Polymorphisms associated with taxane sensory neuropathy

Replication of Genetic Polymorphisms Reported to be Associated with Taxane-Related Sensory Neuropathy in Early Breast Cancer Patients treated with Paclitaxel.

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Translational Relevance:

Taxanes are chemotherapeutic agents used in a wide variety of cancers. The main non-haematological 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 which gives an indication of the likelihood of true associations within the cohort of genetic variants assessed. The ability to predict for TRSN prior to taxane treatment would allow modification of dose or treatment based on each individual’s risk of TRSN.
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Abstract:

Purpose

Associations between taxane-related sensory neuropathy (TRSN) and single nucleotide polymorphisms (SNPs) 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 breast cancer patients.

Experimental Design

We investigated 73 SNPs in 50 genes for their contribution to TRSN risk, using genotype data from 1303 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%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 nine SNPs were significant at p-value ≤0.05.

Conclusion
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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,
genres and SNPs involved in TRSN susceptibility.
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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 two 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 stabilisation, 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 non-haematological 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 (TRS N) are variable depending on factors such as the dose of taxane given, concomitant neurotoxic drugs and pre-existing co-morbidities. The prevalence of National Cancer Institute common toxicity criteria for adverse events (NCI CTCAE) grade 2 neuropathy is 50–80% in stage I-III breast cancer patients (2-4) whereas the prevalence of grade 3 or grade 4 neuropathy is 5–30% (2,5). Longer-term neurotoxicity complications (beyond 2 years post-chemotherapy) 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 disorganised 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).
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There is evidence to show that chemotherapy related toxicity is a heritable trait. Watters et al (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 et al (10) used the functional characterisation of two closely related drugs e.g. carboplatin and cisplatin to demonstrate that the genetic variations associated with chemotherapeutic toxicity may be similar for closely related drugs i.e. these are likely to be drug class effects.

A predictive biomarker of TRSN would allow stratification by TRSN risk and potentially pre-treatment 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 (Figure 1). The most commonly reported candidate genes associated with TRSN include ABCB1 (MDR1) and CYP2C8 (Supplementary Table 1). 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 1) and inadequate adjustment for multiple testing. In addition, the treatment regimen has included two 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 have been sufficiently robust to develop into reliable biomarkers.
Polymorphisms associated with taxane sensory neuropathy 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 (SNPs) using genotype data from a large cohort of stage I-III breast cancer patients. The promising SNPs reported in published GWAS (2,11) have also been assessed.

Method:

Patients

The study population comprised patients participating in the “Pharmacogenetics of Breast Cancer Chemotherapy (PGSNPS)” study that were recruited from four randomized controlled adjuvant/neoadjuvant breast cancer chemotherapy trials; NEAT (12), BR960 (12), tAnGo (13), and Neo-tAnGo (14). Clinical trial flow diagrams for the paclitaxel-containing trials that contributed to this analysis, including eligibility criteria, are given in Figure 2. In this analysis only patients treated with paclitaxel, from the tAnGo and Neo-tango trials, were included. Clinical information regarding treatment response, toxicity, and outcome is available for all patients recruited to PGSNPS. Drug regimens and dosages are given in Supplementary Tables 2a. Supplementary Table 2b shows TRSN grade by trial. The characteristics of the paclitaxel treated PGSNPS patients are given in Supplementary Table 3. 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 prior to publication, in order to reduce the likelihood of false positive results, and are therefore not

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Polymorphisms associated with taxane sensory neuropathy included in this article. 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 1335 samples (1227 bloods and 108 salivas) were collected from 1335 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 centre. The calling algorithm used for the PGSNPS GWAS analysis was CRLMM provides a measure of confidence for each genotype call as well as quality metrics for SNPs, samples, and hybridization batches. Standard quality control applied 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 1303 patients in the analysis. The 1303 samples included 1195 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,
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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 polymorphisms” 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 1) (2,11,16-29). 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 regarding the putative functional variant and the strength of association were missing or inadequate. Any tumour 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 1000 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
Polymorphisms associated with taxane sensory neuropathy of 5 Mb and default parameters) (33,34). The imputation reference set consisted of 2,186 phased haplotypes from the full 1000 Genomes Project data set (March 2012). All genomic locations are given in NCBI Build 37/UCSC hg19 coordinates. We estimated the accuracy of imputation by calculating the estimated r² between the imputed and actual SNP. SNPs with r²<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 odds ratios and 95% CI were estimated. Non-genetic factors associated with TRSN such as trial, performance status, age, menopausal 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 two 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 χ² distribution on 1 degree of freedom). The resulting genomic inflation factor is 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 ≥2 and time was defined as cumulative paclitaxel dose (mg/m²) at the event. For patients not experiencing any event, the total study paclitaxel drug exposure was used. Cumulative dose data was unavailable for 35 patients, so the sample size for this analysis was 1268 patients. The association between SNPs and cumulative dose levels was assessed using Cox regression including first two eigenvalues, BMI and trial as covariates. The 73 SNPs under investigation were also assessed in this analysis (Supplementary Table 4).
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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 to the alternative that one 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 1) (2,11,16-29). TRSN assessments were performed in most studies using a variety of NCI CTCAE versions and the majority of them classified cases (TRSN grade ≥2) and controls (TRSN grade ≤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 Table 1 and Table 2. 11 SNPs were not included due to high correlation with other SNPs in the dataset. Of the remaining 62 SNPs, one SNP could not be found in our imputed dataset and two 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 was imputed from the 1000 Genomes Project (31).

Maximum TRSN analysis:
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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, nine of the 59 uncorrelated SNPs were associated with TRSN at a nominal P<0.05 compared to the 3 SNPs expected under the null hypothesis (Table 1 and Figure 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) (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 5.
Supplementary Figure 1: Presents the percentage of patients with TRSN ≥ grade 2 by genotype, for three 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 4. Nine of the eleven 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 two 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 Figure 2a-c: Shows Kaplan-Meier curves for each SNP, by genotype, the probability of TRSN grade 0-1 (i.e. the probability of not experiencing moderate/severe TRSN) as the cumulative dose increases.

In both Supplementary Figure 1 and 2, data is 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
Polymorphisms associated with taxane sensory neuropathy (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=1303). 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 pre-select 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 sub-optimal 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 nine most strongly associated SNPs, six 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 et al (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 et al, 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 et al (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
Polymorphisms associated with taxane sensory neuropathy appear to play a role in susceptibility to TRSN. However, it is unclear what proportion of the total inter-individual 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 inter-individual 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 et al (29), have investigated the role of tubulin gene polymorphisms in TRSN using lymphocytes from peripheral blood samples from patients exposed to taxanes in various tumour types and found that polymorphisms in certain genes led to a reduction in susceptibility to TRSN. The biological basis for tubulin gene polymorphisms contributing to TRSN susceptibility hinges on the concept that the effect of taxanes is via beta-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) (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 appear 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.
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If we assume a prior odds for association of 1 in 100 and a 95 per cent probability that the true odds ratio is less than 1.5 then there is a 74 per cent chance that the association between rs9501929 and TRSN is a false positive. However, we identified nine SNPs with P<0.05. The probability that all nine are false positives is 2.8 per cent, thus there is a 97 per cent 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 et al\(^2\), 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 co-morbidity data relating to diseases that could contribute to the extent of neuropathy such as diabetes. The prevalence of sub-acute neuropathy pre-treatment in the early breast cancer population is unclear. Another potential limitation was the difference in the schedule of the paclitaxel treatment (Supplementary Table 2a - paclitaxel 175mg/m2 given 3 weekly and paclitaxel 175mg/m2 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 2b). Information regarding 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
Polymorphisms associated with taxane sensory neuropathy 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 e.g. weekly versus three weekly taxanes can be accounted for in multivariate analysis prior to 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.

Acknowledgements

We would like to thank: the women participating in the PGSNPS study, the clinicians who recruited patients into the study; Sue Shadbolt for her help with the development of the study protocol and Philip Ball for his help with the design of the figures used in this article.
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Author Contributions

JEA conceived the study idea. JEA, CC, PDPP and HME designed the study. JEA and LD reviewed the literature. QG, LD, JT, JEA and PDPP planned and performed the analyses. JEA, QG, SI, RH, ALV, LJ, RB, SJB, JAD and LH collected, cleaned and developed a database for the clinical data. JEA drafted the manuscript. All authors reviewed the article. JEA, QG, CC, HME, LD, LH and PDPP edited the manuscript.

Disclosures

The authors declare that they have no relevant disclosures.
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Tables & Figures Legends

1. **Figure 1: Taxane Pathway**

   **Figure 1 Legend**:

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Description</th>
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<tr>
<td>SLCO1B3</td>
<td>Solute carrier organic anion transporter family, member 1B3</td>
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<td>Cytochrome P450, family 3, subfamily A, polypeptide 4</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>Cytochrome P450, family 3, subfamily A, polypeptide 5</td>
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<td>CYP2C8</td>
<td>Cytochrome P450, family 2, subfamily C, polypeptide 8</td>
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<td>ABCG2</td>
<td>ATP-binding cassette, sub-family G (MDR/TAP), member 2</td>
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2. **Figure 2: Randomised Controls Clinical Trials contributing to TRSN analysis**

   Footnote: * Only patients receiving paclitaxel (taxane) in the tAnGo and Neo-tAnGo patients were included in this analysis.

3. **Figure 3: Q-Q plot for replicated polymorphisms associated with taxane neuropathy**

   **Figure 3 Legend**: Only the SNPs reaching statistical significance (p≤0.05) are labelled with rs numbers

4. **Table 1: Statistically Significant SNPs Replicated in the PGSNPS dataset**

   **(phenotype: maximum TRSN)**

   Footnote:
   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
   ORs and 95% CIs represent the per-allele effect of the minor allele for any particular SNP
   §§rs6473187 in KIAA0146 is in complete LD with rs2632496 in KIAA0146, with rs4873774 in UBE2V2 and with rs8178108 in PRKD.
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rs3829306 is in complete LD with rs4149013 and rs4149023, both in SLCO1B1.

5. Table 2: Statistically Significant SNPs Replicated in the PGSNPS dataset (phenotype: TRSN cumulative dose analysis)

Footnote:
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

◊ HRs and 95% CIs represent the per-allele effect of the minor allele for any particular SNP

rs3829306 is in complete LD with rs4149013 and rs4149023, both in SLCO1B1.
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Table 1 - Statistically Significant SNPs Replicated in the PGSNPS dataset (phenotype: maximum TRSN)

<table>
<thead>
<tr>
<th>GENE</th>
<th>VARIANT</th>
<th>SNP RS NUMBER</th>
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<th>TYPE of SNP</th>
<th>ODDS RATIO (OR)</th>
<th>95% CONFIDENCE INTERVALS (95%CI)</th>
<th>P-VALUE</th>
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Table 2 - Statistically Significant SNPs Replicated in the PGSNPS dataset (phenotype: TRSN cumulative dose analysis)

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<th>MAJOR/MINOR ALLELE</th>
<th>TYPE OF SNP</th>
<th>Genotyped (G)</th>
<th>Imputed (I)</th>
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<th>95% CONFIDENCE INTERVALS (95%CI)</th>
<th>P-VALUE</th>
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</table>

Polymorphisms associated with taxane sensory neuropathy
Figure 1-Taxane Pathway
Figure 2 – Paclitaxel-Containing Randomised Controls Clinical Trials Contributing to PGSNPS
**tAnGo n=872**

**Abbreviated Eligibility Criteria**
- (i) Histologically confirmed invasive breast cancer
- (ii) T2, T3 or locally advanced
- (iii) Any nodal status
- (iv) Any hormone receptor status

**SURGERY**

**Stratification**
- (i) Country of randomising hospital: England; Scotland; Wales; N. Ireland & Ireland
- (ii) Nodal status: negative; 1-3; ≥4
- (iii) Age: ≤50 years; >50 years
- (iv) ER status: -ve; weakly +ve; +ve
- (v) Radiotherapy: no; yes
- (vi) HER-2 status: (+++); (0,1,2+); (unmeasured)

**Randomisation**

Arm A n=444
- 4 x Epirubicin (E) 90mg/m² q 3 weekly & Cyclophosphamide (C) 600mg/m² q3 weekly
- Followed by 4 x Paclitaxel (T) 175mg/m² q3 weekly

Arm B n=428
- 4 x Epirubicin (E) 90mg/m² q 3 weekly & Cyclophosphamide (C) 600mg/m² q3 weekly
- Followed by 4 x Paclitaxel (T) 175mg/m² q3 weekly & Gemcitabine (G) 1250mg/m² day1 & 8

**Neo- tAnGo n=431**

**Abbreviated Eligibility Criteria**
- (i) Histologically confirmed invasive breast cancer
- (ii) T2, T3 or locally advanced;
- (iii) Diameter >20mm; T4 any size

**SURGERY**

**Stratification**
- (i) Age: ≤50 years; >50 years
- (ii) ER status: -ve; +ve
- (iii) Tumour size: ≤50mm; >50mm
- (iv) Clinical Axillary Node involvement: yes; no
- (v) Inflammatory or locally advanced: no; yes

**Randomisation**

Arm A1 n=104
- 4 x EC
- As for tango
- Followed by 4 x T 175mg/m² q2 weekly

Arm A2 n=110
- 4 x T
- 175mg/m² q2 weekly
- Followed by 4 x EC

Arm B1 n=108
- 4 x EC
- As for tango
- Followed by 4 x TG 175mg/m² q2 weekly
- T: 175mg/m² q2 weekly
- G: 2000mg/m² q2 weekly

Arm B2 n=109
- 4 x T G
- T: 175mg/m² q2 weekly
- G: 2000mg/m² q2 weekly
- Followed by 4 x EC
- As for tango
Figure 3: Q-Q Plot of replicated Polymorphisms Associated with Taxane-related Sensory Neuropathy
Replication of Genetic Polymorphisms Reported to be Associated with Taxane-Related Sensory Neuropathy in Early Breast Cancer Patients treated with Paclitaxel.

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

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Supplementary Material  Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2014/03/07/1078-0432.CCR-13-3232.DC1

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