



## ORIGINAL ARTICLE

# Genetic variation in *EPHA* contributes to sensitivity to paclitaxel-induced peripheral neuropathy

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**Aims:** Chemotherapy-induced peripheral neuropathy (PN) is a treatment limiting toxicity of paclitaxel. We evaluated if *EPHA* genetic variation (*EPHA4*, *EPHA5*, *EPHA6*, and *EPHA8*) is associated with PN sensitivity by accounting for variability in systemic paclitaxel exposure (time above threshold).

**Methods:** Germline DNA from 60 patients with breast cancer was sequenced. PN was measured using the 8-item sensory subscale (CIPN8) of the patient-reported CIPN20. Associations for 3 genetic models were tested by incorporating genetics into previously published PN prediction models integrating measured paclitaxel exposure and cumulative treatment. Significant associations were then tested for association with PN-related treatment disruption.

**Results:** *EPHA5* rs7349683 (minor allele frequency = 0.32) was associated with increased PN sensitivity ( $\beta$ -coefficient = 0.39, 95% confidence interval 0.11–0.67,  $p = 0.007$ ). Setting a maximum tolerable threshold of CIPN8 = 30, optimal paclitaxel exposure target is shorter for rs7349683 homozygous (11.6 h) than heterozygous (12.6 h) or wild-type (13.6 h) patients. Total number of missense variants (median = 0, range 0–2) was associated with decreased PN sensitivity ( $\beta$ -coefficient:  $-0.42$ , 95% confidence interval  $-0.72$  to  $-0.12$ ,  $P = .006$ ). No association with treatment disruption was detected for the total number of missense variants or rs7349683.

**Conclusion:** Isolating toxicity sensitivity by accounting for exposure is a novel approach, and rs7349683 represents a promising marker for PN sensitivity that may be used to individualize paclitaxel treatment.

## KEYWORDS

breast cancer, genetic polymorphism, pharmacodynamics, pharmacogenomics, pharmacokinetics

## 1 | INTRODUCTION

Paclitaxel is commonly used to treat early breast cancer and improves overall survival in this treatment setting<sup>1</sup>. Weekly paclitaxel used in early breast cancer is similarly efficacious to every 2-week or every 3-week regimens<sup>2,3</sup>. Although it is highly efficacious, weekly paclitaxel has a dose-limiting toxicity, paclitaxel-induced peripheral neuropathy (PN), which is characterized by numbness, tingling, and pain in the hands and feet that can negatively impact long-term quality of life<sup>4</sup>. Paclitaxel-induced PN necessitates dosing delays, decreases, or even premature treatment discontinuation<sup>5</sup>. These treatment disruptions decrease therapy effectiveness<sup>6,7</sup>. Consequently, there is substantial interest in identifying patient-specific predictors of PN that could be used to individualize paclitaxel therapy to prevent PN-necessitated treatment disruptions and improve treatment efficacy.

Paclitaxel exposure (i.e. the amount of time paclitaxel systemic concentrations remain above a threshold plasma concentration of 0.05  $\mu\text{M}$ ) is an established predictor of PN<sup>8-11</sup>. Individualized paclitaxel dosing to achieve a target exposure substantially decreases the occurrence of PN, but some patients experience severe PN despite receiving treatment at the target exposure<sup>10</sup>, suggesting these patients have an inherent predisposition to PN (i.e. PN sensitivity). PN sensitivity is probably determined by a combination of genetic and clinical factors. These PN-sensitivity biomarkers could be discovered by conducting analyses that account for exposure variability, which would isolate the PN sensitivity phenotype.

Previous research has attempted to discover genetic predictors of paclitaxel-induced PN<sup>12-14</sup> or replicate previous findings<sup>15-17</sup>. These important discoveries need to be fully validated to establish clinical utility. Pioneering studies exploring these associations mostly utilized a case-control approach with the endpoint of occurrence of PN<sup>18</sup>. More recent studies have explored PN susceptibility by accounting for the cumulative dose at PN occurrence<sup>12,13,19,20</sup>. Within these studies, variants in genes encoding ephrin (*EPHA*) receptors from the receptor tyrosine kinase family, which have a role in neuronal development<sup>21</sup>, have been observed to increase risk of paclitaxel-induced PN (i.e. *EPHA4* rs17348202, *EPHA5* rs7349683, *EPHA6* rs301927)<sup>12,15-17,19,22</sup>. However, replication has been challenging, probably because genetic variants should be considered alongside other predictive variables when explaining the multifactorial PN endpoint. A recent study attempted to impute exposure from dosing data<sup>23</sup>, but no prior analyses have incorporated measured paclitaxel exposure to isolate PN sensitivity. Accounting for actual cumulative exposure at PN occurrence is a novel approach to isolate PN sensitivity for use as a phenotype for PN biomarker discovery and validation.

We previously created models that explain the trajectory of patient-reported PN during paclitaxel treatment using cumulative treatment and measured paclitaxel exposure<sup>11</sup>. Clinical factors associated with PN were previously explored in this cohort<sup>11</sup>. The purpose of this study is to utilize these previously published models to determine whether genetic variation in *EPHA* genes are associated with increased PN sensitivity.

### What is already known about this subject

- Peripheral neuropathy (PN) is a treatment limiting adverse effect of paclitaxel that is determined primarily by drug exposure.
- Replication has been a challenge for genetic predictors of paclitaxel-induced PN, including *EPHA* variants.
- Accounting for pharmacokinetic variability could isolate genetic PN predisposition (i.e. PN sensitivity) for genetic replication studies.

### What this study adds

- This study supports *EPHA5* rs7349683 as a biomarker of PN sensitivity.
- rs7349683 may be utilized to estimate the optimal paclitaxel exposure target for individual patients, preventing toxicity and improving treatment outcomes.
- Isolating toxicity sensitivity by accounting for drug exposure is a novel analytical approach for pharmacogenetic discovery and replication.

## 2 | METHODS

### 2.1 | Patient population and clinical data

Patients aged >18 years without PN or prior neurotoxic chemotherapy scheduled to receive 12 weekly infusions of paclitaxel 80 mg/m<sup>2</sup> for curative treatment of breast cancer were enrolled in a prospective, observational clinical cohort registry (UMCCC 2014.002, NCT0233815). Detailed information about these patients, their treatment, pharmacokinetic sampling time points, PN data collection, and the primary analysis of the association between pharmacokinetics and PN severity have been previously reported and are described briefly below<sup>11</sup>. All patients included 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.

### 2.2 | Pharmacokinetic sampling

Blood samples were collected 16–24 h after the start of the first paclitaxel infusion. Plasma samples were stored at  $-20^{\circ}\text{C}$  until measurement of paclitaxel in plasma via liquid chromatography–mass spectrometry. A previously published population-pharmacokinetic model was used to estimate each patient's paclitaxel time above threshold ( $T_c > 0.05$ ), defined as the amount of time in h that the patient's plasma concentration remains above 0.05  $\mu\text{M}$ , utilizing the

measured paclitaxel concentration and the amount of time since the beginning of infusion<sup>24,25</sup>.

### 2.3 | PN measurement

PN was quantified utilizing the Quality of Life Questionnaire Chemotherapy-Induced Peripheral Neuropathy (CIPN20) from the European Organisation for Research and Treatment of Cancer. Patients completed the CIPN20 prior to their first paclitaxel dose and weekly until the end of treatment. Since paclitaxel primarily causes sensory PN, this analysis used the 8 sensory items of the CIPN20 (CIPN8), excluding the ototoxicity question, as was reported in the primary analysis of this cohort<sup>26,27</sup>. CIPN8 raw scores were linearly translated to a 0–100 scale, with higher scores representing greater PN, as recommended by the European Organisation for Research and Treatment of Cancer scoring manual<sup>28</sup>.

### 2.4 | Pharmacogenomic sampling and DNA isolation

A 5 mL whole blood sample was collected prior to the first cycle in a lavender-top EDTA tube and stored at  $-20^{\circ}\text{C}$ . Germline DNA was isolated from buffy coat using QIAamp DNA Mini Kits using the spin protocol with manufacturer's instructions (QIAGEN, Valencia, CA, USA). Sample quantity and quality was assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

### 2.5 | Genotyping and single nucleotide variant functional assessment

DNA sequencing techniques have been previously described in detail<sup>29</sup>. Briefly, targeted exon sequencing was conducted for chemotherapy-induced PN genes and genes previously associated with PN. The isolated DNA was sheared and subjected to end repair with target fragment size of 300–400 base pairs. It was sequenced twice and sequencing reads were aligned to a reference genome (grch37). Single nucleotide variants were ranked by variant quality score recalibration according to the variant quality log-odds, and only single nucleotide variants that had a specificity of >99.9% and sensitivity of >90% were included. The annotations included are based on Ensemble GRCh37.75. *Posthoc* verification of rs7349683 genotype calls in sequencing data was conducted via TaqMan allelic discrimination assay, as previously described<sup>30</sup>.

This primary analysis of genetic PN predisposition was conducted using only the sequencing data from the 4 *EPHA* genes (*EPHA4*, *EPHA5*, *EPHA6*, *EPHA8*). The variants were categorized based on predicted consequence on the encoded protein. Coding variants

include all variants (missense and synonymous) located in the translated region of DNA. Predicted functional consequence of variants was determined by 2 different bioinformatics tools: CADD<sup>31,32</sup> and PROVEAN<sup>33</sup>. For coding variants, CADD PHRED-like scaled C-score rankings  $\geq 15$  and PROVEAN scores  $< -2.5$  were considered functionally consequential.

### 2.6 | *Posthoc* exploration of variants in linkage disequilibrium

All positive associations were further explored using HaploReg for variants in linkage disequilibrium (LD) using the American population for the 1000G Phase 1 population for the LD calculation<sup>34</sup>. The LD threshold was set at 0.8, and the variant position was described relative to GENCODE genes. Variants found to be in LD were explored using the previously described bioinformatics tools for coding variants. Each variant was also explored using GTex to determine if it is an expression quantitative trait locus ( $P < .005$ ) in neuron-related tissues (brain or tibial nerve)<sup>35</sup>.

### 2.7 | Statistical analyses

The analysis was conducted by testing whether *EPHA* genetics significantly contribute to a previously developed CIPN8 prediction model. The base model from our prior publication included baseline CIPN8 (0–100), cumulative dose ( $\text{mg}/\text{m}^2$ , actual-weight body surface area adjusted), and relative dose intensity (defined as the proportion of cumulative planned doses received to expected cumulative dose, in order to account for delays and decreases)<sup>11</sup>. An interaction term with  $T_c > 0.05$  and cumulative dose was included based on our previous findings that CIPN8 increased more quickly with continued dosing in patients with longer  $T_c > 0.05$ . This PN sensitivity model, which incorporates paclitaxel exposure ( $T_c > 0.05$ ), allows for direct testing of genetic associations with the isolated phenotype of PN sensitivity.

The association between PN sensitivity and 3 different *EPHA* genetic predictors were analysed independently: (i) missense variants: the total number of missense variants per patient; (ii) functionally consequential coding variants: the total number of coding variants per patient predicted to be of functional consequence; and (iii) rs7349683: the additive genetic effect of rs7349683, which was selected for independent inclusion based on multiple prior reports of its association with PN<sup>12,16,19</sup>.

Genetic predictors that were significantly associated with PN sensitivity were then tested for an association with the clinically relevant endpoint of sensitivity to PN-induced treatment disruption (i.e. paclitaxel dose decrease, dose delay, or discontinuation due to PN, as previously defined), using our previously published predictive model that includes baseline CIPN8, cumulative dose, and  $T_c > 0.05$  without an interaction term<sup>11</sup>. A *posthoc* analysis testing rs7349683 in a dominant genetic model was conducted to affirm an additive model best represents the genetic consequence of rs7349683.

As a *posthoc* analysis, the predicted CIPN8 at the end of standard, undisrupted treatment (weekly dose = 80 mg/m<sup>2</sup>, number of doses = 12, relative dose intensity = 1) was estimated for a typical patient (baseline CIPN8 = 0) with each rs7349683 genotype (wild-type, heterozygous, homozygous variant) and a range of paclitaxel exposures ( $T_{c > 0.05}$ ) of 0–16 h. These model results were then used to identify the optimal exposure ( $T_{c > 0.05}$ ) at which a patient with each genotype would experience a maximum CIPN8 score of 10, 20, 30 or 40.

### 3 | NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

## 4 | RESULTS

### 4.1 | Patient demographics, pharmacokinetics and genetics

Sixty patients were enrolled in this prospective cohort study. One patient who received a 3-h paclitaxel infusion was excluded and time above threshold ( $T_{c > 0.05}$ , defined as the amount of time in h that the patient's plasma concentration remains above 0.05 µM) could not be calculated for 1 patient (Figure 1). Patients included in this analysis had a mean age of 52.5 years (range: 28–71, Table 1), had mean body surface area of 1.83 m<sup>2</sup> (standard deviation [SD:] 0.21) and the majority (93.1%) were Caucasian. The average  $T_{c > 0.05}$  was 10.72 h (SD: 2.73). As previously reported, CIPN8 was low at baseline (mean = 1.29, SD = 3.04) and increased throughout treatment (mean maximum

**TABLE 1** Patient demographic and genetic information

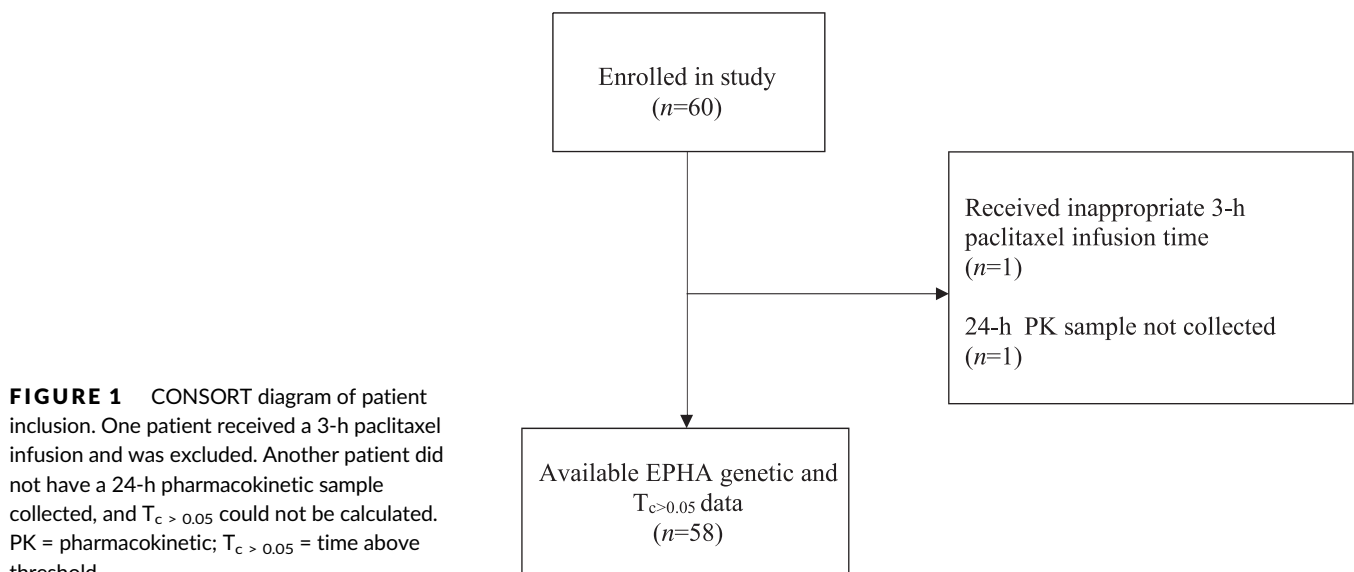
Patient demographics	n (%) or mean (standard deviation)
Age (y)	52.52 (10.31)
Body surface area (m <sup>2</sup> )	1.83 (0.21)
Race (Caucasian)	54 (93.1%)
$T_{c > 0.05}$ (h)	10.72 (2.73)
Baseline CIPN8 (range: 0–100)	1.29 (3.04)
Cumulative dose (mg)	883.95 (163.82)
Relative dose intensity	0.95 (0.01)
rs7349683	Heterozygous: 23 (39.7%) Homozygous variant: 7 (12.1%)

CIPN8 = 13.26, SD = 1.76). Detailed patient demographics and CIPN8 data have been previously described<sup>11</sup>.

The 32 coding variants detected are included in Appendix A with the results of the *in silico* prediction tools and the determination of functional consequence. A total of 13 missense variants were observed in this cohort: rs45498698, rs144329757, rs999765, rs569320402, rs62618734, rs147795823, rs149515751, rs200304246, rs768964879, rs36050417, rs33932471, 2:222347192 and 3:96706703. Four coding variants were considered functionally consequential for this analysis (Table 2). The synonymous variant rs7349683 was considered functionally consequential based on prior literature<sup>12,16,19</sup>. In the *posthoc* genotype verification of rs7349683, sequencing and TaqMan genotyping results were 100% concordant.

### 4.2 | Genetic associations with PN sensitivity

The genotype distribution for rs7349683 was consistent with Hardy-Weinberg equilibrium (minor allele frequency = 0.32,



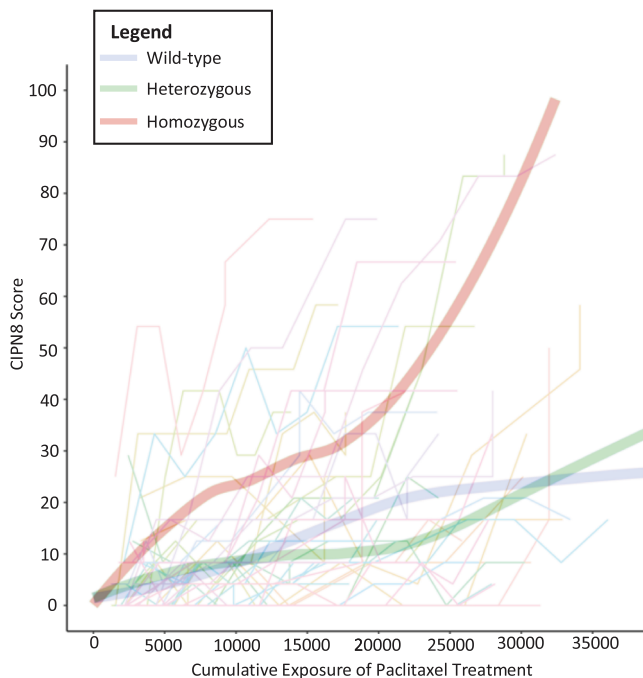
**TABLE 2** Variants included in the 3 genetic models with predicted functional consequence

Gene	Chr:Pos	Ref	Variant type	Existing variation	CADD - PHRED	PROVEAN	Functional consequence	WT/V n (%)	V/V n (%)	Models tested (missense, coding, or rs7349684)
EPHA4	2:222320321	A	Missense	rs768964879	23.5	Neutral	No	1 (1.72)	0 (0)	Missense
EPHA4	2:222347192	C	Missense	----	17.69	Neutral	No	1 (1.72)	0 (0)	Missense
EPHA5	4:66197804	T	Synonymous	rs7349683	10.61	Neutral	Yes <sup>a</sup>	23 (39.66)	7 (12.07)	Coding, rs7349683
EPHA5	4:66231686	T	Missense	rs36050417	22.8	Neutral	No	5 (8.62)	0 (0)	Missense
EPHA5	4:66509085	G	Missense	rs33932471	23.4	Neutral	No	7 (12.07)	0 (0)	Missense
EPHA6	3:96706703	A	Missense	----	22.5	Neutral	No	1 (1.72)	0 (0)	Missense
EPHA8	1:22895820	A	Missense	rs45498698	25.6	Deleterious	Yes	3 (5.17)	0 (0)	Missense, coding
EPHA8	1:22923859	A	Missense	rs144329757	17.56	Neutral	No	3 (5.17)	0 (0)	Missense
EPHA8	1:22923873	C	Missense	rs999765	22.1	Neutral	No	7 (12.07)	1 (1.72)	Missense
EPHA8	1:22927298	T	Missense	rs569320402	25.1	Deleterious	Yes	1 (1.72)	0 (0)	Missense, coding
EPHA8	1:22927503	A	Missense	rs62618734	24.8	Deleterious	Yes	2 (3.45)	0 (0)	Missense, coding
EPHA8	1:22927812	A	Missense	rs147795823	22.2	Neutral	No	1 (1.72)	0 (0)	Missense
EPHA8	1:22927906	A	Missense	rs149515751	24.7	Neutral	No	1 (1.72)	0 (0)	Missense
EPHA8	1:22928229	G	Missense	rs200304246	16.12	Neutral	No	1 (1.72)	0 (0)	Missense

Chr = chromosome; Pos = position; ref = reference; WT = wild type; V = variant  
<sup>a</sup>supported by prior data <sup>12,16,19</sup>

$P = .51$ , Table 1). In the PN sensitivity model, which included  $T_c > 0.05$  and the interaction term, rs7349683 was associated with greater PN sensitivity ( $\beta$ -coefficient: 0.39, 95% confidence interval [CI] 0.11–0.67,  $P = .007$ , Table 2 and Figure 2A). In a secondary analysis assuming a dominant genetic effect, rs7349683 was not associated with PN sensitivity ( $\beta$ -coefficient: 0.35, 95% CI –0.04 to 0.74,  $P = .08$ ).

Carrying a greater number of missense variants (median = 0, range 0–2,) was associated with decreased PN sensitivity ( $\beta$ -coefficient: –0.42, 95% CI –0.72 to –0.12,  $P = .006$ , Table 3). The number of coding (median = 1 range: 0–2) functional variants was not associated with CIPN8 sensitivity (data not shown).



**FIGURE 2** CIPN8 score by cumulative exposure (cumulative dose \*  $T_c > 0.05$ ) stratified by rs7349683 wild-type, heterozygous and homozygous patients. Patients homozygous for rs7349683 had a greater increase in CIPN8 scores with increasing cumulative exposure than heterozygous and wild-type patients<sup>a</sup>.  $T_c > 0.05$  = time above threshold. <sup>a</sup>Solid lines represent lines of best fit

**TABLE 3** Model containing  $T_c > 0.05$  and rs7349683 or total missense variants

Clinical variable	rs7349683		Missense variants	
	Beta-coefficient (95% CI)	P-value	Beta-coefficient (95% CI)	P-value
Baseline CIPN8	0.20 (0.13–0.26)	<.0001	0.20 (0.14–0.27)	<.0001
Cumulative dose <sup>a</sup>	–0.12 (–0.55 to 0.31)	.57	–0.13 (–0.56–0.31)	.56
Dose intensity	–1.80 (–3.28 to –0.31)	.018	–1.61 (–3.10 to –0.13)	.034
Time above threshold	–0.29 (–0.51 to –0.07)	.011	–0.22 (–0.43 to –0.001)	.049
Interaction term with $T_c > 0.05$ and cumulative dose	0.14 (0.04–0.24)	.009	0.14 (0.03–0.25)	.009
Genetic variant	0.39 (0.11–0.67)	.007	–0.42 (–0.72 to –0.12)	.006

<sup>a</sup>Cumulative dose is significant ( $P < .0001$ ) without the interaction term but is not significant in the model with the interaction, as expected.

### 4.3 | Genetic associations with PN-induced treatment disruptions

As previously reported, 19 patients experienced at least 1 treatment disruption during the study<sup>11</sup>. Neither rs7349683 (odds ratio = 0.46, 95% CI = 0.17–1.26,  $P = .13$ ) or total missense variants (odds ratio = 1.04, 95% CI 0.35–3.06,  $P = .94$ ) were associated with sensitivity to PN-induced treatment disruption.

### 4.4 | Posthoc exploration of variants in LD with rs7349683 and missense variants

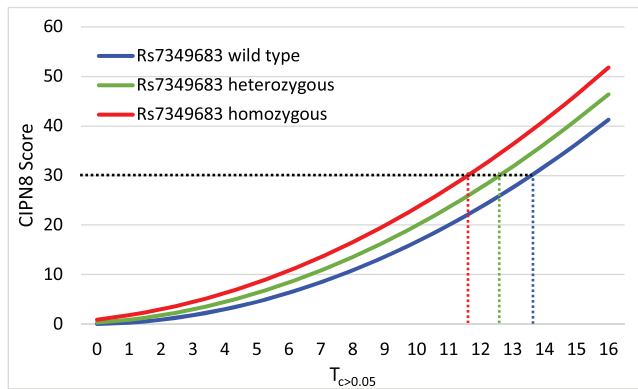
Forty-six variants were found to be in LD with rs7349683 using HaploReg. All variants in LD were located in noncoding or intronic regions. None of the variants exceeded the thresholds for in silico-predicted functional consequence (See Methods) of  $\geq 15$  for the CADD PHRED-like scaled C-score rankings. None of the variants were associated with tissue-specific gene expression in GTex for neuron-related tissue.

For 4 of the 13 missense variants detected by sequencing, rs569320402, rs768964879, 2:22347192 and 3:96706703, LD information was unable to be determined. Ten noncoding or intronic variants were identified that are in LD with these remaining 9 missense variants. Two of the missense variants, rs45498698 and rs144329757, were in complete LD. None of the variants were associated with neuron-related tissue as determined by GTex.

### 4.5 | Posthoc exploration of optimal paclitaxel exposure by CIPN8 score

Using our final PN model parameters, the  $T_c > 0.05$  that causes a typical patient to experience several thresholds of CIPN8 during treatment were estimated. Patients homozygous for rs7349683 have greater predicted CIPN8 than wild-type or heterozygous patients for  $T_c > 0.05$  (Figure 3). For example, using a threshold of CIPN8 = 30, the optimal exposure target to reduce PN-related treatment disruption for a rs7349683 homozygous patient is 11.6 h, whereas the optimal





**FIGURE 3** CIPN8 score by fixed cumulative exposure stratified by rs7349683 wild-type, heterozygous and homozygous patients. Maximum tolerated time above threshold ( $T_c > 0.05$ ) and cumulative exposure (cumulative dose \*  $T_c > 0.05$ ) was estimated for CIPN8 scores of 10, 20, 30, and 40. A CIPN8 clinically relevant threshold of 30 is indicated by dotted lines. Homozygous rs7349683 subjects tolerated the lowest cumulative exposure and shortest  $T_c > 0.05$ . Heterozygous rs7349683 and wild-type subjects were able to tolerate higher paclitaxel exposure and longer  $T_c > 0.05$ .  $T_c > 0.05$  = time above threshold

exposure target for a heterozygous or wild-type patient would be 12.6 or 13.6 h, respectively.

## 5 | DISCUSSION

Paclitaxel-induced PN is a common, debilitating and treatment-limiting adverse effect that is determined by both paclitaxel exposure and a patient's predisposition to PN. PN sensitivity is probably influenced by clinical and genetic factors, such as *EPHA* variants previously associated with increased PN risk<sup>12,16,17,19</sup>. Using our previously published PN models that incorporate cumulative paclitaxel treatment and measured exposure, this proof of concept analysis found evidence that the *EPHA5* synonymous variant rs7349683 is associated with increased PN sensitivity.

Previous studies have observed associations between variants in the ephrin (*EPHA*) genes and increased PN severity or occurrence. In genome-wide association studies conducted by Baldwin *et al.* and Leandro-Garcia *et al.*, rs7349683 was associated with lower cumulative dose at the time of National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade 2 or higher PN occurrence<sup>12,19</sup>. Additionally, in a candidate variant study, rs7349683 was found more frequently in PN cases than controls, as defined by the phenotype extremes using trajectories of CIPN20 increase<sup>16</sup>. Another candidate variant analysis did not detect an association for rs7349683 with NCI-CTCAE grade 1 or higher PN<sup>20</sup>, possibly due to their inability to account for interpatient variability in paclitaxel exposure or to the use of a lower PN threshold that is not as accurately classified. The number of independent studies finding a directionally consistent association for rs7349683 and increased PN, including ours, diminishes the

likelihood that this is a spurious finding. In our secondary analysis rs7349683 was not associated with PN-related treatment disruption, probably due to the modest number of patients experiencing treatment disruption ( $n = 19$ ). Patient-reported outcomes are more sensitive than CTCAE to changes in PN<sup>36</sup>; however, for a genetic predictor to be translated into practice it must predict a clinically relevant endpoint, such as irreversible PN, PN-induced treatment disruption, PN-related falls or diminished quality of life<sup>36,37</sup>.

We also unexpectedly found that patients carrying a greater number of missense variants had lower risk of PN, opposite to a previous report, where low-frequency missense variants in *EPHA5*, *EPHA6* and *EPHA8* increased PN risk<sup>17</sup>. *EPHA4*, *EPHA5*, *EPHA6* and *EPHA8* receptors are involved in neural development; however, pinpointing the precise function of each receptor has been challenging due to hypothesized partial redundancy in action<sup>21</sup> complicating the ability to draw conclusions about the impact of variants on function. Alternatively, our finding may be a false positive, as these variants were not found to be in LD with any putatively consequential variants.

Continued PN biomarker validation is necessary to enable integration of genetic, clinical and pharmacokinetic data to guide personalized paclitaxel therapy. Exposure-guided paclitaxel dosing has been demonstrated to reduce PN occurrence in nonsmall cell lung cancer patients receiving paclitaxel every 3 weeks<sup>10</sup>. However, no prospective trials have been conducted in patients receiving smaller, weekly paclitaxel doses, similar to this cohort of patients. If rs7349683 is validated as a PN sensitivity biomarker, this exposure target would need to be personalized. Based on the results of this analysis, and assuming the maximum CIPN8 score a patient can tolerate is 30 based on our previous findings<sup>11</sup>, a rs7349683 homozygous subject could only tolerate a  $T_c > 0.05$  of 12 h whereas carriers or wild-type patients could tolerate a  $T_c > 0.05$  of 13 and 14 h, respectively. Translation of this proof of concept personalized exposure-targeted dosing approach into clinical care will require prospective trials assessing the effect of rs7349683-guided dosing on PN and efficacy.

Strengths to our approach include the novel inclusion of paclitaxel exposure data ( $T_c > 0.05$ ) in the statistical model to elucidate *EPHA* variants associated with PN sensitivity, use of patient reported outcomes instead of NCI-CTCAE grade, and use of prospectively collected data. In this novel proof of concept analysis, several limitations are worth considering. These findings may not be generalizable to other patient populations such as male patients with breast or other cancer types, or patients treated with other paclitaxel dosing regimens or other neurotoxic chemotherapeutic agents. The bioinformatics tools may have incorrectly predicted which of the variants detected by sequencing are or are not functionally consequential<sup>33,38-40</sup>. Despite their limitations, the bioinformatics tools have moderate specificity predicting functional consequence of coding variants<sup>41</sup>. Noncoding variants were also assessed using these tools; however, those data were not included due concerns about the quality of the predictions. Additionally, it is unclear from our bioinformatics analysis how the synonymous rs7349683 variant or the combined missense variants impact

*EPHA5* expression or function, leading to their effects on PN sensitivity. In the missense and coding models, low-frequency variants were included in the analysis. This assumes that all variants were similarly consequential to function, which might not be the case. Another limitation is the lack of statistical correction for the 3 genetic models, increasing the possibility of false positive findings. Despite this fact, the results are significant after multiple comparisons correction.

Using a novel, sensitive approach for biomarker science that isolates PN sensitivity by accounting for measured systemic paclitaxel exposure, this study supports prior evidence that *EPHA5* rs7349683 is associated with increased PN, and suggests that this association is due to its direct effect on increasing PN sensitivity. Additional clinical pharmacogenetics studies with measured exposure are needed to confirm the association with clinically meaningful endpoints, followed by integration of rs7349683 into personalized treatment approaches and prospective demonstration of improved therapeutic outcomes from paclitaxel treatment. Finally, researchers should collect drug exposure data when conducting biomarker studies for multifactorial toxicities, and use our novel approach of isolating toxicity sensitivity for biomarker discovery, validation and translation.

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#### COMPETING INTERESTS

D.L. Hertz has an informal, unpaid collaborative relationship with Saladax Biomedical Inc., a company that offers CLIA-approved paclitaxel measurement. Saladax was not involved in the design, conduct, analysis, or sponsorship of this trial, and had no contribution to the writing of this manuscript. N.L. Henry has contracted research to her institution from AbbVie, Innocrim Pharmaceuticals, and Pfizer.

#### CONTRIBUTORS

The observational clinical study (UMCCC 2014.002, NCT0233815) was designed by D.L. Hertz, N.L. Henry and E.M. Lavoie Smith. K. Vangiupram, M.L. Burness, J.J. Griggs, C. Van Poznak, D.F. Hayes and N.L. Henry recruited patients and collected samples and data. The secondary analysis was designed by L.A. Marcath and D.L. Hertz. Genotyping and sequencing was conducted by C.L. Gersch, J.M. Rae and A.S. Beutler and sequencing data cleaning was conducted by L.A. Marcath. Statistical analysis was conducted by K.M. Kidwell. This manuscript was drafted by

L.A. Marcath and D.L. Hertz. All coauthors reviewed and approved this manuscript prior to submission.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A: | CODING VARIANT CLASSIFICATION OF FUNCTIONAL CONSEQUENCE AND NUMBER OF PATIENTS CARRYING VARIANTS INCLUDED IN THE MODELS TESTED

Gene	Chr:Pos	Ref	Variant Type	Existing variation	CADD - PHRED	PROVEAN	Functional consequence	Heterozygous n (%)	Homozygous n (%)	Models Tested (Missense, Coding, or rs7349684)
EPHA4	2:222290829	T	Synonymous	rs35860178	8.295	Neutral	no	----	----	----
EPHA4	2:222320321	A	Missense	rs768964879	23.5	Neutral	no	1 (1.72)	0 (0)	Missense
EPHA4	2:222347192	C	Missense	---	17.69	Neutral	no	1 (1.72)	0 (0)	Missense
EPHA4	2:222365855	G	Synonymous	rs144077750	11.51	Neutral	no	----	----	----
EPHA5	4:66197696	A	Synonymous	rs371489573	9.564	Neutral	no	----	----	----
EPHA5	4:66197804	T	Synonymous	rs7349683	10.61	Neutral	yes*	23 (39.66)	7 (12.07)	Coding, rs7349683
EPHA5	4:66231686	T	Missense	rs36050417	22.8	Neutral	no	5 (8.62)	0 (0)	Missense
EPHA5	4:66356159	G	Synonymous	rs145994427	11.72	Neutral	no	----	----	----
EPHA5	4:66467732	A	Synonymous	rs55983743	0.632	Neutral	no	----	----	----
EPHA5	4:66509085	G	Missense	rs33932471	23.4	Neutral	no	7 (12.07)	0 (0)	Missense
EPHA5	4:66535284	A	Synonymous	rs55860557	20.6	Neutral	no	----	----	----
EPHA6	3:96533683	T	Synonymous	rs77729199	2.033	Neutral	no	----	----	----
EPHA6	3:96706703	A	Missense	----	22.5	Neutral	no	1 (1.72)	0 (0)	Missense
EPHA6	3:96963004	T	Synonymous	rs9835140	7.81	Neutral	no	----	----	----
EPHA6	3:97167522	A	Synonymous	rs56147502	1.919	Neutral	no	----	----	----
EPHA6	3:97194242	T	Synonymous	rs16838751	5.269	Neutral	no	----	----	----
EPHA8	1:22895820	A	Missense	rs45498698	25.6	Deleterious	yes	3 (5.17)	0 (0)	Missense, Coding
EPHA8	1:22902772	T	Synonymous	rs56218493	7.584	Neutral	no	----	----	----
EPHA8	1:22903102	T	Synonymous	rs55844027	11.83	Neutral	no	----	----	----
EPHA8	1:22915674	A	Synonymous	rs56044578	0.417	Neutral	no	----	----	----
EPHA8	1:22919904	T	Synonymous	rs209696	14.47	Neutral	no	----	----	----
EPHA8	1:22920094	G	Synonymous	rs56157621	2.64	Neutral	no	----	----	----
EPHA8	1:22923859	A	Missense	rs144329757	17.56	Neutral	no	3 (5.17)	0 (0)	Missense

(Continues)

Gene	Chr:Pos	Ref	Variant Type	Existing variation	CADD - PHRED	PROVEAN	Functional consequence	Heterozygous n (%)	Homozygous n (%)	Models Tested (Missense, Coding, or rs7349684)
EPHA8	1:22923873	C	Missense	rs999765	22.1	Neutral	no	7 (12.07)	1 (1.72)	Missense
EPHA8	1:22925420	C	Synonymous	rs755679171	5.455	Neutral	no	----	----	----
EPHA8	1:22927298	T	Missense	rs569320402	25.1	Deleterious	yes	1 (1.72)	0 (0)	Missense, Coding
EPHA8	1:22927408	A	Synonymous	rs56055011	15.23	Neutral	no	----	----	----
EPHA8	1:22927503	A	Missense	rs62618734	24.8	Deleterious	yes	2 (3.45)	0 (0)	Missense, Coding
EPHA8	1:22927812	A	Missense	rs147795823	22.2	Neutral	no	1 (1.72)	0 (0)	Missense
EPHA8	1:22927906	A	Missense	rs149515751	24.7	Neutral	no	1 (1.72)	0 (0)	Missense
EPHA8	1:22928229	G	Missense	rs200304246	16.12	Neutral	no	1 (1.72)	0 (0)	Missense
EPHA8	1:22927385	T	Splice	rs368425196	5.762	----	**	----	----	----

Chr = chromosome, Pos = position, Ref = reference, WT = wild type, V = variant

\*supported by prior data(12, 16, 19); \*\* functional consequence not determined since not scored by PROVEAN