/PR unknown	2 (<1%)	—	—	—	—
HER2 status	Positive	1,505 (78%)	790 (77%)	660 (77%)	120 (78%)	88 (75%)
	Negative	361 (19%)	195 (19%)	163 (19%)	27 (18%)	26 (22%)
	Unknown	74 (4%)	38 (4%)	32 (4%)	7 (5%)	3 (3%)
Assigned number of cycles	4	1,151 (59%)	572 (56%)	471 (55%)	139 (90%) ^e	75 (64%)
	6	789 (41%)	451 (44%)	384 (45%)	15 (10%)	42 (36%)

^a Randomized refers to all patients enrolled in CALGB 40101 and assigned to the paclitaxel treatment arm.

^b Post quality control refers to patients with whole genome data passing quality control ($n = 1,029$) and excluding patients without evaluable phenotype data ($n = 6$).

^c Discovery cohort is all patients with Northwestern European ancestry and evaluable phenotype data.

^d Identified using principal components analysis of whole genome data.

^e This reflects the early closure of the 6 cycle arm of the study.

both drugs were closed after enrolling 3,172 patients. Arms were stratified by menopausal, estrogen receptor (ER), progesterone receptor (PR), and HER2 status. Patient demographics are shown in Table 1. Premedication recommendations for the initial dose were 12.5 to 50 mg diphenhydramine and 50 mg ranitidine, 300 mg cimetidine, or 20 mg famotidine administered i.v. 30 to 60 minutes before paclitaxel. Dexamethasone was given as a 10 mg i.v. dose within 60 minutes of paclitaxel or alternatively, as a 10 mg or 20 mg oral dose more than one hour before paclitaxel. To facilitate the 14-day dosing

schedule, filgrastim was recommended on days 3 to 10 of each cycle (5 µg/kg rounded to either 300 or 480 µg). Sargramostim (250–500 µg/m², days 3–10) or pegfilgrastim (6 mg s.c., 24–36 hours after paclitaxel) could be used in place of filgrastim. The treating physician could omit granulocyte colony-stimulating factor (G-CSF) treatment when confident neutrophils would recover within 14 days; however, if treatment could not be delivered on schedule, then a G-CSF was required in subsequent cycles. Erythropoietin was permitted at the discretion of the treating physician. Patients positive for HER2 by either

immunohistochemical 3⁺ staining or gene amplification by FISH could initiate adjuvant trastuzumab concurrent with paclitaxel (weekly administration) or at the completion of paclitaxel (weekly or every 3 weeks). Weekly trastuzumab consisted of a 4 mg/kg i.v. loading dose followed by weekly doses of 2 mg/kg and the 3-week schedule of a loading dose of 8 mg/kg and 6 mg/kg every 3 weeks for a total duration of one year.

Genotyping and quality control

A summary of the steps included in sample and single nucleotide polymorphism (SNP) quality control and in principal components analysis (PCA) is illustrated in Supplementary Fig. S1. A total of 1,040 paclitaxel-treated patients with informed consent and a DNA sample (obtained from peripheral blood) available as of July 1, 2009 were included in the primary study. Genomic DNA was genotyped using the HumanHap610-Quad Genotyping BeadChip (Illumina), which interrogated 592,532 SNPs. Subjects with call rates less than 0.98 ($n = 5$) or with suboptimal genotype clustering performance ($n = 1$) were excluded followed by reassessment of genotypes within the remaining subjects. SNPs with call rates less than 0.95, poor genotype clustering performance, more than 1 replicate or Mendelian discordance, relative minor allele frequency (MAF) less than 0.005, nondiploid (e.g., Y or mitochondrial chromosomes), or deemed unreliable by Illumina ($n = 4,106$; Tech Note: Infinium Genotyping Data Analysis, 2007) were excluded, leaving 572,745 SNPs. Identity-by-descent (IBD) analysis verified the absence of closely related individuals (proportion IBD > 0.15) and identified one unintended duplicate pair, which was removed and later confirmed to be due to a DNA plating error (PLINK version 1.07; ref. 7). Evaluation of X-chromosome heterozygosity identified 3 genetic males that were also removed and similarly confirmed to be due to a DNA plating error (8). PCA, as implemented by EIGENSOFT version 3.0, was used to visualize the genetic ancestry of the 1,029 individuals passing quality control (9). PCA was conducted using genotypes from study subjects combined with genotypes of unrelated individuals from the HapMap Project representing Northwest European (CEU, $n = 73$), African (YRI, $n = 77$), and Chinese (CHB, $n = 75$) ancestries and genotyped using the same platform by Illumina (Supplementary Fig. S2; ref. 10). To address the potential bias arising from population stratification, we chose to focus our primary analysis on individuals of Northern European descent. A second PCA was conducted using only 1,029 study subjects. Mean values for the first 3 eigenvectors within all patients self-declaring "White" race and "Non-Hispanic" ethnicity were determined. "Genetic Northwest Europeans" (herein called Europeans) were defined as individuals with each of their first 3 eigenvectors within 2 SDs of each mean value irrespective of self-declared race and ethnicity. A total of 859 individuals were identified and identical results were obtained when repeated with the inclusion of HapMap individuals (data not shown). These 859 individuals were the focus of the primary analysis (Supplementary Fig. S2).

Imputation of genotypes was conducted within the 859 Europeans using MACH 1.0 (11) and reference haplotypes from unrelated CEU individuals from either HapMap (r22) or the 1000 Genomes Project (June 2010 release). Before imputation, study genotypes were more stringently filtered and limited to autosomal SNPs with MAF 0.01 or more and exact Hardy-Weinberg P values ≥ 0.001 in control subjects. To address any potential stranding inconsistencies between study genotypes and the reference haplotypes, all symmetric SNPs (A/T or C/G) with MAF more than 0.40, and therefore difficult to resolve, were removed leaving 548,596 and 547,465 SNPs for imputation using the HapMap and 1000 Genomes reference haplotypes, respectively. Imputed SNPs with MAF less than 0.01 or R^2 less than 0.5 were excluded. Genotyping within the replication cohorts (described below) was conducted using TaqMan Allelic Discrimination assays (Applied Biosystems), and individual assays are shown in Supplementary Tables S1 and S2.

One hundred fifty nine self-declared "White" individuals with either "Non-Hispanic" or "Unknown" ethnicity, who enrolled in the CALGB 40101 pharmacogenetic companion study subsequent to the genotyping of the original 1,040 subjects, were used as a replication cohort. Within the discovery set, these criteria accurately identified 98.7% of the 859 Europeans with a false-positive rate of 2.4%. An additional 100 individuals of African ancestry were also identified from within the group of 1,029 individuals passing sample quality control. African ancestry was defined using individuals who self-declared "Black/African American" race with either "Non-Hispanic" or "Unknown" ethnicity. Any individual with their first 3 eigenvectors within 3 SDs of each eigenvector mean value were considered to be of African descent. This self-declared race/ethnicity criteria identified 94.2% of individuals with African ancestry and incorrectly identified 2.0%. The final African American replication cohort consisted of the 100 patients of African descent with genome-wide data and an additional 20 self-declared "Black/African American" individuals with either "Non-Hispanic" or "Unknown" ethnicity, who enrolled after the original genotyping.

Statistical analysis

The primary objective was the identification of SNPs associated with the occurrence of sensory peripheral neuropathy. The analyses were carried out using 2 complementary endpoints: (i) the cumulative dose level triggering the first grade II or higher treatment related sensory peripheral neuropathy episode and (ii) the maximum observed treatment-related sensory peripheral neuropathy grade. The adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 2.0. The timing of sensory peripheral neuropathy was assessed with a time-to-event approach in which an event was defined as the first incidence of a grade II or higher neuropathy and the time as cumulative paclitaxel exposure (mg/m^2). For patients not experiencing any event, the total study paclitaxel drug exposure was used. These patients are effectively right-censored at the cumulative dose

level. The marginal associations were tested using the Cox score test (12). The severity of sensory peripheral neuropathy, defined as the maximum grade neuropathy observed during paclitaxel treatment or within 30 days following the last dose, was evaluated using ordinal logistic regression. Cumulative dose (mg/m^2) was log-transformed and incorporated into the ordinal regression model. For both cases, the marginal null sampling distribution was approximated using asymptotics. These analyses were powered for an additive genetic model. To minimize type I error due to sparseness, SNPs within the European discovery set were constrained to relative MAF of 1% or more and the observation of a minimum of 2 minor allele homozygous genotypes leaving 521,600 evaluable SNPs. Imputed genotypes were represented as allele dosages bound between 0.0 and 2.0. All analyses were conducted using the *R* statistical environment version 2.12 with the cumulative dose-to-event and ordinal analyses implemented using functions from the survival and MASS extension packages (13–16). Quantile-quantile plots of the marginal asymptotic *P* values were evaluated for potential remaining population stratification or inflation of significance levels. Each SNP with a marginal *P* value $\leq 10^{-5}$ was evaluated further for potential errors by checking its MAF (vs. HapMap), Hardy–Weinberg Equilibrium within unaffected subjects, and potentially informative missing rates; they were also visually inspected for genotype clustering performance.

On the basis of the combined results of the time-to-event and ordinal regression analyses of the 859 European patients, a replication plan delineating SNPs, regression model, genetic model (the most plausible model suggested from Kaplan–Meier estimates), and effect direction for one-sided testing was drafted *a priori* to any data collection within the replication cohorts. Three SNPs from the genes *FZD3*, *EPHA5*, and *FGD4* (rs7001034, rs7349683, and rs10771973) were selected for replication based on marginal significance levels, biologic plausibility, and estimated effect size (as detailed in the Results). An additional 10 SNPs with *P* values $< 10^{-5}$ and/or previously implicated in congenital sensory peripheral neuropathies (*NDRG1*) were also evaluated with the specified limitation of being constrained to exploratory analyses. Genotypes for the *FZD3* SNP rs7001034 were captured indirectly using a proxy SNP (rs7833751; $R^2 = 1.0$ CEU HapMap r27) due to the absence of acceptable TaqMan assays to evaluate the locus directly. Because of the impracticality of capturing the *FZD3* linkage disequilibrium (LD) block to the same extent as the European group, this locus was not evaluated in the African ancestry replication group. Direct sequencing was used to capture the *FGD4* rs10771973 genotypes within the replication cohorts. To limit the overall type I error rate for the validation study at the one-sided 0.05 level, we tested each of the 3 SNPs at the marginal 0.01 level. Because the *FGD4* locus replicated in both populations and there are significant differences in LD structure between the European and African American populations, an additional 4 coding region SNPs were chosen from the approximately 30 kb LD block containing rs10771973 to further extend this

finding. In addition, to evaluate the independence of the identified association in rs10771973, the time-to-event analysis was repeated with rs10771973 as a covariate. This analysis was conducted using the *R* extension package GenABEL (13). A haplotype-based association test was also conducted for the 3 genes containing the top hits (*EPHA5*, *FZD3*, and *FGD4*), using all genotyped SNPs within 100 kb of the transcription start and stop sites for each gene. Phase for each SNP set was estimated using fastPHASE v 1.1 in all samples combined (17). Haplotype block boundaries using the method of Gabriel and colleagues were generated in Haploview v4.2 using HapMap v3 r2 CEU samples (18). For each haplotype block that included an allele with a per SNP association signal of less than 10^{-3} , individual haplotypes were extracted from fastPHASE output, and haplotypes with frequency less than 5% were combined. Association with outcome was analyzed on a per haplotype basis using time-to-event or maximum grade as described above.

Results

Of the 859 individuals with European ancestry randomly assigned to paclitaxel treatment, 4 withdrew before study treatment and were therefore excluded (Supplementary Fig. S1). Patient characteristics of the CALGB 40101 paclitaxel treatment arm, the genotyped samples, and the discovery and replication cohorts are listed in Table 1. The menopausal, ER, PR and HER2 status, and the assigned number of cycles were not different between the genotyped paclitaxel cohort and the European discovery cohort. The genotyped sample was also representative of all patients randomized to paclitaxel treatment in CALGB 40101. One exception is a fewer number of samples from the 6 cycle paclitaxel arm in the European replication cohort, which reflects the early closure of the 6 cycle arm and the later study enrollment of this group of patients. Peripheral sensory neuropathy was the major dose limiting toxicity in the paclitaxel arm, and the distribution of toxicity grades within the 855 patients in the primary analysis, stratified for number of treatment cycles assigned, is shown in Table 2. Sensory peripheral neuropathy was dose dependent with 17% of the patients randomized to 4 cycles of paclitaxel experiencing a grade II or greater event as compared with 33% of those randomized to 6 cycles of treatment. The cumulative incidence of sensory peripheral neuropathy was similar between the entire cohort randomized to paclitaxel treatment and the discovery set (Supplementary Fig. S3), and between the discovery set and both replication groups (data not shown). There was no effect of age on cumulative dose triggering a grade II or greater peripheral neuropathy event (data not shown).

Among the SNPs analyzed in the GWAS for association with the initial onset of sensory peripheral neuropathy, none reached genome-wide significance although 7 had a marginal significance level of $P < 10^{-5}$ (Table 3 and Supplementary Fig. S4). Inspection of the quantile-quantile plot of the marginal *P* values (Supplementary Fig. S5A) indicates the absence of any remaining population substructure ($\lambda = 1.01$). Of these top SNPs, biologic relevance

Table 2. Incidence of sensory peripheral neuropathies in study groups

	Sensory peripheral neuropathy grade					Event rate ^b
	0	1	2	3	4	
Discovery set						
4 Cycles	181 (38%) ^a	209 (44%)	66 (14%)	15 (3%)	0 (0%)	17%
6 Cycles	99 (26%)	160 (42%)	81 (21%)	44 (11%)	0 (0%)	33%
European replication						
4 Cycles	44 (32%)	67 (48%)	24 (17%)	4 (3%)	0 (0%)	20%
6 Cycles	6 (40%)	4 (27%)	3 (20%)	2 (13%)	0 (0%)	33%
African American Replication						
4 Cycles	23 (31%)	33 (44%)	12 (16%)	7 (9%)	0 (0%)	25%
6 Cycles	9 (21%)	19 (45%)	7 (17%)	6 (14%)	1 (2%)	33%

^a Number of patients and percentage (in parentheses) of all patients in the discovery or replication cohort assigned to 4 or 6 cycles of dose dense paclitaxel.

^b Event rate is the incidence of a grade 2 or greater sensory peripheral neuropathy.

was apparent for polymorphisms in *EPHA5* (rs7349683; per allele HR, 1.63; 95% CI, 1.34–1.98; $P = 9.6 \times 10^{-7}$; Fig. 1A) and *FGD4* (rs10771973; per allele HR, 1.57; 95% CI, 1.30–1.91; $P = 2.6 \times 10^{-6}$; Fig. 1B). *EPHA5* encodes an ephrin receptor gene implicated in the process of neuronal regeneration following nerve injury and *FGD4* encodes a Rho-GTPase guanine nucleotide exchange factor previously implicated in congenital peripheral neuropathies (19–22). The *FGD4* (Table 3; Supplementary Fig. S6A and S6B) and *EPHA5* (Table 3; Supplementary Fig. S7A and S7B) SNPs were tested in replication cohorts, and association for the former was confirmed in both the European and African American samples (Europeans: rs10771973; per allele HR, 1.72; 95% CI, 1.06–2.80; $P = 0.013$; African Americans: rs10771973; per allele HR, 1.93; 95% CI, 1.13–3.28; $P = 6.7 \times 10^{-3}$). Considering the high minor allele frequency of this risk allele in Europeans, 42% of patients are expected to have a 1.6-fold increased risk and 9% a 2.6-fold increased risk of peripheral neuropathy; in African Americans (MAF 17%), the increased risk is 1.9- and 3.7-fold, respectively. Inspection of the Kaplan–Meier genotype stratified time to neuropathy distributions suggests that an allele dose–effect assumption for *FGD4* rs10771973 is appropriate (Fig. 1B).

No haplotypes in *FGD4* or *EPHA5* showed stronger association with time to sensory peripheral neuropathy than the single SNP analyses in these regions (data not shown). After conditioning the time-to-event analysis on rs10771973, no other genotyped markers at the *FGD4* locus showed association with time to peripheral neuropathy (data not shown). Using imputation to infer additional untyped markers and visualizing the LD structure within the HapMap CEU population revealed an approximately 30 kb region of high LD within the *FGD4* locus showing a strong and reproducible association with the onset of sensory peripheral neuropathy (Supplementary Fig. S8). Approximately, 16 SNPs are strongly linked ($R^2 \geq 0.80$) with rs10771973, 5 of which are synonymous variants within the coding region.

Ordinal logistic regression analyses were used to identify SNPs associated with the severity of sensory peripheral neuropathy. Four SNPs were associated with toxicity grade with a significance level of $P < 1 \times 10^{-5}$ (Table 4 and Supplementary Fig. S4). As with the Cox analysis, a quantile-quantile plot of the normalized marginal P values (Supplementary Fig. S5B) suggests the absence of any remaining population substructure ($\lambda = 0.986$). A SNP within the Frizzled 3 homolog WNT signaling receptor gene (*FZD3*) (rs7001034; $P = 3.1 \times 10^{-9}$; OR, 0.57; 95% CI, 0.48–0.69) and showed a clear relationship between allele dosage and sensory peripheral neuropathy grade (Fig. 2). However, none of these top SNPs from the ordinal regression analysis replicated in either the European or African American populations (Table 4).

Discussion

A small subset of patients exposed to paclitaxel have significant and occasionally protracted neuropathy that has a major impact on quality of life. If we could prospectively identify these patients before administration of paclitaxel, they might be otherwise equally well served with alternative nonpaclitaxel containing regimens. Using a genome-wide association study of CALGB 40101, we have identified several genetic loci associated with the onset or severity of paclitaxel-induced sensory peripheral neuropathy. One of these novel markers associated with early-onset, paclitaxel-induced sensory peripheral neuropathy (*FGD4*, rs10771973) was replicated in both Europeans and African Americans and resides within a gene with a clearly established role in the hereditary peripheral neuropathy Charcot–Marie–Tooth disease (CMT). These findings will inform studies to test the application of genetic markers for optimization of paclitaxel selection, dosing, and adverse event management. Several features of the study design and analysis support the robustness of our findings, including

Table 3. Top SNPs from cumulative dose to event analysis

SNP	Chr	Gene ^a	Alleles	Discovery (n = 855)			European replication (n = 154) ^d			African American replication (n = 117) ^d		
				MAF ^b	HR (95% CI)	P ^c	MAF	HR (95% CI)	P	MAF	HR (95% CI)	P
rs7349683	4	EPHA5	C/T	0.36	1.63 (1.34–1.98)	9.6 × 10 ⁻⁷	0.32	0.96 (0.57–1.60)	0.43	0.13	1.16 (0.55–2.42)	0.35
rs4737264	8	XKR4	A/C	0.22	1.68 (1.36–2.09)	1.9 × 10 ⁻⁶	0.24	1.84 (1.02–3.33)	0.021	0.18	1.23 (0.69–2.21)	0.24
rs10771973	12	FGD4	G/A	0.31	1.57 (1.30–1.91)	2.6 × 10 ⁻⁶	0.33	1.72 (1.06–2.80)	0.013	0.17	1.93 (1.13–3.28)	6.7 × 10 ⁻³
rs16948748	17	PITPNA	T/G	0.04	2.37 (1.63–3.44)	2.7 × 10 ⁻⁶	0.02	2.65 ^e (0.63–11.1)	0.083	0.07	1.07 ^e (0.41–2.77)	0.45
rs16916932	10	CACNB2	C/T	0.06	2.08 (1.51–2.87)	4.3 × 10 ⁻⁶	0.06	0.38 ^e (0.09–1.58)	0.082	0.08	1.13 ^e (0.47–2.74)	0.39
rs17781082	12	GRIP1/CAND1	C/T	0.42	1.60 (1.31–1.96)	4.3 × 10 ⁻⁶	0.43	1.22 (0.74–1.99)	0.22	0.21	1.32 (0.76–2.30)	0.16
rs1903216	3	BCL6/	G/A	0.48	1.59 (1.30–1.95)	5.6 × 10 ⁻⁶	0.41	2.08 ^f (0.99–4.37)	0.024	0.03	3.02 ^f (1.04–8.73)	0.016
rs2233335	8	NDRG1	T/G	0.38	0.65 (0.52–0.80)	5.2 × 10 ⁻⁵	0.39	0.94 (0.58–1.53)	0.41	0.23	1.40 (0.75–2.60)	0.14

^a Intergenic SNPs are denoted by the closest flanking annotated gene(s).^b MAF was calculated within the indicated cohort.^c P values are 2-sided for discovery analysis and one-sided for replication.^d As stated in the replication plan, analyses were exploratory for all except the *EPHA5* and *FGD4* SNPs.^e Analysis assumed a dominant model.^f Analysis assumed a recessive model.

the prospective design, a large cohort of patients with primary breast cancer who are chemotherapy naive and treated with single-agent paclitaxel, careful collection of sensory peripheral neuropathy and covariate data, strict censoring for dose and cycle reductions for other adverse reactions and preexisting neuropathy, and the use of cumulative dose to the initial incidence of grade 2 toxicity to account for the established effect of total drug exposure on sensory peripheral neuropathy.

The current finding that *FGD4* plays a role in the development of paclitaxel-induced sensory peripheral neuropathy and/or the repair response of peripheral nerves following paclitaxel injury is consistent with the known functions of the gene. *FGD4* encodes for the protein FGD1-related F-actin binding protein (Frabin), and previous studies have shown specific point mutations in *FGD4* can cause the congenital peripheral neuropathy CMT (CMT4H; refs. 21–24). The disease is characterized by a slow progressive demyelination of peripheral sensory and motor neurons accompanied by distal muscle weakness and atrophy, sensory loss, hyporeflexia, and skeletal deformity (25). Paclitaxel-induced peripheral neuropathy shares some of these characteristics, including sensory loss and secondary demyelination (26–28). Frabin is a guanine nucleotide exchange factor for cdc42, a Rho-GTPase that regulates cellular morphogenesis, including myelination. Several hypotheses have been proposed to explain how mutations in *FGD4* might lead to demyelinating CMT4H disease, including disruption of the actin/microtubule cytoskeleton, loss of c-Jun-NH-terminal kinase (JNK) activation signals, and disruption of phosphoinositide signaling pathways, all of which could affect Schwann cell myelination and/or the bidirectional communication between Schwann cells and axons (21).

The observed association between the *FGD4* SNP rs10771973 and paclitaxel-induced sensory peripheral neuropathy is consistent with the hypothesis that common *FGD4* polymorphisms subtly affect the development and/or maintenance of Schwann cell function. In this case, carriers of common *FGD4* polymorphisms would have preexisting subclinical abnormalities and a predisposition for toxicity. This is supported by increased risk for paclitaxel-induced sensory peripheral neuropathy in asymptomatic patients with diabetes, previous platinum drug exposures and alcohol use (3), and early Schwann cell activation in response to paclitaxel administration (29). Alternatively, *FGD4* polymorphisms could lead to impaired repair processes such as Schwann cell remyelination and/or axonal regeneration after paclitaxel exposure. Genetic variation in *FGD4* could also directly affect the response of Schwann cells to axonal injury via its ability to activate JNK (30). A neuronal protective role for activated JNK in cultured dorsal root ganglion cells exposed to oxaliplatin has been reported (31). Whether changes in frabin activity or expression lead to a decreased neuronal regenerative capacity and/or an increased sensitivity to paclitaxel-induced sensory peripheral neuropathy requires further study. Interestingly, *FGD4* was identified through a genome-wide siRNA screen in lung

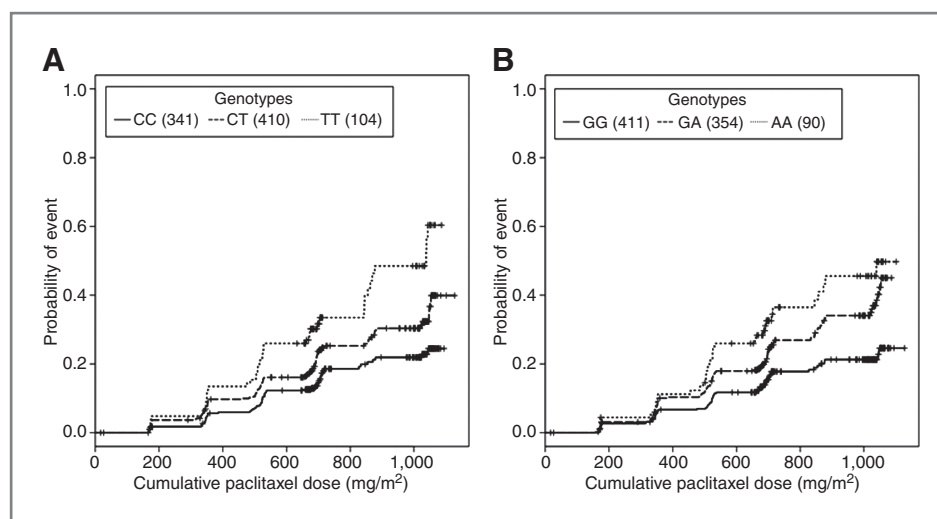


Figure 1. The *EPHA5* rs7349683 C>T and *FGD4* rs10771973 G>A polymorphisms are associated with an increased probability of developing paclitaxel-induced grade 2 or greater sensory peripheral neuropathy. The probability of the first instance of grade 2 or greater neuropathy as a function of cumulative paclitaxel dose (corrected for body surface area) is shown for each genotype. Results are shown for (A) rs7239683 (per allele HR = 1.63; 95% CI, 1.34–1.98; $P = 9.6 \times 10^{-7}$) and (B) rs10771973 (per allele HR, 1.57; 95% CI, 1.30–1.91; $P = 2.6 \times 10^{-6}$) in the discovery set. The number of individuals with each genotype is noted in parentheses.

cancer cell lines as a paclitaxel chemosensitizer. The chemosensitizing properties of *FGD4* are related, at least in part, to its ability to prevent mitotic progression (32). Whether a similar mechanism is involved in the repair response to paclitaxel-induced peripheral neuropathy is unknown.

The *FGD4* rs10771973 SNP is located in the intronic region and is in tight LD with a number of other SNPs. Computational analysis of the genomic region surrounding this SNP found that rs10771972, another intronic SNP in high LD with rs10771973 in both the European and African populations, is predicted to alter conserved transcription factor-binding sites for Myc-Max and USF (data not shown). One could speculate that disruption of either one or both of these transcription factor-binding sites in patients carrying the rs10771973 SNP could lead to altered expression and therefore function of *FGD4*/Frabin.

The other 2 top hits from the genome-wide analysis are also of potential interest for the paclitaxel-induced sensory peripheral neuropathy phenotype. In the time-to-toxicity analysis, the most significant SNP was in *EPHA5*, which

encodes for an ephrin receptor involved in axonal guidance and regeneration following injury. Recent studies have shown that in mice, *Epha5* mRNA is rapidly upregulated in response to a sciatic nerve lesion (20) and that *Epha5* signaling during synaptogenesis is transduced via *cdc42* (19), the Rho-GTPase involved in Frabin signaling. A common SNP in *FZD3* reached genome-wide significance in the ordinal analysis. *FZD3* encodes a Wnt receptor with reported roles in neurite outgrowth (33). In light of the biologic relevance of *EPHA5* and *FZD3* and the limited size of the replication cohorts available for these studies, it will be necessary to further explore the role of these 2 genes in larger populations of paclitaxel-treated patients. Additional studies are also warranted for other top hits, including rs2233335 in the N-myc downstream-regulated gene 1 (*NDRG1*; Supplementary Table S1). Rare mutations in *NDRG1* are also associated with a different subtype of CMT (CMT4D; ref. 34).

Until the availability of genome-wide approaches for identifying genetic predictors of paclitaxel-induced peripheral neuropathy, candidate gene approaches focused mostly on drug metabolizing enzymes and transporters implicated in paclitaxel exposure. These candidate gene studies yielded no replicated associations of SNPs with paclitaxel-induced sensory peripheral neuropathy, and most were complicated by a very small number of subjects, a retrospective analysis of toxicity, and chemotherapy with multiple agents (35–38). In the current analysis, no significant associations were observed for any SNPs residing in the candidate genes known to influence paclitaxel exposure (Supplementary Table S3), providing further evidence that factors contributing to the function and repair of peripheral nerves are more important than alterations in paclitaxel pharmacokinetics for determining genetic susceptibility to this toxicity. Interestingly, recent analyses of peripheral neuropathy induced by treatment with bortezomib, thalidomide, and vincristine have provided evidence that genes involved in repair mechanisms, inflammation, peripheral nervous system development, and

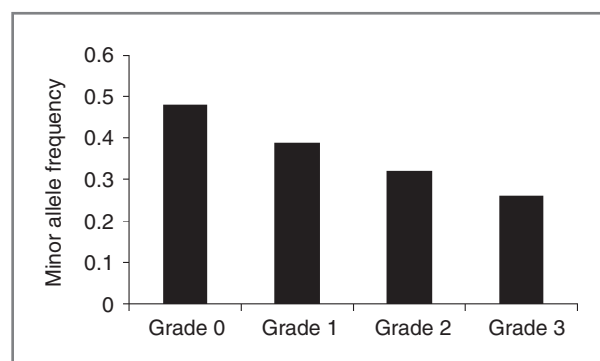


Figure 2. Association of *FZD3* SNP rs7001034 with sensory peripheral neuropathy. The minor allele frequency of rs7001034 in the European discovery cohort is expressed as a function of maximal grade of sensory peripheral neuropathy in 855 individuals.

Table 4. Top SNPs from ordinal analysis

SNP	Chr	Gene ^a	Alleles	Discovery (n = 855)			European Replication (n = 154) ^d			African American Replication (n = 117) ^d		
				MAF ^b	OR (95% CI)	P ^c	MAF	OR (95% CI)	P	MAF	OR (95% CI)	P
rs7001034 ^e	8	FZD3	G/T	0.40	0.57 (0.48–0.69)	3.1×10^{-9}						
rs7833751 ^f	8	FZD3	G/T	0.40	0.58 (0.49–0.70)	7.5×10^{-9}	0.41	1.67 ^f (0.88–3.19)	0.058			
rs5934683	X	/SHROOM2	C/T	0.36	1.61 (1.33–1.93)	6.0×10^{-7}	0.33	0.99 ^g (0.54–1.82)	0.49	0.30	1.96 ^g (0.49–7.89)	0.17
rs2941627	8	/ZFPM2	A/G	0.13	1.91 (1.40–2.51)	3.5×10^{-6}	0.15	1.37 ^g (0.68–2.73)	0.19	0.25	0.49 ^g (0.24–0.97)	0.021
rs7973533	12	/BCAT1	T/G	0.46	0.66 (0.55–0.79)	8.4×10^{-6}	0.44	0.75 (0.47–1.19)	0.11	0.27	1.13 (0.65–1.94)	0.33

^a Intergenic SNPs are denoted by the closest flanking annotated gene.^b MAF was calculated within the indicated cohort.^c P values are 2-sided for discovery analysis and one-sided for replication.^d As stated in the replication plan, all analyses were exploratory except for the FZD3 SNP.^e There was no available TaqMan assay for rs7001034 to use in replication studies.^f This SNP tags rs7001034 in the European population but not in the African American population.^g Analysis assumed a dominant model.

mitochondrial dysfunction could influence an individual patient's risk of developing toxicity (39–42). However, there was no overlap of implicated genes with the current study (Supplementary Table S3), suggesting that the mechanisms underlying this common toxicity might be drug specific.

To assess the potential translational implications of this finding to clinical practice, we estimated the cumulative dose level triggering an event for each *FGD4* rs10771973 genotype. Considering the data in Fig. 1B, to control the probability of experiencing a neuropathy event at a critical threshold of 33%, the tolerated cumulative dose level for patients with 2 copies of the risk allele is 710 mg/m². The corresponding expected critical dose level for patients with one copy of the risk allele is increased to 877 mg/m². Patients with no copies of the risk allele are expected to tolerate more than 1047 mg/m², corresponding to the full dose of paclitaxel for 6 cycles. If these thresholds are prospectively validated and further refined in follow-up studies, they may be used to estimate tolerable dose levels based on genotype and to tailor the treatment regimen.

While this pharmacogenetic study has several advantages over previous studies on paclitaxel pharmacogenetics, including a large cohort of treatment-naïve patients receiving single-agent paclitaxel and a genome-wide approach to discovery, several limitations also exist. The most significant limitation is the sole use of the NCI-CTC for assessment of sensory peripheral neuropathy. It is widely recognized that detailed patient-reported symptom data and a quality of life assessment more accurately describes this phenotype and that physician-reported NCI-CTC grading underreports peripheral neuropathy (43–45). However, it remains difficult to apply these techniques across the multiple sites and large sample sizes required for the sufficient power for pharmacogenetic analyses. In a recent phase III study of 1,060 women treated with taxanes, the Patient Neurotoxicity Questionnaire and the Functional Assessment of Cancer Therapy-General were administered to only the first 300 patients in the study (46). The only use of patient-reported toxicity data and symptom measurements for pharmacogenetic analysis of taxane peripheral neuropathy is limited by the very small sample size of the study (38). While it will be important in follow-up studies to validate these findings using additional instruments, it should be noted that despite its limitations, the NCI-CTC scores are widely accepted for primary evaluation of treatment toxicity in large phase III studies such as CALGB 40101. A second limitation of the current study is the small sample size of the replication cohorts, a common issue confronting almost all pharmacogenetic studies (47).

In summary, our findings support the use of prospective pharmacogenetic analyses of well-phenotyped data sets collected under controlled clinical trial settings and unbiased genome-wide genetic approaches for the identification of novel genes involved in drug efficacy and toxicity. Using a prospective design for validation and replication and a well-controlled single-agent clinical study, we have identified an SNP in *FGD4* associated with increased risk of developing paclitaxel-induced sensory peripheral neuropathy. The

involvement of *FGD4* in CMT disease, a congenital peripheral neuropathy, provides strong evidence for the biologic significance of this finding. The fact that a common *FGD4* SNP is associated with an increased risk of paclitaxel-induced sensory peripheral neuropathy in patients with both European and African ancestry makes it of potentially broad clinical significance. Additional SNPs in *EPHA5* and *FZD3* were also identified as potential risk factors for the onset and severity of sensory peripheral neuropathy. Additional samples for extension and validation of these findings are currently being collected in ongoing CALGB clinical trials of paclitaxel in the setting of metastatic breast cancer.

Disclosure of Potential Conflicts of Interest

The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute. No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.M. Baldwin, K. Owzar, H.L. McLeod, P.N. Friedman, C.A. Hudis, E.P. Winer, L. Shulman, M.J. Ratain, D. Kroetz

Development of methodology: R.M. Baldwin, K. Owzar, H. Zembutsu, M. Kubo, E. Jorgenson, J.S. Witte, D. Kroetz

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.M. Baldwin, H. Zembutsu, R.J. Eclow, C.A. Hudis, E.P. Winer, L. Shulman, Y. Nakamura, D. Kroetz

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.M. Baldwin, K. Owzar, H. Zembutsu, A. Chhibber, M. Kubo, C. Jiang, R.J. Eclow, J. Mefford, H.L. McLeod, C.A. Hudis, E. Jorgenson, J.S. Witte, L. Shulman, D. Kroetz

Writing, review, and/or revision of the manuscript: R.M. Baldwin, K. Owzar, A. Chhibber, C. Jiang, H.L. McLeod, P.N. Friedman, C.A. Hudis, E.P. Winer, E. Jorgenson, J.S. Witte, L. Shulman, M.J. Ratain

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.M. Baldwin, K. Owzar, M. Kubo, D. Watson, H.L. McLeod, P.N. Friedman, C.A. Hudis

Study supervision: C.A. Hudis, L. Shulman, Y. Nakamura, M.J. Ratain, D. Kroetz

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R. Michael Baldwin, Kouros Owzar, Hitoshi Zembutsu, et al.

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