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Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Replication of Genetic Polymorphisms Reported to Be Associated with Taxane-Related Sensory Neuropathy in Patients with Early Breast Cancer Treated with Paclitaxel

Jean E. Abraham[1,2,4], Qi Guo[1], Leila Dorling[1], Jonathan Tyrer[1], Susan Ingle[2], Richard Hardy[2], Anne-Laure Vallier[2], Louise Hiller[5], Russell Burns[2], Linda Jones[2], Sarah J. Bowden[6], Janet A. Dunn[5], Christopher J. Poole[5], Carlos Caldas[2,3,4], Paul P.D. Pharoah[1], and Helena M. Earl[2,4]

Abstract

Purpose: Associations between taxane-related sensory neuropathy (TRSN) and single-nucleotide polymorphisms (SNP) have previously been reported, but few have been replicated in large, independent validation studies. This study evaluates the association between previously investigated SNPs and TRSN, using genotype data from a study of chemotherapy-related toxicity in patients with breast cancer.

Experimental Design: We investigated 73 SNPs in 50 genes for their contribution to TRSN risk, using genotype data from 1,303 European patients. TRSN was assessed using National Cancer Institute common toxicity criteria for adverse events classification. Unconditional logistic regression evaluated the association between each SNP and TRSN risk (primary analysis). Cox regression analysis assessed the association between each SNP and cumulative taxane dose causing the first reported moderate/severe TRSN (secondary analysis). The admixture likelihood (AML) test, which considers all SNPs with a prior probability of association with TRSN together, tested the hypothesis that certain SNPs are truly associated.

Results: The AML test provided strong evidence for the association of some SNPs with TRSN ($P = 0.023$). The two most significantly associated SNPs were rs3213619(*ABCB1*) [OR = 0.47; 95% confidence interval (CI), 0.28–0.79; $P = 0.004$] and rs9501929(*TUBB2A*) (OR = 1.80; 95% CI, 1.20–2.72; $P = 0.005$). A further 9 SNPs were significant at *P*-value ≤ 0.05.

Conclusion: This is currently the largest study investigating SNPs associated with TRSN. We found strong evidence that SNPs within genes in taxane pharmacokinetic and pharmacodynamic pathways contribute to TRSN risk. However, a large proportion of the inter-individual variability in TRSN remains unexplained. Further validated results from GWAS will help to identify new pathways, genes, and SNPs involved in TRSN susceptibility. *Clin Cancer Res; 20(9); 2466–75. ©2014 AACR.*

Authors' Affiliations: [1]Department of Oncology and Strangeways Research Laboratory, University of Cambridge; [2]Cambridge Breast Unit and NIHR Cambridge Biomedical Research Centre, University of Cambridge NHS Foundation Hospitals; [3]Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way; [4]Cambridge Experimental Cancer Medicine Centre, Cambridge; [5]Warwick Clinical Trials Unit, University of Warwick; and [6]Cancer Research UK Clinical Trials Unit (CRCTU), University of Birmingham, Birmingham, United Kingdom

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

J.E. Abraham and Q. Guo contributed equally to this article.

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Corresponding Authors: Paul P.D. Pharoah, Department of Oncology and Strangeway's Research Laboratory, University of Cambridge, 2 Worts Causeway, Cambridge CB1 8RN, UK. Phone: 44-1223-761926; E-mail: paul.pharoah@medschl.cam.ac.uk and Carlos Caldas, Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK. Phone: 44-1223-769650; E-mail: carlos.caldas@cruk.cam.ac.uk

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Introduction

Taxanes have been in routine use in cancer therapy for over a decade. In the management of breast cancer, they are used in the neo-adjuvant, adjuvant, and metastatic setting. The 2 main taxanes in clinical use are paclitaxel and docetaxel. Paclitaxel was originally isolated from the bark of the Pacific yew tree, *Taxus brevifolia* and docetaxel, is a semi-synthetic analogue of paclitaxel. The mechanism of action is microtubule assembly stabilization, which ultimately inhibits mitotic cell division. The taxane binding sites on β-tubulin (functional domains) have been identified and are similar for both taxanes (1).

The main nonhematologic toxicity associated with taxanes is sensory peripheral neuropathy. Peripheral neuropathy can be functionally debilitating and may lead to dose reduction, dose delay, and early cessation of taxane treatment. This may affect treatment efficacy and potentially clinical outcome. Reported rates of taxane-related sensory neuropathy (TRSN) are variable depending on factors such as the dose of taxane given, concomitant neurotoxic

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Translational Relevance

Taxanes are chemotherapeutic agents used in a wide variety of cancers. The main nonhematologic side effect that limits dose density and treatment duration is taxane-related sensory neuropathy (TRSN). Multiple studies have investigated many putative functional variants, mostly derived from the taxane pharmacokinetic and pharmacodynamic pathways. The results of these studies have been conflicting and the studies have frequently had inadequate statistical power to provide a conclusive answer.

This is a large and comprehensive study, which has investigated the role of genetic variants associated with TRSN. In addition, the study provides a global test that gives an indication of the likelihood of true associations within the cohort of genetic variants assessed. The ability to predict for TRSN before taxane treatment would allow modification of dose or treatment based on each individual's risk of TRSN.

drugs, and preexisting comorbidities. The prevalence of National Cancer Institute common toxicity criteria for adverse events (NCI CTCAE) grade 2 neuropathy is 50% to 80% in patients with stage I to III breast cancer (2–4) whereas the prevalence of grade 3 or grade 4 neuropathy is 5% to 30% (2, 5). Longer-term neurotoxicity complications (beyond 2 years postchemotherapy) have also been reported (6).

The mechanism underlying sensory neurotoxicity associated with taxane therapy is unclear. Many different hypotheses have been postulated, including excessive microtubule assembly, which causes abnormal accumulation of disorganized microtubules and disrupts normal cellular activities such as axonal transport and Schwann cell function (7). It is likely that neuropathy secondary to taxanes occurs through a different mechanism, to that of neuropathy secondary to platinum agents and other neurotoxic chemotherapy agents (8).

There is evidence to show that chemotherapy-related toxicity is a heritable trait. Watters and colleagues (9) used lymphoblastoid cells derived from Centre d'Etude du Polymorphisme Humain reference pedigrees to show that cytotoxicity to the mechanistically different chemotherapy agents 5-fluorouracil (5FU) and docetaxel are heritable traits. Heritability values, for a variety of cytotoxic doses of 5FU and docetaxel, ranged from 0.26 to 0.65 for (5FU) and 0.21 to 0.70 (docetaxel), respectively. In addition, Nicolae and colleagues (10) used the functional characterization of 2 closely related drugs, for example carboplatin and cisplatin to demonstrate that the genetic variations associated with chemotherapeutic toxicity may be similar for closely related drugs, that is these are likely to be drug class effects.

A predictive biomarker of TRSN would allow stratification by TRSN risk and potentially pretreatment modification of taxane therapy to avoid serious and potentially permanent neurotoxicity. Previously published TRSN functional candidate genes have generally been associated with the taxane pharmacokinetic pathways (Fig. 1). The most commonly reported candidate genes associated with TRSN include *ABCB1* (*MDR1*) and *CYP2C8* (Supplementary Table S1). Variants of *ABCB1* have been reported to show an association with both increased and decreased risk of TRSN. *CYP2C8* variants have been reported most often as positively associated with increased risk of neurotoxicity (*P*-values $\sim 10^{-2}$). Examples of other genes investigated include *CYP3A4*, *CYP3A5*, *CYP1B1*, *ABCC1*, *ABCC2*, and *ABCG2*.

However, most studies have been underpowered, with small samples sizes (sample sizes by study; Supplementary Table S1) and inadequate adjustment for multiple testing. In addition, the treatment regimen has included 2 neurotoxic drugs in some studies. For example, in the treatment of ovarian cancer, taxanes and platinum drugs are used together, which complicates defining the causative genetic changes that contribute to drug induced neuropathy. None of the candidate genes identified has been sufficiently robust to develop into reliable biomarkers of TRSN. More recently, genome-wide association studies (GWAS) have been used to identify the genetic determinants of neurotoxicity (2, 4, 11), but again no definitive associations have been found.

The purpose of this study was to evaluate the association of previously investigated candidate single-nucleotide polymorphisms (SNP) using genotype data from a large cohort of patients with stage I to III breast cancer. The promising SNPs reported in published GWAS (2, 11) have also been assessed.

Patients and Methods

Patients

The study population comprised patients participating in the "Pharmacogenetics of Breast Cancer Chemotherapy (PGSNPS)" study that were recruited from 4 randomized controlled adjuvant/neoadjuvant breast cancer chemotherapy trials; NEAT (12), BR960 (12), tAnGo (13), and NeotAnGo (14). Clinical trial flow diagrams for the paclitaxel-containing trials that contributed to this analysis, including eligibility criteria, are given in Fig. 2. In this analysis, only patients treated with paclitaxel, from the tAnGo and Neotango trials, were included. Clinical information about treatment response, toxicity, and outcome is available for all patients recruited to PGSNPS. Drug regimens and dosages are given in Supplementary Tables S2A. Supplementary Table S2B shows TRSN grade by trial. The characteristics of the patients with paclitaxel-treated PGSNPS are given in Supplementary Table S3. The TRSN study population was entirely European in ethnicity (see "Statistical Analysis" section). The PGSNPS study used a GWAS approach to assess for TRSN. The full GWAS results will require validation before publication, in order to reduce the likelihood of false positive results, and are therefore not included in this article.

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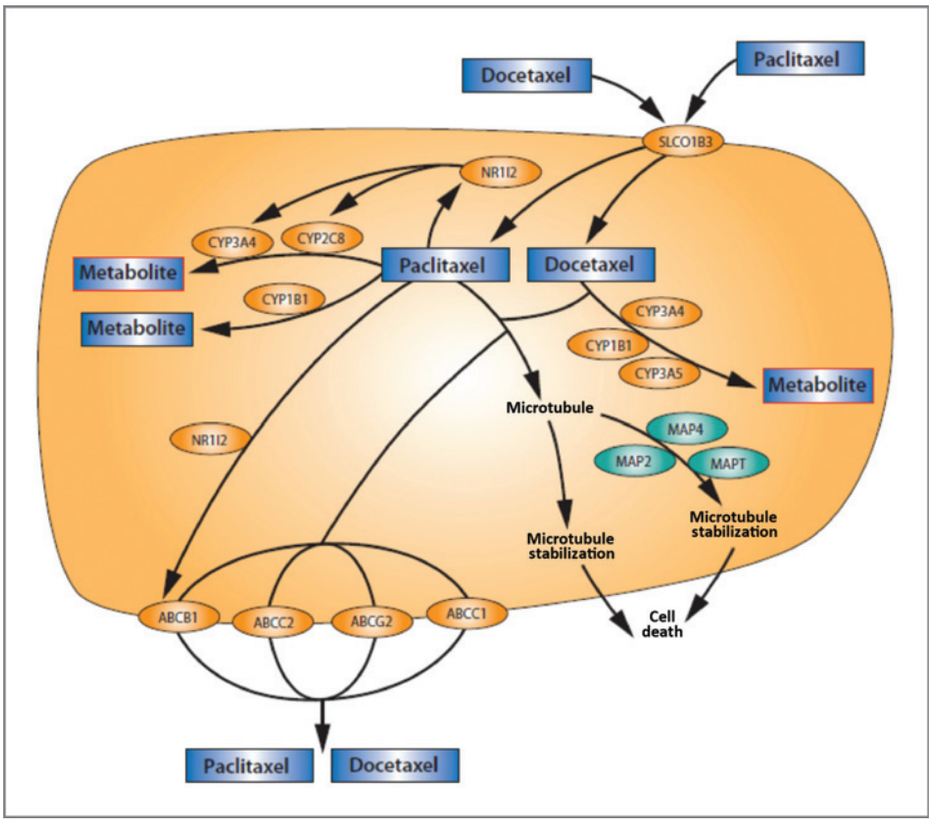

Figure 1. Taxane pathway. Abbreviations: SLCO1B3, Solute carrier organic anion transporter family, member 1B3; CYP3A4, cytochrome P450, family 3, subfamily A, polypeptide 4; CYP3A5, cytochrome P450, family 3, subfamily A, polypeptide 5; CYP1B1, cytochrome P450, family 1, subfamily B, polypeptide 1; CYP2C8, cytochrome P450, family 2, subfamily C, polypeptide 8; NR1I2, nuclear receptor subfamily 1, group I, member 2; MAP4, microtubule-associated protein 4; MAP2, microtubule-associated protein 2; MAPT, microtubule-associated protein tau; ABCB1, ATP-binding cassette, subfamily B (MDR/TAP), member 1; ABCC1, ATP-binding cassette, subfamily C (MDR/TAP), member 1; ABCC2, ATP-binding cassette, subfamily C(MDR/TAP), member 2; ABCG2, ATP-binding cassette, subfamily G (MDR/TAP), member 2.

The PGSNPS study received ethical approval in 2005 from the Cambridgeshire and Huntingdon research ethics committee.

Samples, genotyping, and quality control

During the period 2005 to 2009, a total of 1,335 samples (1,227 bloods and 108 salivas) were collected from 1,335 patients who had been or were being treated with taxanes in the tAnGo and neo-tAnGo trials. DNA was extracted and stored at −40°C. Genotyping was carried out using the Affymetrix SNP6.0 array in a single center. The CRLMM calling algorithm used for the PGSNPS GWAS analysis provides a measure of confidence for each genotype call as well as quality metrics for SNPs, samples, and hybridization batches. Standard quality control for SNPs and samples were applied. SNPs were excluded according to the following criteria: minor allele frequency (MAF) < 0.01; MAF < 0.05 and call rate < 0.99; MAF ≥ 0.05 and call rate < 0.95; deviation of genotype frequencies from those expected under Hardy Weinberg equilibrium $P < 10^{-5}$. Samples with a call rate <0.9 or outliers for heterozygosity were excluded leaving 1,303 patients in the analysis. The 1,303 samples included 1,195 blood samples and 108 saliva samples.

Chemotherapy-related neurotoxicity was assessed using the NCI CTCAE version 2. The worst sensory neuropathy grade experienced during any cycle of paclitaxel chemotherapy was used in this analysis. Patients were classified as cases [NCI CTCAE grades 2–4; $n = 360$ (28%)] or as controls [NCI CTCAE grades 0–1; $n = 943$ (72%)].

Candidate gene selection for assessment in PGSNPS

We carried out a systematic review of the literature using the following electronic databases and search engines: PubMed/Medline, the Cochrane library, PharmGKB (15), Ovid/Embase, Google, and Springerlink. The search terms included the medical subject headings (MeSH) terms "neuropathy taxanes pharmacogenetics," "neurotoxicity taxanes pharmacogenetics," "neuropathy neurotoxicity taxanes SNPs," "neuropathy neurotoxicity taxanes single-nucleotide polymorphism," and "sensory peripheral neuropathy taxanes." In addition, searches were performed by author name and using the gene names of the most commonly reported genes. Finally, we hand searched the bibliographies of articles identified through electronic searches. The last search was performed in August 2013.

We only included studies that reported directly on an association between a genetic variation and TRSN (Supplementary Table S1; refs. 2, 11, and 16–29). Any studies investigating genetic variations only associated with pharmacokinetic, outcome, or taxane resistance endpoints but not sensory peripheral neuropathy/neurotoxicity were excluded. Studies were excluded from the analysis if they were reported only in abstract form with limited information or in articles where information about the putative functional variant and the strength of association were

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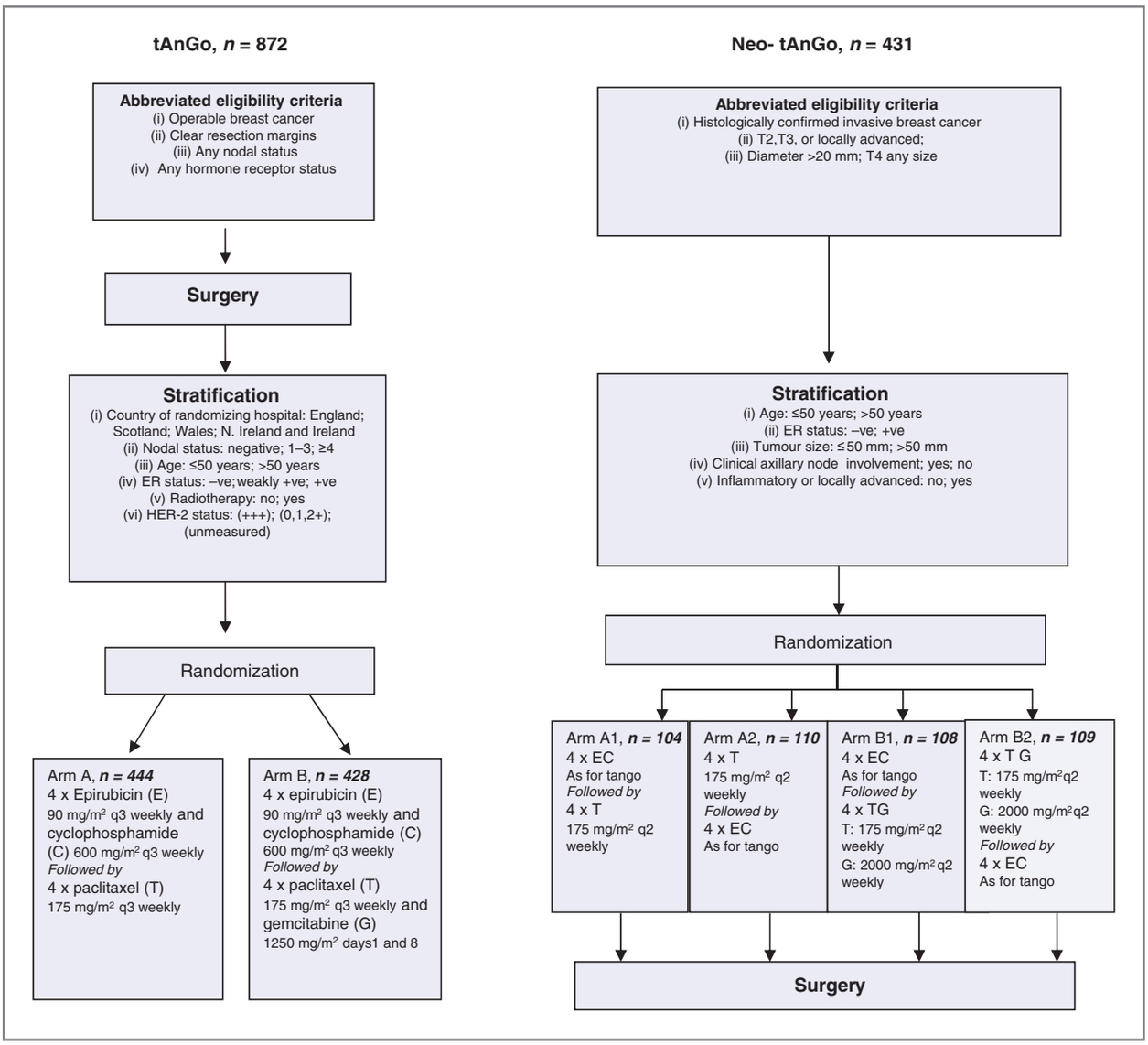

Figure 2. Randomized controlled clinical trials contributing to TRSN analysis. Only patients receiving paclitaxel (taxane) from tAnGo and Neo-tAnGo were included in this analysis.

missing or inadequate. Any tumor types treated with taxanes were considered. Studies that were not available in English were not included.

Statistical analysis

The program LAMP (30) was used to assign intercontinental ancestry based on the 1,000 genomes (31) dataset and all subjects included in the analysis were of European ancestry. Thirty-four samples were excluded from the analysis as the subjects were of non–European ethnicity. We then used a set of 37,000 unlinked markers to perform principal-components analysis within European subgroup (32). Missing genotype data for all the variants were imputed in order to increase genome coverage. Genotype imputation was performed using the prephasing/imputation stepwise approach implemented in IMPUTE2 and SHAPEIT (chunk size of 5 Mb and default parameters; refs. 33 and 34). The imputation reference set consisted of 2,184 phased haplotypes from the full 1,000 Genomes Project dataset (March 2012). All genomic locations are given in NCBI Build 37/UCSC hg19 coordinates. We estimated the accuracy of imputation by calculating the estimated r^2 between the imputed and actual SNP. SNPs with $r^2 < 0.3$ were excluded.

Unconditional logistic regression was used to evaluate the association between each SNP and risk of ≥grade 2 neurotoxicity. A likelihood ratio test was used to test for association and per-allele ORs and 95% confidence intervals (CI) were estimated. Nongenetic factors associated with TRSN such as trial, performance status, age, menopausal

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status, BMI, were assessed. BMI and trial status were significant and included in the multivariate analysis. To enable this analysis on very large sample sizes, we used an in-house program written in C++. The model was adjusted for population substructure by including the first 2 eigenvalues from the principal-components analysis in the model. Inflation of the test statistics (λ) was estimated by dividing the 45th percentile of the test statistic by 0.357 (the 45th percentile for a χ^2 distribution on 1 degree of freedom). The resulting genomic inflation factor was 1.003.

A secondary analysis was undertaken that used a time-to-event approach (2) in which an event is described as the first incidence of TRSN grade $\geq$2 and time was defined as cumulative paclitaxel dose (mg/m^2) at the event. For patients not experiencing any event, the total study paclitaxel drug exposure was used. Cumulative dose data were unavailable for 35 patients, so the sample size for this analysis was 1,268 patients. The association between SNPs and cumulative dose levels was assessed using Cox regression including first 2 eigenvalues, BMI and trial as covariates. The 73 SNPs under investigation were also assessed in this analysis (Supplementary Table S4).

In addition to the single SNP-by-SNP tests for association, we used the admixture maximum likelihood method (35, 36) to perform a global test of the null hypothesis that none of the SNPs are associated with TRSN compared with the alternative that 1 or more of the SNPs are associated. We chose a subset of the SNPs that were uncorrelated to avoid overweighting regions with many correlated SNPs. This was achieved by choosing the SNP with the best *a priori* evidence for association from mutually correlated SNPs. The statistical significance of the result is estimated by a permutation test.

This article adheres to the STREGA/STROBE (37) guidelines.

Results

We identified 17 relevant publications (Supplementary Table S1; refs. 2, 11, and 16–29). TRSN assessments were performed in most studies using a variety of NCI CTCAE versions and the majority of them classified cases (TRSN grade $\geq$2) and controls (TRSN grade $\leq$1) using the same criteria as we have. We identified 73 SNPs that had been previously reported or investigated for an association with TRSN and evaluated their association with TRSN in our own paclitaxel-treated population. The statistically significant SNPs found to be associated with TRSN are shown in Tables 1 and 2. Eleven SNPs were not included in the AML analysis because of high correlation with other SNPs in the dataset. Of the remaining 62 SNPs, 1 SNP could not be found in our imputed dataset and 2 rare SNPs were excluded. Thus, we evaluated the association of 59 (uncorrelated) SNPs with TRSN. Nineteen SNPs were present on the Affymetrix SNP6.0 genotyping array. Imputed genotypes were available for 40 SNPs. Data were imputed from the 1,000 Genomes Project (31).

Maximum TRSN analysis

Table 1 shows 11 SNPs were associated with TRSN at $p < 0.05$ of which 9 were uncorrelated SNPs. We used the AML test (36) as a global experiment-wise test of association based on a subset of uncorrelated SNPs. We found strong evidence for association ($P = 0.023$), suggesting that a subset of these SNPs is truly associated with TRSN. Indeed, 9 of the 59 uncorrelated SNPs were associated with TRSN at a nominal $P < 0.05$ compared with the 3 SNPs expected under the null hypothesis (Table 1 and Fig. 3).

Two SNPs showed a strong association with TRSN, rs3213619 in *ABCB1* ($P = 0.004$), and rs9501929 in *TUBB2A* ($P = 0.005$). The rs3213619 in *ABCB1* ($P = 0.004$; OR = 0.47; 95% CI, 0.28–0.79; MAF = 0.04) was associated with a decreased risk of TRSN. The effect allele (minor allele) of rs9501929 in *TUBB2A* (MAF = 0.04) was associated with an increased risk of TRSN (OR = 1.80; 95% CI, 1.20–2.72).

Several SNPs in *ABCB1* have previously been commonly reported to be associated with TRSN (16–20, 22–26). Two of such SNPs are *ABCB1*, rs1045642 ($P = 0.03$), and rs2032582 ($P = 0.02$). The rs1045642 was not included in the AML analysis as it was highly correlated with another SNP. In rs2032582, the minor allele is associated with an increased risk of TRSN (rs2032582, OR = 1.22; 95% CI, 1.03–1.45).

One of the most commonly reported variants associated with TRSN is *CYP2C8*3* (rs11572080; refs. 16–21). *CYP2C8*3* (rs11572080) was not significantly associated with TRSN risk in our data (OR = 1.22; 95% CI, 0.93–1.59; $P = 0.14$). However, rs1058930 *CYP2C8*4* (OR = 1.48; 95% CI, 1.02–2.15; $P = 0.04$) showed an increased risk of TRSN.

Table 1 shows the statistically significant SNPs associated with maximum TRSN. The full maximum TRSN results are shown in Supplementary Table S5.

Supplementary Fig. S1 presents the percentage of patients with TRSN $\geq$ grade 2 by genotype, for 3 statistically significant SNPs that were genotyped in PGSNPS (maximum TRSN analysis).

Cumulative dose to TRSN analysis

The statistically significant SNPs associated with the cumulative dose TRSN analysis are shown in Table 2. The full results of this analysis are shown in Supplementary Table S4. Nine of the 11 SNPs found to be associated with TRSN risk in the maximum TRSN analysis are similarly statistically significant in this analysis and there are no marked differences between the 2 analyses. The only minor differences in the results for cumulative dose to TRSN in comparison to the maximum TRSN analysis was that the *EPHA6* variant rs301927 becomes modestly more significant ($P = 0.008$; OR = 1.29; 95% CI, 1.07–1.55). In addition, *C19orf21*(rs8110536) shows a borderline association with TRSN ($P = 0.05$; OR = 1.37; 95% CI, 1.01–1.87).

Supplementary Fig. S2A to S2C shows Kaplan–Meier curves for each SNP, by genotype, the probability of TRSN grade 0 to 1 (i.e., the probability of not experiencing moderate/severe TRSN) as the cumulative dose increases.

Table 1. Statistically significant SNPs replicated in the PGSNPS dataset (phenotype: maximum TRSN)

Gene	Variant	SNP rs number	Chromosome	Position DBSNP build 37	Major/ minor allele	Type of SNP genotyped (G) imputed (I)	Imputation R^2	OR[a]	95% CI[a]	*P*-value	Included in AML yes (Y) no (N)
CYP2C8	*4	rs1058930	10	96818119	G/C	G		1.48	1.02–2.15	0.04	Y
ABCB1	—	rs2032582	7	87160618	C/A	I	0.99	1.22	1.03–1.45	0.02	Y
ABCB1	—	**rs1045642	7	87138645	A/G	G		0.83	0.70–0.98	0.03	N [high correlation with other SNP(s)]
ABCB1	—	rs3213619	7	87230193	A/G	I	0.99	0.47	0.28–0.79	0.004	Y
ABCC2	—	rs8187710	10	101611294	G/A	I	1	0.63	0.42–0.93	0.02	Y
ABCC2	—	**rs17222723	10	101595996	T/A	I	1	0.66	0.44–1.01	0.05	N [high correlation with other SNP(s)]
CYP1B1	*3	rs1056836	2	38298203	G/C	G		0.81	0.68–0.96	0.02	Y
TUBB2A	—	rs9501929	6	3157854	T/C	I	0.96	1.80	1.20–2.72	0.005	Y
KIAA0146-PRKD[b]	—	rs6473187	8	48483958	A/G	I	0.91	1.48	1.01–2.17	0.04	Y
SLCO1B1[c]	—	rs3829306	12	21292280	C/T	I	0.99	0.66	0.44–1.01	0.05	Y
EPHA6	—	rs301927	3	97346618	A/G	I	0.99	1.35	1.07–1.70	0.01	Y

Not all variants described in each study as having "no association with neuropathy" are reported in the table.

OR > 1 indicates an increased risk of TRSN.

OR < 1 indicates a decreased risk of TRSN.

[a]ORs and 95% CIs represent the per-allele effect of the minor allele for any particular SNP.

[b]rs6473187 in *KIAA0146* is in complete LD with rs2632496 in *KIAA0146*, with rs4873774 in *UBE2V2*, and with rs8178108 in *PRKD*.

[c]rs3829306 is in complete LD with rs4149013 and rs4149023, both in *SLCO1B1*.

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Table 2. Statistically significant SNPs replicated in the PGSNPS dataset (phenotype: TRSN cumulative dose analysis)

Gene	Variant	SNP rs number and nucleotide change	Chromosome	Position DBSNP build 37	Major/ minor allele	Type of SNP genotyped (G) imputed (I)	Imputation R^2	HR[a]	95% CI[a]	*P*-value	Included in AML yes (Y) no (N)
CYP2C8	*4	rs1058930	10	96818119	G/C	G		1.38	1.03–1.86	0.04	Y
ABCB1	—	rs2032582	7	87160618	C/A	I	0.99	1.19	1.04–1.38	0.02	Y
ABCB1	—	**rs1045642	7	87138645	A/G	G		0.83	0.72–0.95	0.009	N [high correlation with other SNP(s)]
ABCB1	—	rs3213619	7	87230193	A/G	I	0.99	0.51	0.31–0.85	0.004	Y
ABCC2	—	rs8187710	10	101611294	G/A	I	1	0.71	0.50–1.02	0.05	Y
CYP1B1	*3	rs1056836	2	38298203	G/C	G		0.83	0.72–0.96	0.01	Y
TUBB2A	—	rs9501929	6	3157854	T/C	I	0.96	1.60	1.18–2.18	0.005	Y
SLCO1B1[b]	—	rs3829306	12	21292280	C/T	I	0.99	0.67	0.46–0.97	0.02	Y
C19orf21	—	rs8110536	19	756985	T/G	I	0.37	1.37	1.01–1.87	0.05	Y
EPHA6	—	rs301927	3	97346618	A/G	I	0.99	1.29	1.07–1.55	0.008	Y

Not all variants described in each study as having "no association with neuropathy" are reported in the table.

OR > 1 indicates an increased risk of TRSN.

OR < 1 indicates a decreased risk of TRSN.

[a]HRs and 95% CIs represent the per-allele effect of the minor allele for any particular SNP.

[b]rs3829306 is in complete LD with rs4149013 and rs4149023, both in *SLCO1B1*.

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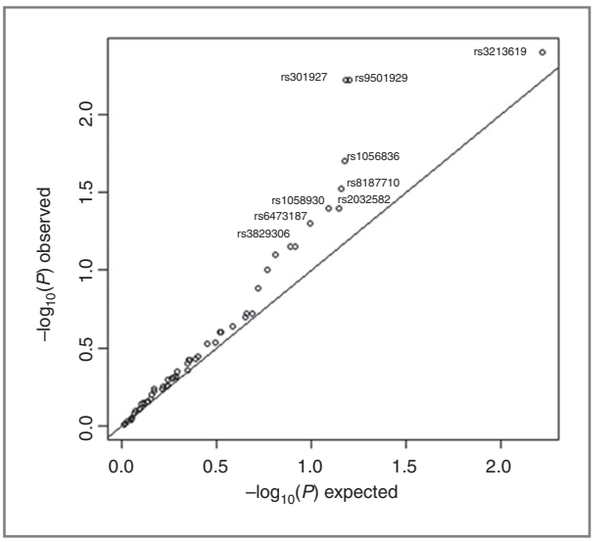

Figure 3. Q–Q plot for replicated polymorphisms associated with taxane neuropathy. Only the SNPs reaching statistical significance ($P \leq 0.05$) are labeled with rs numbers.

In both Supplementary Figures S1 and S2, data are only shown for statistically significant genotyped SNPs (not for imputed SNPs).

Discussion

Over the past decade, many studies have evaluated genetic variants in multiple genes for an association with TRSN, but few definite risk alleles have been found at stringent levels of statistical significance. This may be because the variants studied are not associated with TRSN or because studies with small sample sizes lacked statistical power to detect the effect of these variants. This is the largest reported study investigating TRSN ($n = 1{,}303$). We have completed a comprehensive review of previously published genes and SNPs investigated for an association with TRSN and identified 17 studies involving 73 SNPs in 50 genes. Of these 73 SNPs, 49 SNPs have previously reported a significant association with TRSN in either direction. We used the data from a genome-wide association study of chemotherapy related toxicity to assess the contribution of all the variants to TRSN. We did not preselect solely for SNPs with reported significant associations as many of the contributing studies were limited in sample size or number of SNPs investigated and were therefore suboptimal in their design.

We found moderately strong evidence that, overall, this set of variants is associated with TRSN. The overall association signal was driven by the 9 most strongly associated SNPs, 6 of which are in genes in the taxane pharmacokinetic pathway and one is in a gene in the pharmacodynamic pathway. The *EPHA6* gene variant rs301927 has been described in Leandro-Garcia and colleagues (11), as being associated with an increased risk of TRSN. In our data, rs301927 also shows an association with increased risk of TRSN ($P = 0.01$). Other *EPHA* genes have been identified in the paclitaxel induced neuropathy GWAS study by Baldwin and colleagues (2), as also being associated with TRSN risk. The Ephrin A genes encode for a family of receptors, which are activated by the binding of ephrin. This pathway activation has been associated with axonal regulation in embryonic development (38) and pathways involved in neural repair (39).

One recently published study by de Graan and colleagues (40) identified *CYP3A4*22* as having an association with an increased risk of developing grade 3 neurotoxicity. This SNP was neither genotyped, nor imputed within our dataset and was not included in our analysis.

When taking into consideration the results of this study with previously published studies, candidates genes in the pharmacokinetic and pharmacodynamic pathways of taxanes do seem to play a role in susceptibility to TRSN. However, it is unclear what proportion of the total interindividual genetic variability in susceptibility to TRSN is accounted for by candidate genes in the pharmacokinetic/pharmacodynamics pathways involved in taxane metabolism. It is possible that other, as yet undiscovered, pathways involved in neuronal or axonal development may also account for a proportion of the interindividual variability in TRSN risk.

Relatively few of the previously reported germline candidate gene studies have focused on pharmacodynamic pathways. However, somatic mutations in tubulins have been investigated as a cause of taxane resistance (41). Leandro-Garcia and colleagues (29) have investigated the role of tubulin gene polymorphisms in TRSN using lymphocytes from peripheral blood samples from patients exposed to taxanes in various tumor types and found that polymorphisms in certain genes led to a reduction in susceptibility to TRSN. The biologic basis for tubulin gene polymorphisms contributing to TRSN susceptibility hinges on the concept that the effect of taxanes is via β-tubulin binding in the microtubules (29) and that functional variants in regulatory genes such as the promoter *TUBB2A* may affect binding, thus affecting risk of TRSN. In our study, *TUBB2A* (rs9501929) was associated with an increased risk of TRSN ($P = 0.005$; OR = 1.80; 95% CI, 1.20–2.72). This allele has previously been reported to be associated with an increased risk of TRSN, but the effect was not statistically significant (personal communication; ref. 29).

In our study, although most of the variants investigated did not show a significant association with susceptibility to TRSN, the SNPs rs3213619 ($P = 0.004$) and rs9501929 ($P = 0.005$) do seem to influence TRSN risk. These associations may not be considered definitive.

Wakefield (42) presents a framework for evaluating the importance of a defined statistical association based on an estimation of the Bayes factor from standard frequentist statistics and the prior odds of the null hypothesis.

If we assume a prior odds for association of 1 in 100 and a 95% probability that the true odds ratio is less than 1.5 then there is a 74% chance that the association between rs9501929 and TRSN is a false positive. However, we identified 9 SNPs with $P < 0.05$. The probability that all

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9 are false positives is 2.8%, thus there is a 97% chance that at least one of these SNPs is truly associated with TRSN.

Although the strategy of investigating pharmacokinetic/pharmacodynamics pathways has been successful in identifying functional SNPs, there are still many unidentified pathways and genes that may contribute to TRSN susceptibility. Baldwin and colleagues, have used a GWAS approach to identify new pathways and genes. GWAS used in other clinical phenotypes have identified regions outside genes and within regulatory areas or desert regions, which the candidate gene approach would not capture. Other recent approaches used to investigate the polygenic architecture of complex pharmacogenetic traits include use of cellular models of chemotherapeutic toxicity (43).

One limitation of the study was the lack of comorbidity data relating to diseases that could contribute to the extent of neuropathy such as diabetes. The prevalence of subacute neuropathy pretreatment in the early breast cancer population is unclear. Another potential limitation was the difference in the schedule of the paclitaxel treatment (Supplementary Table S2A, paclitaxel 175 mg/m^2 given 3 weekly and paclitaxel 175 mg/m^2 given 2 weekly) received by the patients included in the study. However, the rates of TRSN did not significantly differ between patients receiving the different schedules of paclitaxel, either in overall TRSN or in TRSN by grade (Supplementary Table S2B). Information about concomitant medication prescribed outside trial protocol is unavailable.

A limiting factor in many of the previously published studies is sample size. Many studies have had inadequate statistical power to make a meaningful comment on the potential effect of the variants under investigation. The use of meta-analysis may help improve the power to detect relevant SNPs, genes, and pathways. Emphasis in meta-analysis should be placed on including all relevant data. Factors such as the dosing strategy of the taxane included, for example, weekly versus 3 weekly taxanes can be accounted for in multivariate analysis before inclusion in the meta-analysis model. It is likely that when looking for common variants with small effect sizes, the biggest barrier to finding the real variants of interest may be sample size and study power.

Accurately defining the phenotype under consideration is important. Most clinical trials have used NCI CTCAE scoring to assess TRSN. There may be better methods of neurotoxicity assessment that would detect TRSN at an earlier stage (44). However, this has to be balanced with the practical issues surrounding assessment of chemotherapy related toxicity within the confines of busy clinical settings.

In conclusion, our study reports that some of the previously identified candidate genes may contribute to the overall risk of TRSN. However, the results of further GWAS will help to highlight new loci and pathways involved in the mechanism of TRSN that have not previously been considered. In addition, the role of meta-analysis in elucidating new loci will be important. The routine collection of DNA samples and relevant clinical data for pharmacogenetic analysis within clinical trials would be an important step in providing sufficient clinical data and samples to allow more powerful analyses in order to identify new variants of interest.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.E. Abraham, Q. Guo, C. Caldas, P.P.D. Pharoah, H.M. Earl

Development of methodology: J.E. Abraham, Q. Guo, C. Caldas, P.P.D. Pharoah

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.E. Abraham, S. Ingle, R. Burns, L. Jones, S.J. Bowden, C.J. Poole, C. Caldas, H.M. Earl

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.E. Abraham, Q. Guo, L. Dorling, J. Tyrer, L. Hiller, R. Burns, J.A. Dunn, P.P.D. Pharoah

Writing, review, and/or revision of the manuscript: J.E. Abraham, Q. Guo, L. Dorling, J. Tyrer, L. Hiller, L. Jones, S.J. Bowden, J.A. Dunn, C.J. Poole, C. Caldas, P.P.D. Pharoah, H.M. Earl

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.E. Abraham, L. Dorling, S. Ingle, R. Hardy, R. Burns, L. Jones, S.J. Bowden, C. Caldas

Study supervision: J.E. Abraham, A.-L. Vallier, C. Caldas, H.M. Earl

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Jean E. Abraham, Qi Guo, Leila Dorling, et al.

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