Research Article

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Pharmacogenomics

Genetic variation in Charcot–Marie–Tooth genes contributes to sensitivity to paclitaxel-induced peripheral neuropathy

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Aim: This study explored whether inherited variants in genes causing the hereditary neuropathy condition Charcot–Marie–Tooth disease are associated with sensitivity to paclitaxel-induced peripheral neuropathy (PN). **Patients & methods:** Hereditary neuropathy genes previously associated with risk of paclitaxel-induced PN were sequenced in paclitaxel-treated patients. Eight putative genetic predictors in five hereditary neuropathy genes (*ARHGEF10*, *SBF2*, *FGD4*, *FZD3* and *NXN*) were tested for association with PN sensitivity after accounting for systemic exposure and clinical variables. **Results:** *FZD3* rs7833751, a proxy for rs7001034, decreased PN sensitivity (additive model, β = -0.41; 95% CI: -0.66 to -0.17; p = 0.0011). None of the other genetic predictors were associated with PN sensitivity. **Conclusion:** Our results support prior evidence that *FZD3* rs7001034 is protective of PN and may be useful for individualizing paclitaxel treatment to prevent PN.

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Keywords: *ARHGEF10* • *FZD3* • paclitaxel • peripheral neuropathy • pharmacogenomics

Paclitaxel is a critical component of combination chemotherapy treatment for patients with breast cancer [1]. About 25% of patients treated with weekly paclitaxel experience ≥ grade 2 peripheral neuropathy (PN) based on the National Cancer Institute Common Terminology Criteria for Adverse Events grading scale [2]. PN causes symptoms including numbness, tingling, allodynia, hyperalgesia or loss of proprioception in the hands or feet [3,4]. In order to avoid severe PN that can have long-term effects [5], nearly a quarter of patients require dose reductions, delays or discontinuations, which can decrease therapy effectiveness [6–9]. As there are no agents for preventing PN or treating nonpainful PN symptoms [9,10], identification of predictive PN biomarkers could help identify patients likely to experience PN, so their treatment can be adjusted accordingly. Previous research from our group and others have demonstrated that the primary determinant of PN is the patient's systemic paclitaxel exposure, as estimated by the amount of time (in hours) the patient's systemic concentration remains above 0.05 μmol/l ('time above threshold', $T_{c>0.05}$) [7,11]. However, up to 10% of patients treated with individualized paclitaxel doses to achieve a target paclitaxel exposure still experience severe PN [12]. These patients must be inherently PN-sensitive, which may be related to their inherited genetics [13].

Charcot–Marie–Tooth (CMT) disease, the most common inherited PN condition, is caused by more than 1000 inherited variants in 80 genes responsible for various processes in neuronal development and function [14–17]. CMT patients have been reported to be particularly susceptible to paclitaxel-induced PN [18]. Genome-wide association studies (GWAS) conducted in large cohorts of paclitaxel-treated patients have repeatedly identified variants in genes linked to CMT that may affect PN risk [19–22]. Lower PN risk were observed in patients carrying variants in *ARHGEF10* [19], *FZD3* [21] and *NXN* [22], which have a role in peripheral nerve myelination [15], neurite growth [17] and neuronal development [23], respectively. Higher PN risk was reported in carriers of variants in *EPHA* receptors, *FGD4* [21] and *SBF2* [20], which are involved in synaptogenesis, neuronal regeneration following nerve injury [24,25], and autosomal recessive demyelinating forms of CMT disease (CMT subtype 4) [26,27].

Despite this vast biomarker discovery effort, no genetic predictors of PN have been validated in independent replication attempts [28,29], which is necessary prior to clinical translation. The inability to validate genetic PN biomarkers is likely due to the multifactorial nature of PN and the failure of prior studies to account for variability in systemic paclitaxel exposure, which is the critical determinant of paclitaxel-induced PN [7,11,12,30]. Accounting for interindividual pharmacokinetic variability is the only way to isolate a patient's true PN-sensitivity, which can then be used as a quantitative end point for pharmacogenetic association testing. We previously used our PN sensitivity model [7], which accounts for cumulative paclitaxel systemic exposure and clinical factors, to demonstrate that *EPHA5* rs7349683 increases PN sensitivity [31]. Since PN sensitivity is likely a polygenic trait [32], the objective of this study was to use a similar approach to investigate whether inherited variants in five CMT-linked genes that have been previously associated with PN risk are associated with PN sensitivity after accounting for cumulative systemic paclitaxel exposure.

Materials & methods

Patients, PN & paclitaxel pharmacokinetics

UMCCC 2014.002 (clinicaltrials.gov; NCT0233815) is a previously reported prospective observational clinical study to investigate predictors of PN [7,31]. Participants enrolled in this clinical trial were >18 years old, with stage I–III or oligometastatic breast cancer, without PN or previous exposure to neurotoxic chemotherapy, and scheduled to receive 12 weekly infusions of paclitaxel 80 mg/m^2 for curative treatment of breast cancer. Detailed information about patient demographics, cancer treatment, paclitaxel pharmacokinetic sample collection and analysis, neuropathy assessment, germline DNA collection and sequencing of CMT-linked gene have been reported [7,31] and are briefly described below. All participants in this study signed written informed consent. This study was approved by the University of Michigan IRBMed and was conducted in accordance with recognized ethical guidelines.

Patients answered the quality of life questionnaire chemotherapy-induced peripheral neuropathy (CIPN20) from the European Organisation for Research and Treatment of Cancer [33] before their first paclitaxel dose and weekly until the end of treatment. The raw scores from eight sensory items (numbness, tingling and burning/shooting pain, difficulty in standing or walking and difficulty in distinguishing between hot and cold water), excluding the ninth sensory item on ototoxicity, were summed and linearly translated to a 0–100 scale (CIPN8) [7,31] with a higher CIPN8 score indicating greater PN symptoms.

Blood samples were collected 16–26 h after the start of the paclitaxel infusion to measure plasma paclitaxel concentration via liquid chromatography–mass spectrometry (LCMS) by the University of Michigan College of Pharmacy Pharmacokinetics Core (MI, USA). This single measurement was used to estimate time above threshold ($T_{c>0.05}$) using a previously published population-pharmacokinetic model [34,35].

CMT gene sequencing & identification of CMT genes of interest

A whole blood sample was collected prior to the first infusion for isolation of germline DNA. Targeted exonic sequencing of genes known to cause CMT was conducted followed by alignment to a reference genome (grch37), as previously described [19,29,31]. Although exonic regions were targeted, some nonexonic regions were sequenced as a byproduct [36]. Identified SNV were ranked by variant quality score recalibration according to the variant quality log-odds, and only SNV that had a specificity of >99.9% and sensitivity of >90% were included [19,31]. The annotations included are based on Ensemble GRCh37.75.

From the CMT genes sequenced, genes and SNVs of interest were selected based on a literature review of previously published pharmacogenetic studies of paclitaxel-induced PN. For *ARHGEF10,* two individual SNVs (rs9657362 and rs17683288) and the overall SNV gene burden were previously reported to decrease PN risk [19,29]. For *SBF2,* five individual SNVs (rs149501654, rs117957652, rs141368249, rs146987383 and rs7102464), and

SNV gene burden have been reported to increase PN risk in African–American patients [20]. A GWAS reported rs10771973 in *FGD4* and rs7001034 in *FZD3* were associated with increased and decreased PN, respectively [21]. Finally, rs910920 in *NXN* was reported to be protective of PN [22]. These ten SNVs in five CMT-linked genes that were previously reported to be associated with PN occurrence were selected as candidate genetic predictors for this analysis.

Genetic data cleaning & selection of genetic predictors

The following describes the process for selecting which potential genetic predictors of chemotherapy-induced neuropathy to include in the analysis. Starting with the five CMT-linked genes described above, many potential genetic predictors, either individual SNV or gene-burden tests, were considered. Most of these potential genetic predictors were rejected prior to analysis based on a requirement that any tested predictor has at least ten patients in each genotype group. The ten-patient threshold was used to ensure that each group had sufficient patients for meaningful association testing but was not based on a formal power analysis. After this filtering process, pharmacogenetic association testing was only conducted for the remaining eight genetic predictors described in this manuscript.

Each SNV analysis was conducted either based on the presence of a single variant or the total number of variant alleles carried by the patient, based partially on the previously reported genetic effect and ensuring an adequate number of patients for analysis (>10). Six candidate SNVs of interest that were previously associated with PN risk (rs149501654, rs141368249, rs146987383, rs10771973, rs7001034 and rs910920) were not detected by our sequencing. HaploReg was used to identify proxy variants in linkage disequilibrium (LD) >0.8 in the American population to be analyzed as tagging SNVs [37], where possible.

Similarly, for gene-based analyses, patients were classified by the presence of any missense variant, any functional variant, or by the total number of functional variant alleles the patient carried. Functional variants refer to genetic variants that are predicted to affect protein activity, including by affecting protein expression. Whether a variant has functional consequences was determined by three predictive bioinformatics tools: combined annotation-dependent depletion (CADD) [38,39], GWAVA [40] and PROVEAN [41], similar to our previous analysis of *EPHA* genes [31]. Coding variants were functional if they had CADD PHRED-like scaled C-score rankings ≥15 and PROVEAN scores <-2.5. Noncoding variants were functional if their CADD rankings ≥15 and GWAVA transcription start site scores ≥0.5. Functional noncoding variants that were located upstream or downstream of the candidate gene were only included, if they were an expression quantitative trait loci (eQTL) ($p < 0.005$) of their target gene in the GTex database [42]. Analyses of the total number of functional variants include both coding and noncoding functional variants. HaploReg was used to ensure each SNV included within any analysis was independent (LD <0.8), to prevent double counting.

Since *ARHGEF10* SNV rs9657362 and rs17683288 have been replicated as protective for PN [19,29], our *a priori* defined primary hypothesis was that patients carrying an rs9657362 or rs17683288 variant have decreased PN sensitivity (#1 in Table 3). After genetic data cleaning, seven additional genetic predictors were selected for secondary analyses, each with a prespecified direction of effect on PN sensitivity: carrying any *ARHGEF10* missense SNV (2), carrying *SBF2* rs117957652 or rs7102464 (3), the number of functional *SBF2* SNV alleles a patient carried (4), carrying *FGD4* rs10844253 (tag SNV for rs10771973) (5), carrying any functional *FGD4* SNV (6), the number of *FZD3* rs7833751 alleles a patient carried (tag SNV for rs7001034) (7) and carrying any functional *NXN* SNV (8).

Statistical analysis

A previously developed PN sensitivity prediction model [7] was used to analyze the contribution of our eight genetic predictors with PN severity, as defined by the square root of CIPN8. This PN sensitivity model includes baseline CIPN8 (0–100), cumulative dose (mg/m^2, actual-weight body surface area adjusted), relative dose intensity (proportion of cumulative planned dose received to expected cumulative dose, to account for delays and decreases), measured systemic paclitaxel exposure ($T_{c>0.05}$) and an interaction term with $T_{c>0.05}$ and cumulative dose. Each putative genetic predictor was introduced into the model independently to determine whether it has a significant contribution to PN sensitivity, using an uncorrected significance threshold ($\alpha = 0.05$). Significant associations were then tested in the model including the *EPHA5* SNV rs7349683 to investigate whether these were independent genetic predictors of PN sensitivity [31]. All analyses were conducted in SAS v.9.4.

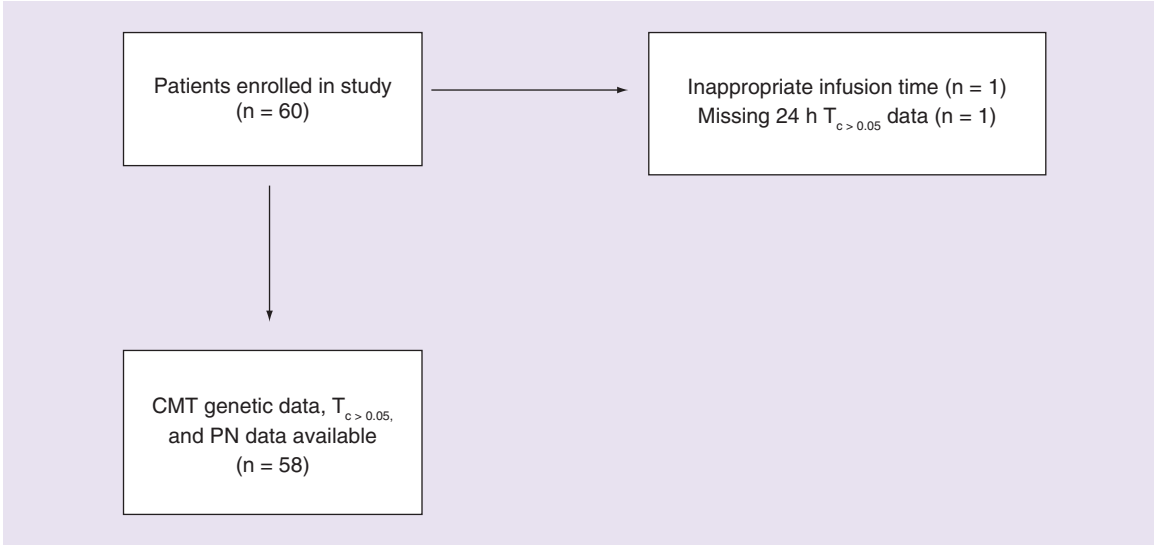

Figure 1. Patient flow from observational study into this analysis.
CMT: Charcot–Marie–Tooth; PN: Peripheral neuropathy.

Table 1. Demographic and treatment information (n = 58).

Patient demographics	n or mean (% or SD)
Age (years)	52.52 (10.31)
BSA (m^2)	1.83 (0.21)
Race (Caucasian)	54 (93.1%)
$T_{c>0.05}$ (h)	10.72 (2.73)
Baseline CIPN8 (range: 0–100)	1.29 (3.04)
Cumulative dose (mg/m^2)	883.95 (163.82)
Relative dose intensity	0.95 (0.01)

BSA: Body surface area; SD: Standard deviation.

Results

Patient demographics & clinical data

Detailed information about the 58 patients enrolled in this prospective cohort study that are included in this secondary pharmacogenetic analysis (Figure 1) has been previously reported [7,31]. Patients included in this analysis had a mean age of 52.5 years (range: 28–71), mean body surface area of 1.83 m^2 (standard deviation [SD]: 0.21) and 93.1% were Caucasian (Table 1). The average $T_{c>0.05}$ was 10.72 h (SD: 2.73). As previously reported, CIPN8 was low at baseline (mean = 1.29 ± 3.04) and increased throughout treatment (mean maximum CIPN8 = 13.26 ± 1.76).

Genetic variants included in each genetic predictor

Each SNV included in any analysis is listed in Table 2. The primary analysis included two *ARHGEF10* SNV (rs9657362 and rs17683288). The secondary analysis of carrying any *ARHGEF10* missense SNV allele included seven independent missense variants. Six *SBF2* SNVs were considered functionally consequential. In the analysis of *SBF2*, patients were classified as to whether they carried *SBF2* rs117957652 or rs7102464 or by the number of functional *SBF2* SNV alleles. The analyses of *FGD4* were conducted on the basis of carrying *FGD4* rs10844253 (tag SNV for rs10771973, r^2 = 0.92), or carrying any functional *FGD4* SNV. Two functional *FGD4* SNVs were identified: rs11539445 and rs10844308, but due to LD only rs11539445 was considered a functional SNV in the analysis. In the analysis of *FZD3*, patients were classified by the number of *FZD3* rs7833751 alleles (tag SNV for rs7001034, r^2 = 0.98). In the *NXN* analysis, rs11247571 was the only functionally consequential SNV identified and patients were classified by whether they carried this functional SNV.

Table 2. All variants included in genetic analysis.

Gene	rs ID	Chromosomal position	Reference allele	Variant type	Reason for variant inclusion (candidate SNV, tag SNV, missense or functional)	Corresponding genetic predictor†
ARHGEF10	rs9657362	8:1833801	G	Missense	Candidate, missense	1, 2
	rs17683288	8:1877480	T	Missense	Candidate, missense	1, 2
	rs141069028	8:1851564	C	Missense	Missense	2
	rs2294039	8:1857591	G	Missense	Missense	2
	rs201516531	8:1905361	C	Missense	Missense	2
	rs887797448	8:1900880	G	Missense	Missense	2
	rs139515492	8:1905048	C	Missense	Missense	2
SBF2	rs117957652	11:9861208	C	Missense	Candidate	3
	rs7102464	11:9879838	T	Missense	Candidate	3
	rs59613534	11:9800552	C	3 prime UTR	Functional (CADD: 19.22, GWAVA: 0.71)	4
	rs60154961	11:9800566	G	3 prime UTR	Functional (CADD: 18.12, GWAVA: 0.67)	4
	rs360126	11:9800346	G	3 prime UTR	Functional (CADD: 17.02, GWAVA: 0.68)	4
	rs360125	11:9800650	G	3 prime UTR	Functional (CADD: 17.01, GWAVA: 0.63)	4
	rs1045634	11:9800450	T	3 prime UTR	Functional (CADD: 15.01, GWAVA: 0.70)	4
	rs146366305	11:9989990	A	Missense	Functional (CADD: 25.3, PROVEAN: -2.53	4
FGD4	rs10844253	12:32764184	A	Synonymous	Tag SNV of rs10771973 (r^2 = 0.92)	5
	rs11539445	12:32908237	A	Regulatory	Functional (CADD: 24.2, GWAVA: 0.50, GTEx p = 0.00012)	6
	rs10844308	12:32854366	C	Regulatory	Functional (CADD: 17.03, GWAVA: 0.54, GTEx p = 0.00012)	Excluded due to LD‡
FZD3	rs7833751	8:28362792	G	Intron	Tag SNV of rs7001034 (r^2 = 0.98)	7
NXN	rs11247571	17:908502	G	Regulatory	Functional (CADD: 17.79, GWAVA: 0.51, GTEx p = 0.0000049)	8

†Corresponding genetic predictor: the genetic predictor (Table 3) in which each SNV was included.
‡Variant excluded from analysis due to LD with rs11539445 (r^2 = 0.93).
CADD: Combined annotation-dependent depletion; LD: Linkage disequilibrium.

Table 3. Genetic associations with peripheral neuropathy sensitivity.

Entry	Genetic predictor	Genetic predictor distribution	Expected effect on PN Sensitivity	Beta†	95% CI	p-value
1	*ARHGEF10*: carrying rs9657362 or rs17683288	Yes: 21/58 = 36.2%	Lower	-0.27	-0.68–0.14	0.20
2	*ARHGEF10*: carrying any missense SNV	Yes: 30/58 = 51.7%	Lower	0.23	-0.16–0.63	0.25
3	*SBF2*: carrying rs117957652 or rs7102464	Yes: 18/58 = 31.0%	Higher	-0.32	-0.75–0.10	0.14
4	*SBF2*: carrying more functional SNV alleles	0: 21/58 = 36.2% 1: 26/58 = 44.8% 2: 11/58 = 19.0%	Higher	0.12	0.15–0.40	0.39
5	*FGD4*: carrying rs10844253‡	Yes: 36/58 = 62.1%	Higher	0.31	-0.09–0.71	0.13
6	*FGD4*: carrying any functional SNV	Yes: 17/58 = 29.3%	Higher	-0.07	-0.51–0.36	0.75
7	*FZD3*: carrying more rs7833751‡ alleles	0: 13/58 = 22.4% 1: 20/58 = 34.5% 2: 25/58 = 43.1%	Lower	-0.41§	-0.66 to -0.17§	0.0011§
8	*NXN*: carrying any functional SNV	Yes: 42/58 = 72.4%	Lower	-0.23	-0.66–0.20	0.29

†Positive β-coefficient indicates higher PN sensitivity, negative indicates lower PN sensitivity. Bold indicates statistical significance ($p < 0.05$).
‡These alleles are tagging SNV of the variant of interest (*FGD4*: rs10771973 and *FZD3*: rs7001034).
§Statistical significance $p < 0.05$.
PN: Peripheral neuropathy.

Genetic associations with PN sensitivity

Table 3 lists each genetic predictor analyzed, the distribution of that genetic predictor in the cohort, the expected direction of effect on PN sensitivity, and the association for that genetic predictor when introduced in the PN sensitivity model that accounts for cumulative treatment, systemic paclitaxel exposure, and clinical factors. In the primary analysis, carrying either rs9657362 or rs17683288 in *ARHGEF10* was not associated with lower PN

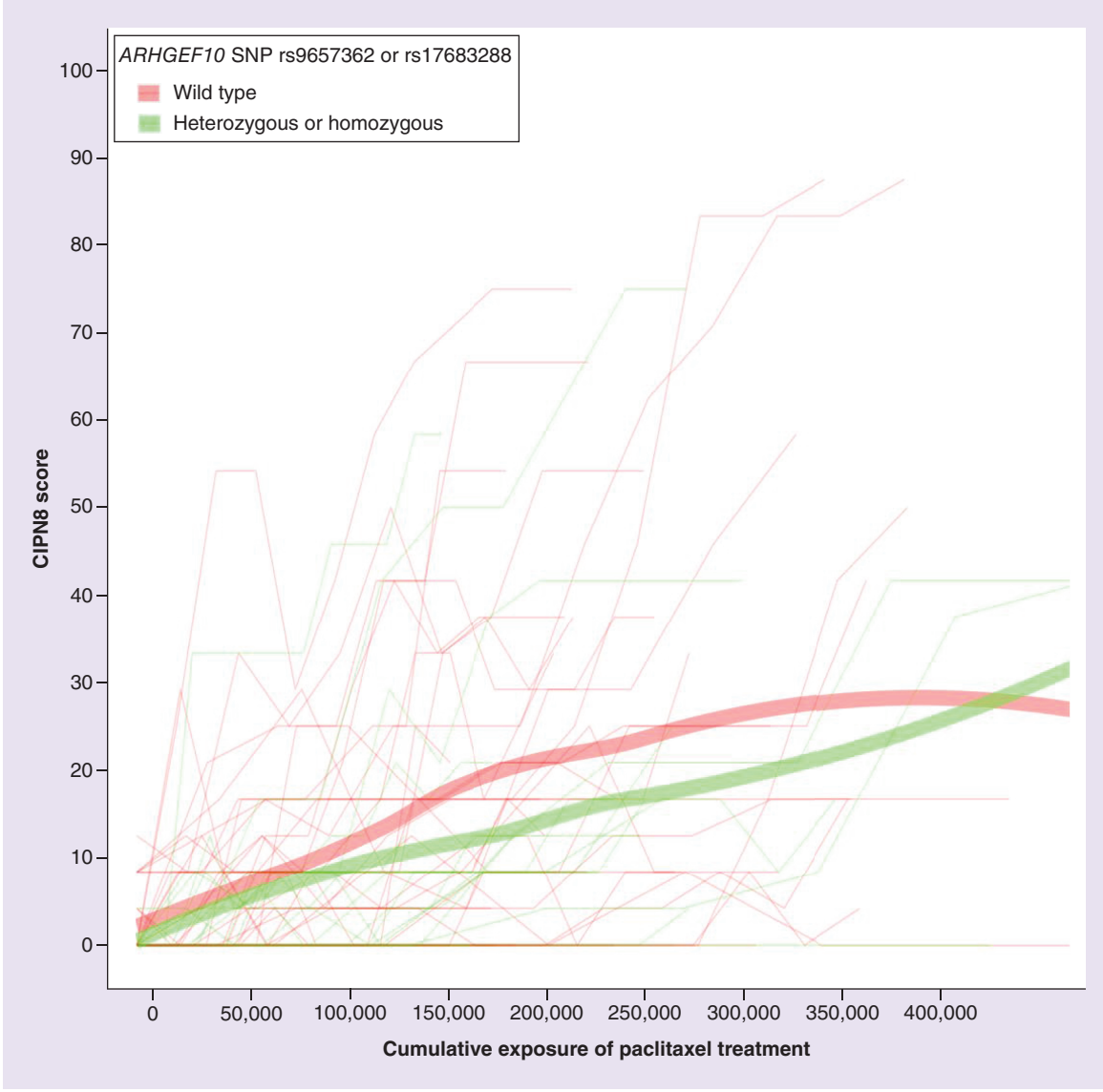

Figure 2. CIPN8 score by cumulative exposure stratified by whether a patient carried either *ARHGEF10* SNV rs9657362 or rs17683288 (green line) or not (red line). Carrying *ARHGEF10* rs9657362 or rs17683288 was not associated with peripheral neuropathy sensitivity. Wider lines represent lines of best fit.

sensitivity (β-coefficient: -0.27, 95% CI: -0.68–0.14; p = 0.20; Table 3 & Figure 2). In a secondary analysis, each additional *FZD3* rs7833751 variant allele a patient carried decreased her PN sensitivity (additive β-coefficient = -0.41; 95% CI: -0.66 to -0.17; p = 0.0011; Figure 3), which is consistent with the expected direction of effect. Although the exploratory secondary analyses were not corrected for multiple comparisons, this association does retain significance after strict Bonferonni multiple comparisons testing correction (α = 0.05/8 = 0.00625). The PN sensitivity model parameter estimates for all clinical covariates with *FZD3* rs7833751 alone, or including *EPHA5* rs7349683, are reported in Table 4. The final model indicates that both variants were independently associated with PN sensitivity, though with opposing direction of effect. None of the other six genetic predictors tested in secondary analyses was associated with PN sensitivity.

Discussion

PN is a common, debilitating, sometimes irreversible side effect of paclitaxel treatment [5,8,43]. PN is primarily determined by cumulative systemic paclitaxel exposure [7,11], but there also seems to be an inherent sensitivity

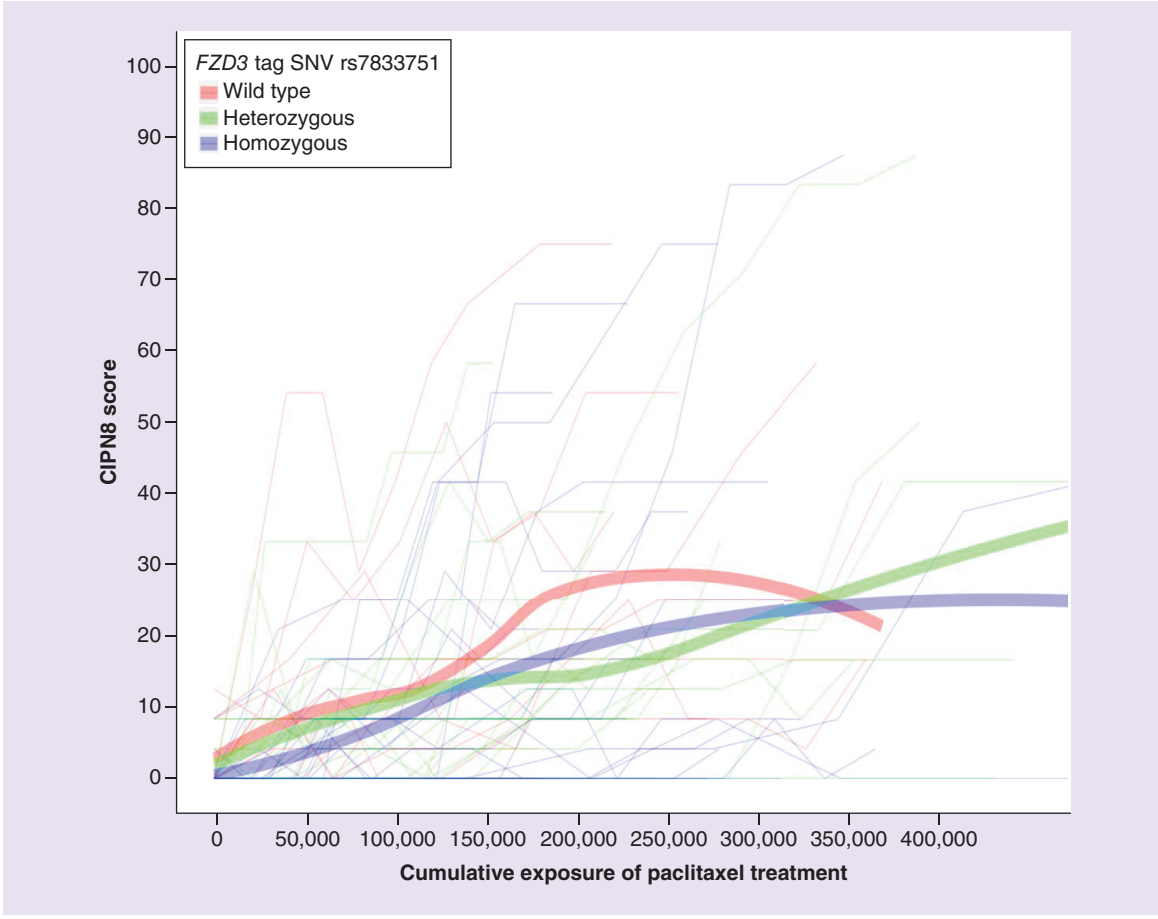

Figure 3. CIPN8 score by cumulative dose (cumulative dose * $T_{c>0.05}$) stratified by whether a patient carried 0 (red line), 1 (green line) or 2 (blue line) *FZD3* tag SNV rs7833751 alleles. Peripheral neuropathy sensitivity was decreased for each rs7833751 variant allele a patient carried (β-coefficient = -0.41; 95% CI: -0.66 to -0.17; p = 0.0011). Thick lines represent lines of best fit.

Table 4. Final peripheral neuropathy sensitivity model including clinical and genetic predictors.

Predictor	PN sensitivity model with *FZD3* rs7833751			PN sensitivity model with *FZD3* rs7833751 and *EPHA5* rs7349683			Ref.
	Beta†	95% CI	p-value	Beta†	95% CI	p-value	
Each additional *FZD3* rs7833751 variant allele a patient carried	-0.41	-0.66 to -0.17	0.0011	-0.47	-0.72 to -0.23	<0.001	
Baseline CIPN8	0.19	0.12–0.25	<0.001	0.18	0.12–0.24	<0.0001	
Cumulative dose	-0.13	-0.55–0.29	0.55	-0.14	-0.56–0.28	0.52	
Relative dose intensity	-1.53	-3.01 to -0.04	0.04	-1.44	-2.91–0.03	0.06	
$T_{c>0.05}$	-0.23	-0.45 to -0.02	0.03	-0.31	-0.53 to -0.10	<0.01	
Cumulative dose $T_{c>0.05}$ interaction†	0.14	0.04–0.25	<0.01	0.14	0.04–0.25	<0.01	
Each additional *EPHA5* rs7349683 variant allele a patient carried	–	–	–	0.47	0.19–0.75	<0.01	[31]

†Positive beta-coefficient indicates higher PN sensitivity, negative indicates lower PN sensitivity.
PN: Peripheral neuropathy.

to PN that may be determined by patient genetics [12,31]. Predictive PN sensitivity biomarkers could be used to individualize paclitaxel dosing or select non-neuropathic alternative regimens, to prevent PN and improve treatment outcomes. Using a previously published PN sensitivity model that accounts for measured cumulative paclitaxel systemic exposure, we were unable to confirm that patients carrying *ARHGEF10* rs9657362 or rs17683288, who

have been previously reported to have lower risk of developing PN [19,29], are less PN-sensitive. In a statistically uncorrected secondary analysis, each additional variant allele of *FZD3* rs7833751, a tag SNV for rs7001034, a patient carried was associated with lower PN sensitivity.

Our results that *FZD3* rs7833751, a proxy for rs7001034, decreases PN sensitivity is consistent with an observation from a previously published GWAS that European patients carrying *FZD3* rs7001034 had lower risk of PN [21]. Our study had to use a proxy SNV of rs7001034 due to lack of intronic coverage on our sequencing panel. Though previous replication studies failed to support the PN protective effect of rs7001034, perhaps due to insufficient study power [28], the consistency of our findings with the original publication warrant additional replication attempts to confirm that rs7001034 decreases PN sensitivity and is protective of PN. *FZD3* encodes a G-protein-coupled receptor involved in Wnt signaling that is important for neurite outgrowth [17] and development of the neural crest [44]. Further biological experiments should be conducted to confirm this variant's functional impact on FZD3 activity and its possible contribution to PN sensitivity. Our results indicate that each rs7001034 variant allele a patient carries could increase their optimal systemic paclitaxel exposure (i.e., $T_{c>0.05}$) [7]. This finding is similar to our previous finding that each *EPHA* rs7349683 variant a patient carriers decreases their optimal exposure by approximately 1 h [31]. Our final model suggests that these two SNVs act independent of each other, and with opposite directions of effect on optimal exposure, and both would need to be considered when selecting an optimal exposure target for a patient. Upon validation of these PN sensitivity biomarkers, and determination of whether they act independently or whether there are gene-by-gene interactions between them, genotype-specific optimal exposure targets would need to be tested in prospective clinical trials to demonstrate the clinical benefit of individualized paclitaxel dosing.

Since PN sensitivity is likely to be a complex polygenic trait, genotype-guided paclitaxel dosing may require consideration of multiple genetic predictors. We investigated four other genes previously reported to be associated with PN risk, however, none of these genes were associated with PN sensitivity in this analysis. Rs9657362 and rs17683288 in *ARHGEF10* were included in the primary analysis because they have been successfully replicated to have protective effects on PN susceptibility [19,29]. *FGD4* SNV rs10771973 was included as a candidate SNV since it was originally reported to be associated with earlier-onset of paclitaxel-induced sensory PN and subsequently replicated to increase PN risk or risk of paclitaxel dose reduction in multiple independent patient cohorts [21,45]. *SBF2* and *NXN* variants were reported to be associated with occurrence of severe PN in individual studies [20,22] but have not been successfully replicated to our knowledge. Our study was not able to detect an association with PN sensitivity for any of these genes, again perhaps due to limited analytical power.

Strengths of this study include the use of a PN sensitivity model that accounted for cumulative systemic paclitaxel exposure to explore genetic PN predisposition, the inclusion of gene-based genetic predictors, and the use of a reliable and valid patient-reported questionnaire for PN assessment [46–48]. There are also some limitations in this study. First, the small sample size limited the statistical powers to detect association with PN sensitivity for several genes that were previously reported to be associated with PN risk, including our primary hypothesis that SNVs in *ARHGEF10* decrease PN sensitivity [19,29], and precludes meaningful analysis of gene-by-gene interactions. Second, our genetic dataset was derived from targeted exonic sequencing, which precluded direct analysis of several previously SNVs previously reported to be associated with PN risk. Although we attempted to include proxy SNVs with high LD, these surrogates may not perfectly represent the previously reported SNVs. Finally, our gene-based hypotheses assume all variants have similar functional consequences, which is unlikely to be true at the level of protein expression or function.

Conclusion

This study supports prior findings that *FZD3* SNV rs7001034 decreases PN risk and indicates that the causal mechanism is by decreasing patients' PN sensitivity. Additional validation studies in larger patient cohorts that account for cumulative paclitaxel exposure are necessary to confirm this predictive PN sensitivity biomarker, followed by prospective clinical trials testing individualized treatment strategies based on the patient's PN sensitivity. This work could enable personalized treatment to prevent PN and improve therapeutic outcomes in patients with cancer.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was approved by the University of Michigan IRBMed and was conducted in accordance with recognized ethical guidelines. All participants in this study signed written informed consent.

Data sharing statement

Data is available upon reasonable request to the corresponding author.

Summary points

- Genetic variation in genes linked to hereditary neuropathy, specifically Charcot–Marie–Tooth (CMT) disease, have been reported to effect the risk of paclitaxel-induced peripheral neuropathy (PN).
- Since PN is primarily determined by cumulative systemic paclitaxel exposure, analyses accounting for exposure can isolate a patient's sensitivity to PN for use as an end point in pharmacogenetic analyses.
- A total of 58 paclitaxel-treated patients were sequenced for a panel of genes linked to CMT. Their PN were measured using the eight sensory items of the patient-reported European Organisation for Research and Treatment of Cancer-Quality of Life Questionnaire (EORTC-QLQ) CIPN20 subscale.
- Eight putative genetic predictors in five CMT *genes (ARHGEF10, SBF2, FGD4, FZD3* and *NXN)* with prespecified expected direction of effect were analyzed.
- Consistent with previous genome-wide association studies findings, each additional variant allele of *FZD3* rs7833751 (a tagging variant of *FZD3* rs7001034) a patient carried decreased her PN sensitivity.
- This study did not find evidence that carrying *ARHGEF10* rs9657362 or rs17683288 was associated with lower PN sensitivity.
- Future biological studies and larger validation studies of rs7001034 and prospective trials that verify the clinical benefit of rs7001034-guided paclitaxel dosing could enable personalized treatment to prevent PN and improve therapeutic outcomes in patients with cancer.
- Consistent replication in independent patient cohorts is necessary prior to clinical translation of pharmacogenetic biomarkers.

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