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Genetic Heterogeneity Beyond CYP2C8*3 Does Not Explain Differential Sensitivity to Paclitaxel-Induced Neuropathy

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Abstract

Purpose—The development of paclitaxel-induced peripheral neuropathy (PIPN) is influenced by drug exposure and patient genetics. The purpose of this analysis was to expand on a previous reported association of CYP2C8*3 and PIPN risk by investigating additional polymorphisms in CYP2C8 and in hundreds of other genes potentially relevant to paclitaxel pharmacokinetics.

Methods—Clinical data was collected prospectively in an observational registry of newly diagnosed breast cancer patients. Patients treated with paclitaxel-containing regimens were genotyped using the Affymetrix DMET™ Plus chip. Patients who carried the CYP2C8*2, *3 or *4 variant were collapsed into a low-metabolizer CYP2C8 phenotype for association with PIPN. Separately, all SNPs that surpassed quality control were assessed individually and as a composite of genetic ancestry for associations with PIPN.

Results—412 paclitaxel-treated patients and 564 genetic markers were included in the analysis. The risk of PIPN was significantly greater in the CYP2C8 low-metabolizer group (HR=1.722, p=0.018), however, the influence of the *2 and *4 SNPs were not independently significant (*2: p=0.847, *4: p=0.408). One intronic SNP in ABCG1 (rs492338) surpassed the exploratory significance threshold for an association with PIPN in the Caucasian cohort (p=0.0008) but not in the non-Caucasian replication group (p=0.54). Substantial genetic variability was observed within

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Conflicts of Interest:
L. Scott Clark is an employee of Gentris Corp. Howard L. McLeod and Alison A. Motsinger-Reif were consultants for Gentris Corp.

self-reported racial groups but this genetic variability was not associated with risk of grade 2+ PIPN.

Conclusions—The pharmacogenetic heterogeneity within a cohort of breast cancer patients is dramatic, though we did not find evidence that this heterogeneity directly influences the risk of PIPN beyond the contribution of CYP2C8*3.

Keywords

paclitaxel; pharmacogenetics; breast cancer; chemotherapy-induced peripheral neuropathy; ABCG1; Affymetrix DMET™ Plus; CYP2C8; race

Introduction

Paclitaxel is a chemotherapeutic agent frequently used in the treatment of a variety of solid tumors including breast, lung, and ovarian cancer. In breast cancer, the sequential addition of paclitaxel to standard anthracycline therapy has improved rates of pathological complete response in neoadjuvant treatment[1] and overall survival in the adjuvant setting[2, 3]. Along with its impressive efficacy, paclitaxel treatment is associated with a variety of severe adverse events, including the development of sensory peripheral neuropathy that can progress to irreversible loss of manual dexterity and balance[4].

Paclitaxel-induced peripheral neuropathy (PIPN) seems to be multifactorial but drug exposure is a primary driver of toxicity development[5–8]. Exposure to paclitaxel is likely determined by the activity of the enzymes and transporters involved in its metabolism and elimination. Several groups have investigated whether single nucleotide polymorphisms (SNPs) within these genes influence drug exposure or PIPN, with inconclusive findings that we recently reviewed[9]. Looking across these studies one of the more plausible candidates as a risk factor for PIPN is the CYP2C8*3 single nucleotide polymorphism (SNP). CYP2C8 is responsible for the majority of paclitaxel elimination[10] and the *in vivo* activity of this enzyme correlates with paclitaxel exposure[11]. The CYP2C8*3 SNP (rs10509681, K399R) has been reported to decrease paclitaxel metabolism *in vitro*[12], decrease drug elimination in patients(13) and increase paclitaxel efficacy and toxicity[14, 15].

Another factor that has been reported to modify PIPN risk in multiple studies is patient race; African-Americans are consistently at greater neuropathy risk than Caucasians[16–18]. It is unknown whether this is due to differences in paclitaxel pharmacokinetics or some underlying sensitivity to neuropathy. We recently reported corroboration of these associations in a cohort of breast cancer patients treated at the UNC Lineberger Comprehensive Cancer Center in which CYP2C8*3 and African-American race independently increased PIPN risk[16].

In this analysis we sought to expand on our previous work by interrogating nearly 2,000 SNPs within genes responsible for Drug Metabolism, Elimination, and Transport[19]. We hypothesized that a broad pharmacogenetic assessment will identify additional common genetic variants that contribute to PIPN risk by first looking within CYP2C8 and then taking a discovery approach to individually assess thousands of SNPs that may influence paclitaxel

pharmacokinetics. Lastly, we combined all of our genetic data to search for evidence that increased PIPN risk in African-Americans is due to germline genetics.

Materials and Methods

Patients and treatments

This cohort, collected from the University of North Carolina Lineberger Comprehensive Cancer Center (UNC LCCC) Breast Cancer Database, has been previously described in detail(16). This observational registry prospectively collects demographic data including treatment and disease characteristics; race was by patient self-report. Toxicity data is collected and described prospectively by the treating physician and retrospectively coded according to NCI CTC AE V4.0[20] biannually. The primary toxicity endpoint for this analysis was grade 2 or higher (grade 2+) neuropathy during paclitaxel treatment. Any individual who was enrolled in an IRB approved clinical trial that collected DNA at enrollment, was treated with paclitaxel in the neoadjuvant and/or adjuvant setting and completed informed consent for genetic studies was eligible for inclusion in this analysis. Patients primarily received contemporary neoadjuvant or adjuvant treatment regimens which define paclitaxel treatment dose, schedule, and duration. Use of HER2 targeted treatment (trastuzumab and/or lapatinib) or agents for neuropathy prevention (glutamine, vitamin B complex, or vitamin B6) or treatment (gabapentin or amitriptyline) were at the discretion of the treating physician and recorded prospectively in the database. The study protocol was approved by the UNC Institutional Review Board.

Genotyping

A 30 mL blood sample was collected from each subject at the time of study enrollment. DNA for genotyping was extracted by the UNC Biospecimen Processing Facility and plated at 60 ng/uL. Genotyping was carried out blinded to clinical data using the Affymetrix DMET™ Plus Chip (Affymetrix Inc., Santa Clara, CA, USA) at Gentris Corp. (Gentris Corp. Morrisville, NC) following the manufacturer's protocol with known genomic DNA controls provided by Affymetrix Inc. to monitor inter- and intra-assay performance. Any patient sample or assay with successful call rate <95% or <90%, respectively, was excluded from analysis. Variants were also excluded if the minor allele frequency was <5% in the entire population or if the p-value for the Fisher's Exact estimate of Hardy-Weinberg proportions was <0.05 in either the Caucasian or non-Caucasian cohort (using stratified analysis). Genotyping of 359 genetic reference samples from the International Hapmap Project[21] (59 Caucasian (CEU), 118 African (YRI), 91 Japanese (JPT), and 90 Chinese (CHB)) was also performed on the Affymetrix DMET™ Plus Chip as described previously[22].

Statistical Analysis

Combined Analysis of CYP2C8 Low-Activity Variants—In addition to the most common CYP2C8 variant (*3) two other somewhat common low-activity variants have been reported. The *2 (rs11572103, I269F) and *4 (rs1058930, I264M) SNPs are non-synonymous variants with diminished metabolic activity[12, 23] found in African (Allele Frequency=0.20) and Caucasian (Allele Frequency=0.07) reference populations, respectively. Any patient who carried any of these three low-activity variants was combined

into a low-metabolizer phenotype. This phenotype was assessed for an association with the cumulative dose at the first occurrence of grade 2+ neuropathy using the log-rank test. Any patient not experiencing grade 2+ neuropathy was censored at the cumulative dose they were administered over the course of therapy. Because the CYP2C8*3 variant had previously been analyzed and reported we then used a likelihood ratio test to determine which of the three SNPs were significantly contributing to PIPN risk.

DMET Discovery—Of the 1,936 genetic markers on the DMET™ Plus chip, a total of 1,372 were excluded from analysis. 1,275 markers were excluded for minor allele frequency <0.05, which is consistent with previously reported DMET™ marker allele frequencies in primarily Caucasian cohorts(24). 30 markers were excluded from the analysis for call rate <90% and 67 were eliminated for significant deviation from Hardy-Weinberg proportions. Thus after appropriate quality control 564 markers (29.1%) were included in the analysis.

Each individual SNP from the DMET™ Plus Chip that passed quality control was tested for an association with the occurrence of grade 2+ neuropathy in the Caucasian patients. This analysis was confined to Caucasians because of the heterogeneity in allele frequency across racial populations and the known influence of race on neuropathy risk. Neuropathy occurrence, in lieu of dose-at-neuropathy, was used in this analysis due to the unacceptable lack of robustness of the latter method when applied to the less common (minor allele frequency 5–10%) SNPs. Because of the previously reported influence of CYP2C8*3 on PIPN in this dataset, the Fisher's exact test was conditioned on the CYP2C8*3 variant. An exploratory significance threshold of α=0.001 was selected for this first-stage discovery analysis, which is consistent with prior pharmacogenetic studies utilizing the DMET™ Plus Chip[24]. Variants that surpassed the exploratory analysis were tested individually in a log-rank analysis similar to that performed for the CYP2C8 low-metabolizer analysis. Any variant with significant findings in the dose-to-event analysis was then tested in a dose-to-grade 2+ analysis using the log-rank test in the non-Caucasian subjects (n=124).

Genetic Estimate of Race—All SNPs that passed quality control were included in the analysis of population sub-structure and admixture. In order to assess population structure from the genetic data, we used two standard approaches, using the reference samples from the Hapmap populations (CEU, YRI and JPT/CHB) to help anchor the results. First, we calculated principal components using Smartpca in EIGENSOFT[25]. Next, the genetic data were processed using STRUCTURE[26] in order to estimate individual ancestry corresponding to three major ancestral population clusters—i.e., Caucasian, African and Asian. In this sense, STRUCTURE essentially provides an estimation of the proportion of the genotypes regions per individual that are derived from each of the major ancestral populations. This analysis can reveal admixture that self-reported race does not fully capture. The results were labeled according to the ancestral background based on the results of the STURCTURE analysis of the reference samples (results not shown). These estimates (q scores for each of the three clusters) were then used as an independent variable in a log-rank test to explore the association between genetically defined race and PIPN risk. All inferential statistical analyses were carried out in R Statistical Software, version 2.13.0 (R Development Core Team, Vienna, Austria).

Results

Patient and Genetic Data

After exclusion of one patient whose sample failed genotyping, 288 Caucasian and 124 non-Caucasian paclitaxel-treated patients were evaluable in these analyses. Demographic data including patient and treatment characteristics for these cohorts can be found in Table 1.

Overall 71 patients experienced grade 2+ neuropathy during paclitaxel treatment (71/412=18%), which is consistent with other studies of paclitaxel treatment in breast cancer[3].

Combined Analysis of CYP2C8 Low-Activity Variants

Any patient who carried a risk allele was collapsed into a CYP2C8 low-metabolizer phenotype and compared with all other patients. As hypothesized, the low-metabolizer group (n=142) had significantly greater risk of PIPN (HR=1.722, 95% CI 1.10–2.70, p=0.018 (Figure 1). Next, a likelihood ratio test was used to determine whether this association was driven entirely by the previously published *3 variant. Removing the *3 variant significantly diminished the performance of the test (p=0.030) while removing either of the other SNPs did not (*2: p=0.847, *4: p=0.408) demonstrating that neither of these SNPs individually contributed significant explanatory information to the overall model. We then performed this analysis in racially stratified subsets but again did not find a significant contribution for either SNP (*2: p=0.914 in self-reported blacks, *4: p=0.297 in self-reported whites). This lack of significance could be due to inadequate power as both the *2 (HR=1.12) and *4 (HR=1.59) variants' risk models were trending in the expected direction (Supplementary Figures 1a and 1b).

DMET Discovery—Results of the exact tests conditioned on CYP2C8*3 for the 10 markers with the strongest association with neuropathy incidence are displayed in Table 2, including one intronic SNP in ABCG1 (rs492338, uncorrected p=0.0008) that surpassed the exploratory significance threshold (α=0.001). A contingency table of neuropathy by genotype for the 285 Caucasian patients with genotype calls at this SNP is presented in Table 3, exhibiting increased neuropathy risk for the minor (T) allele. The results of the secondary analysis using the cumulative dose-at-onset of neuropathy were not meaningfully different from the primary findings (HR (per allele)=2.11, 95% CI: 1.36–3.29, p=0.0008, Figure 2a). In the attempted cross-race replication in non-Caucasian patients, rs492338 was not significantly associated with grade 2+ neuropathy in either the Fisher's exact (p=0.60) or log-rank analysis (p=0.54, Figure 2b).

Genetic Estimate of Population Substructure

Using the results from our STRUCTURE analysis, each patient was categorized by their percentage of Caucasian, African, and Asian ancestry. The percentage of African ancestry was not associated with the risk of grade 2+ PIPN (p=0.744). We then used these estimates to describe the genetic heterogeneity present in this cohort of breast cancer patients. Interestingly, the estimate of Caucasian ancestry in self-reported Caucasians ranged from very high (maximum=99.7%) to surprisingly low (minimum=16.7%). Similar ranges were

found in self-reported African-Americans for regions of the genome directly inherited from Caucasian founders (99%-0.1%) and African founders (99.6%-0.5%). Stacked bar plots of STRUCTURE output which displays the percentage of Caucasian, African, and Asian ancestry, visualized in ascending order of Caucasian genetics, for the cohort stratified by self-reported race can be found in Figure 3 and an unstratified plot is included in Supplementary Figure 2.

An alternative way to visualize the genetic heterogeneity of a sample of breast cancer patients is through principal components analysis. Figure 4 displays the first two principal components for our 412 patients and 359 Hapmap reference samples (YRI=118, JPT=91, CHB=90, CEU=59). Unlike the reference samples, which tightly cluster by self-reported race, our samples again represent a heterogeneous continuum that stretches between the reference samples, as expected for a heterogeneous, admixed population.

Discussion

Paclitaxel-induced peripheral neuropathy is known to be dependent on drug exposure. Within this patient population we previously demonstrated that patients' who carry the low-activity CYP2C8*3 variant are at increased risk of neurotoxicity. We have attempted in the present study to identify additional germline variants that influence risk of neuropathy through a direct effect on drug pharmacokinetics, beyond that of CYP2C8*3. In order to do so we used the Affymetrix DMET™ Plus chip to simultaneously interrogate 1,936 genetic variants within 225 genes that encode for the proteins responsible for Drug Metabolism, Elimination, and Transport (DMET)[19]. This chip has been previously used to identify genetic variants that influence treatment outcomes from various drugs used in cancer and other diseases[27–31].

The strategy of combining uncommon SNPs with similar functional effects has been successfully used in pharmacogenetics; for example, an association for a common SNP in *SLCO1B1* with methotrexate pharmacokinetics was extended to other rare variants within the gene[32]. We attempted to apply this technique to an analysis of additional low-activity non-synonymous CYP2C8 SNPs. Our results for the composite phenotype were consistent with the hypothesis; however, due to the low number of each variant genotype we could not demonstrate that the addition of either the *2 or *4 SNPs significantly improved our findings. This is an inherent limitation of analyses that include rare or uncommon SNPs that can only be overcome through the use of larger patient cohorts.

Despite the use of a genotyping platform that interrogates thousands of SNPs in genes relevant to drug pharmacokinetics, the only hit that surpassed our exploratory significance threshold is located in a gene (ABCG1) not previously identified as a predictor of paclitaxel PK[33]. ABCG1 is an intracellular sterol transporter that is primarily recognized for its role in regulation of intracellular cholesterol levels, particularly in cholesterol-laden macrophages[34]. ABCG1 is also expressed in peripheral neurons[35] where cholesterol is converted to pregnenolone, a conversion that is inhibited by paclitaxel *in vitro*[36]. Pregenenolone is then converted to progesterone, 5α-dihydroprogesterone, and allopregnanolone, which are referred to as neuroactive steroids[37]. Neuroactive steroids are

key regulators of Schwann cell proliferation and myelin formation[38]; processes initiated in response to axonal demyelination, a prominent finding in paclitaxel-induced neurotoxicity both *in vitro*[39] and *in vivo*[40]. Interestingly, co-treatment with neuroactive steroids enhances recovery from docetaxel-induced neuropathy in rats[41].

Pharmacogenetic research has been criticized for the proportion of initial findings that cannot be replicated. Due to the lack of replication of rs492338 in the non-Caucasian patients, in whom this SNP is sufficiently polymorphic, this initial finding should be considered a likely false positive unless successful replication is reported in an independent patient cohort. Likewise, several groups have reported significant SNP associations with paclitaxel exposure or PIPN that we could not reproduce in this cohort, including SNPs in CYP3A4[14] and ABCB1[42]. Alternatively, replication could not be attempted of some intriguing findings including a model that predicts paclitaxel clearance using the DMET™ Plus Chip[33]. We did not attempt validation of this model because we do not have paclitaxel clearance data. What is notable from that model is that the 14 SNPs identified are found in genes which, like ABCG1, were not previously thought to be directly involved in paclitaxel pharmacokinetics. Other SNPs that are not interrogated by the DMET™ Chip, and for which replication of previous associations with PIPN could not be attempted, include recently identified SNPs in CYP2C8[14] and CYP3A4[43] and virtually everything reported in gene or genome-wide studies[44–46].

We also attempted to use a novel approach to elucidate whether the differential PIPN risk across race could be explained by variation in candidate genes. We did not find evidence that the genetic information included on the DMET™ chip, which focuses mainly on enzymes and transporters, explains this risk disparity. This suggests that paclitaxel pharmacokinetics is unlikely to be the underlying cause of increased neuropathy in African-American patients, who may be more sensitive to neuropathy due to comorbidities such as diabetes[47] or other unidentified genetic factors. In support of this hypothesis, African-Americans are known to be at higher neuropathy risk from other etiologies that are unrelated to paclitaxel including HIV and diabetes-associated neuropathy[48, 49].

This analysis revealed an underappreciated aspect of medical care, the substantial genetic heterogeneity found within patients even within a confined geographic region. A subset of patients who self-identify with a given race are genetically quite distinct from that population. We have advocated an approach of using global reference populations to bring pharmacogenetically-guided rational therapy to countries that lack infrastructure for individualized pharmacogenetics[50]. The genetic heterogeneity of breast cancer patients within this cohort, as compared to the reference populations, demonstrates the inferiority of a race-based stratification system compared with true pharmacogenetic screening. As an illustrative example, the CYP2C8*3 allele has not been found in the African or Asian reference populations but was found within the relatively small number of self-reported African patients within our database (minor allele frequency=0.06).

In conclusion, we have attempted to use the DMET™ Plus Chip to discover SNPs, in addition to CYP2C8*3, that modulate risk of PIPN through an effect on drug exposure. The independent influence of other less common low-activity variants in CYP2C8 could not be

verified, though their direction of effect was consistent with that hypothesized. We identified an additional variant in a gene (ABCG1) that is relevant to the regulation of endogenous neuroactive steroids which has not been previously investigated in candidate SNP association studies of PIPN to our knowledge. The finding could not be replicated in the smaller non-Caucasian cohort, suggesting that the effect may be exclusive to Caucasian subjects or may represent a false positive. Finally, though we could not find evidence that the enhanced risk of PIPN in African-American patients is caused by genetic variation in the genes assessed by the DMET™ Plus Chip, we did find dramatic evidence of genetic heterogeneity in breast cancer patients. This illustrates the necessity of developing infrastructure for clinical genetic testing to guide pharmacogenetic treatment individualization for those associations that have demonstrated clinical utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Diéras V, Fumoleau P, Romieu G, Tubiana-Hulin M, Namer M, Mauriac L, et al. Randomized parallel study of doxorubicin plus paclitaxel and doxorubicin plus cyclophosphamide as neoadjuvant treatment of patients with breast cancer. J Clin Oncol. 2004 Dec 15; 22(24):4958–65. [PubMed: 15611510]
2. Martin M, Rodriguez-Lescure Ã, Ruiz A, Alba E, Calvo L, Ruiz-Borrego M, et al. Randomized phase 3 trial of fluorouracil, epirubicin, and cyclophosphamide alone or followed by paclitaxel for early breast cancer. J Natl Cancer Inst. 2008 Jun 04; 100(11):805–14. [PubMed: 18505968]
3. Henderson IC, Berry DA, Demetri GD, Cirrincione CT, Goldstein LJ, Martino S, et al. Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol. 2003 Mar 15; 21(6):976–83. [PubMed: 12637460]
4. Freilich RJ, Balmaceda C, Seidman AD, Rubin M, DeAngelis LM. Motor neuropathy due to docetaxel and paclitaxel. Neurology. 1996 Jul 01; 47(1):115–8. [PubMed: 8710063]
5. Mielke S, Mross K, Gerds TA, Schmidt A, Wasch R, Berger DP, et al. Comparative neurotoxicity of weekly non-break paclitaxel infusions over 1 versus 3 h. Anticancer Drugs. 2003; 14(10):785–92. [PubMed: 14597872]
6. van Gerven JMA, Moll JWB, van den Bent MJ, Bontenbal M, van der Burg MEL, Verweij J, et al. Paclitaxel (taxol) induces cumulative mild neurotoxicity. Eur J Cancer. 1994; 30(8):1074–7. [PubMed: 7654432]
7. Mielke S, Sparreboom A, Steinberg SM, Gelderblom H, Unger C, Behringer D, et al. Association of paclitaxel pharmacokinetics with the development of peripheral neuropathy in patients with advanced cancer. Clin Cancer Res. 2005 Jul 1; 11(13):4843–50. [PubMed: 16000582]
8. de Graan AJ, Elens L, Sprowl JA, Sparreboom A, Friberg LE, van der Holt B, et al. CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. Clin Cancer Res. 2013 May 2.

9. Hertz DL. Germline pharmacogenetics of paclitaxel for cancer treatment. Pharmacogenomics. 2013 Jul 01; 14(9):1065–84. 2013/11. [PubMed: 23837481]
10. Rahman A, Korzekwa KR, Grogan J, Gonzalez FJ, Harris JW. Selective biotransformation of taxol to 6{alpha}-hydroxytaxol by human cytochrome P450 2C8. Cancer Res. 1994 Nov 01; 54(21): 5543–6. [PubMed: 7923194]
11. Hertz DL, Walko CM, Bridges AS, Hull JH, Herendeen J, Rollins K, et al. Pilot study of rosiglitazone as an in vivo probe of paclitaxel exposure. Br J Clin Pharmacol. 2012; 74(1):197–200. [PubMed: 22680343]
12. Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, et al. Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. Pharmacogenetics. 2001 Oct; 11(7):597–607. [PubMed: 11668219]
13. Bergmann TK, Brasch-Andersen C, Green H, Mirza M, Pedersen RS, Nielsen F, et al. Impact of CYP2C8*3 on paclitaxel clearance: A population pharmacokinetic and pharmacogenomic study in 93 patients with ovarian cancer. Pharmacogenomics J. 2011; 11(2):113–20. [PubMed: 20368717]
14. Leskela S, Jara C, Leandro-Garcia L, Martinez A, Garcia-Donas J, Hernando S, et al. Polymorphisms in cytochromes P450 2C8 and 3A5 are associated with paclitaxel neurotoxicity. Pharmacogenomics J. 2011; 11(2):121–9. [PubMed: 20212519]
15. Hertz DL, Motsinger-Reif AA, Drobish A, Winham SJ, McLeod HL, Carey LA, et al. CYP2C8*3 predicts benefit/risk profile in breast cancer patients receiving neoadjuvant paclitaxel. Breast Cancer Res Treat. 2012 Jul; 134(1):401–10. [PubMed: 22527101]
16. Hertz DL, Roy S, Motsinger-Reif AA, Drobish A, Clark LS, McLeod HL, et al. CYP2C8*3 increases risk of neuropathy in breast cancer patients treated with paclitaxel. Ann Oncol. 2013 Jun; 24(6):1472–8. [PubMed: 23413280]
17. Schneider BP, Zhao F, Wang M, Stearns V, Martino S, Jones V, et al. Neuropathy is not associated with clinical outcomes in patients receiving adjuvant taxane-containing therapy for operable breast cancer. J Clin Oncol. 2012 Sep 1; 30(25):3051–7. [PubMed: 22851566]
18. Speck RM, Sammel MD, Farrar JT, Hennessy S, Mao JJ, Stineman MG, et al. Impact of chemotherapy-induced peripheral neuropathy on treatment delivery in nonmetastatic breast cancer. J Oncol Pract. 2013 May 14.
19. Sissung TM, English BC, Venzon D, Figg WD, Deeken JF. Clinical pharmacology and pharmacogenetics in a genomics era: The DMET platform. Pharmacogenomics. 2010 Jan 01; 11(1):89–103. [PubMed: 20017675]
20. Common terminology criteria for adverse events (CTCAE) version 4. 0. Vol. 14. U.S Department of Health and Human Services, National Institutes of Health, National Cancer Insitute; 2010 Jun. p. 2010
21. The international HapMap project. Nature. 2003 Dec 18; 426(6968):789–96. [PubMed: 14685227]
22. He YJ, Shapero MH, McLeod HL. Novel tagging SNP rs1495741 and 2-SNPs (rs1041983 and rs1801280) yield a high prediction of the NAT2 genotype in HapMap samples. Pharmacogenet Genomics. 2012 Apr; 22(4):322–4. [PubMed: 22414877]
23. Singh R, Ting JG, Pan Y, Teh LK, Ismail R, Ong CE. Functional role of Ile264 in CYP2C8: Mutations affect haem incorporation and catalytic activity. Drug Metab Pharmacokinet. 2008; 23(3):165–74. [PubMed: 18574320]
24. Deeken JF, Cormier T, Price DK, Sissung TM, Steinberg SM, Tran K, et al. A pharmacogenetic study of docetaxel and thalidomide in patients with castration-resistant prostate cancer using the DMET genotyping platform. Pharmacogenomics J. 2010; 10(3):191–9. [PubMed: 20038957]
25. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006 Aug; 38(8):904–9. [PubMed: 16862161]
26. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000 Jun 01; 155(2):945–59. [PubMed: 10835412]
27. Iacobucci I, Lonetti A, Candoni A, Sazzini M, Papayannidis C, Formica S, et al. Profiling of drug-metabolizing enzymes/transporters in CD33+ acute myeloid leukemia patients treated with gemtuzumab-ozogamicin and fludarabine, cytarabine and idarubicin. Pharmacogenomics J. 2012 May 15. Epub ahead of print. 10.1038/tpj.2012.13

28. Uchiyama T, Kanno H, Ishitani K, Fujii H, Ohta H, Matsui H, et al. An SNP in CYP39A1 is associated with severe neutropenia induced by docetaxel. Cancer Chemother Pharmacol. 2012 Jun; 69(6):1617–24. [PubMed: 22562553]
29. Di Martino MT, Arbitrio M, Leone E, Guzzi PH, Saveria Rotundo M, Ciliberto D, et al. Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: A DMET microarray profiling study. Cancer Biol Ther. 2011 Nov 01; 12(9):780–7. [PubMed: 21892003]
30. Caldwell MD, Awad T, Johnson JA, Gage BF, Falkowski M, Gardina P, et al. CYP4F2 genetic variant alters required warfarin dose. Blood. 2008 Apr 15; 111(8):4106–12. [PubMed: 18250228]
31. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome P450 genetic polymorphisms and the response to prasugrel: Relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. Circulation. 2009 May 19; 119(19):2553–60. [PubMed: 19414633]
32. Ramsey LB, Bruun GH, Yang W, Trevino LR, Vattathil S, Scheet P, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. Genome Res. 2012 Jan; 22(1):1–8. [PubMed: 22147369]
33. de Graan AJ, Elens L, Smid M, Martens JW, Sparreboom A, Nieuweboer AJ, et al. A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform. Clin Cancer Res. 2013 Sep 15; 19(18):5210–7. [PubMed: 23918604]
34. Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, et al. ABCG1 (ABC8), the human homolog of the drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci U S A. 2000 Jan 18; 97(2):817–22. [PubMed: 10639163]
35. Tarr PT, Edwards PA. ABCG1 and ABCG4 are coexpressed in neurons and astrocytes of the CNS and regulate cholesterol homeostasis through SREBP-2. J Lipid Res. 2008 Jan 01; 49(1):169–82. [PubMed: 17916878]
36. Rainey WE, Kramer RE, Mason JI, Shay JW. The effects of taxol, a microtubule-stabilizing drug, on steroidogenic cells. J Cell Physiol. 1985; 123(1):17–24. [PubMed: 2857725]
37. Schaeffer V, Meyer L, Patte-Mensah C, Mensah-Nyagan A. Progress in dorsal root ganglion neurosteroidogenic activity: Basic evidence and pathophysiological correlation. Prog Neurobiol. 2010 Sep; 92(1):33–41. [PubMed: 20438798]
38. Koenig HL, Schumacher M, Ferzaz B, Thi AN, Ressouches A, Guennoun R, et al. Progesterone synthesis and myelin formation by schwann cells. Science. 1995 Jun 9; 268(5216):1500–3. [PubMed: 7770777]
39. Yang IH, Siddique R, Hosmane S, Thakor N, Hoke A. Compartmentalized microfluidic culture platform to study mechanism of paclitaxel-induced axonal degeneration. Exp Neurol. 2009 Jul; 218(1):124–8. [PubMed: 19409381]
40. Sahenk Z, Barohn R, New P, Mendell JR. Taxol neuropathy: Electrodiagnostic and sural nerve biopsy findings. Arch Neurol. 1994 Jul 1; 51(7):726–9. [PubMed: 7912506]
41. Roglio I, Bianchi R, Camozzi F, Carozzi V, Cervellini I, Crippa D, et al. Docetaxel-induced peripheral neuropathy: Protective effects of dihydroprogesterone and progesterone in an experimental model. J Peripher Nerv Syst. 2009 Mar; 14(1):36–44. [PubMed: 19335538]
42. Sissung TM, Mross K, Steinberg SM, Behringer D, Figg WD, Sparreboom A, et al. Association of ABCB1 genotypes with paclitaxel-mediated peripheral neuropathy and neutropenia. Eur J Cancer. 2006; 42(17):2893–6. [PubMed: 16950614]
43. de Graan AM, Elens L, Sprowl JA, Sparreboom A, Friberg LE, van der Holt B, et al. CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. Clinical Cancer Research. 2013 Jun 15; 19(12):3316–24. [PubMed: 23640974]
44. Baldwin RM, Owzar K, Zembutsu H, Chhibber A, Kubo M, Jiang C, et al. A genome-wide association study identifies novel loci for paclitaxel-induced sensory peripheral neuropathy in CALGB 40101. Clin Cancer Res. 2012 Sep 15; 18(18):5099–109. [PubMed: 22843789]
45. Sucheston L, Zhao H, Yao S, Zirpoli G, Liu S, Barlow W, et al. Genetic predictors of taxane-induced neurotoxicity in a SWOG phase III intergroup adjuvant breast cancer treatment trial (S0221). Breast Cancer Res Treat. 2011; 130(3):993–1002. [PubMed: 21766209]

46. Leandro-Garcia LJ, Leskela S, Jara Sanchez C, Green H, Avall Lundqvist E, Wheeler HE, et al. Regulatory polymorphisms in beta-tubulin IIa are associated with paclitaxel-induced peripheral neuropathy. Clin Cancer Res. 2012 Aug 15; 18(16):4441–8. [PubMed: 22718863]

47. Rowinsky EK, Eisenhauer EA, Chaudhry V, Arbuck SG, Donehower RC. Clinical toxicities encountered with paclitaxel (taxol). Semin Oncol. 1993 Aug; 20(4 Suppl 3):1–15. [PubMed: 8102012]

48. Anziska Y, Helzner EP, Crystal H, Glesby MJ, Plankey M, Weber K, et al. The relationship between race and HIV-distal sensory polyneuropathy in a large cohort of US women. J Neurol Sci. 2012 Apr 15; 315(1–2):129–32. [PubMed: 22123155]

49. Soderstrom LH, Johnson SP, Diaz VA, Mainous AG. Association between vitamin D and diabetic neuropathy in a nationally representative sample: Results from 2001–2004 NHANES. Diabetic Med. 2012; 29(1):50–5. [PubMed: 21726279]

50. Roederer MW, Sanchez-Giron F, Kalideen K, Kudzi W, McLeod HL, Zhang W, et al. Pharmacogenetics and rational drug use around the world. Pharmacogenomics. 2011 Jun; 12(6): 897–905. [PubMed: 21692619]

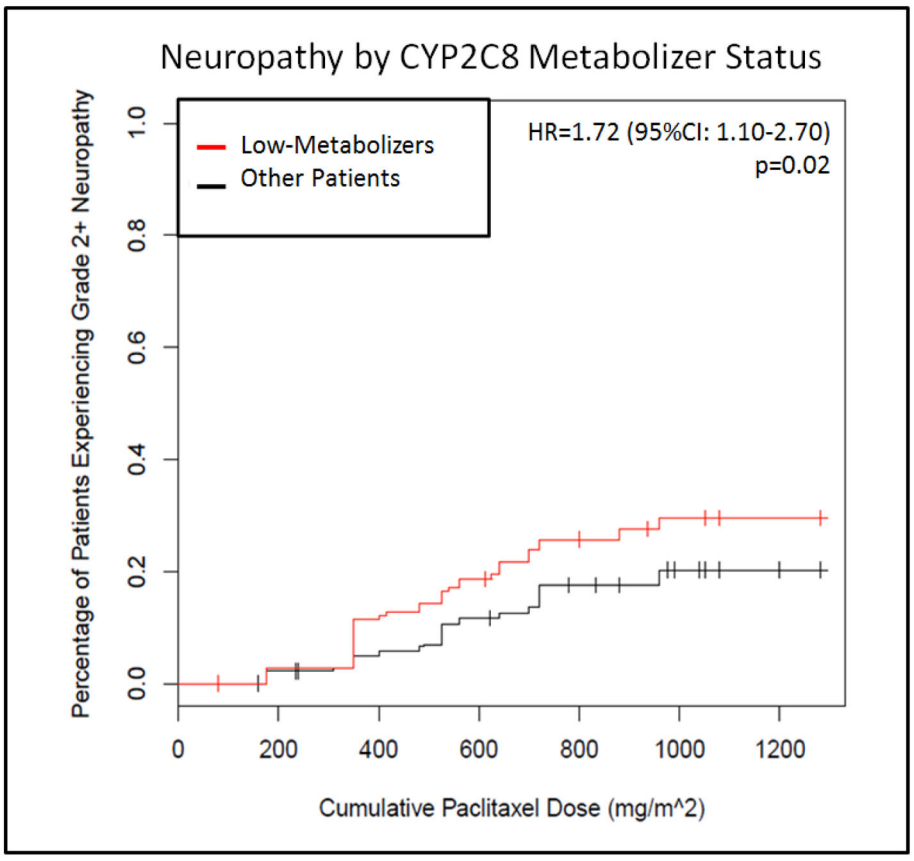

Fig 1.

Incidence of grade 2+ neuropathy by CYP2C8 metabolizer status. All patients carrying a CYP2C8*2, *3 or *4 allele were grouped into a low-metabolizer phenotype. As hypothesized, CYP2C8 low-metabolizers had a greater risk of neuropathy (p=0.02).

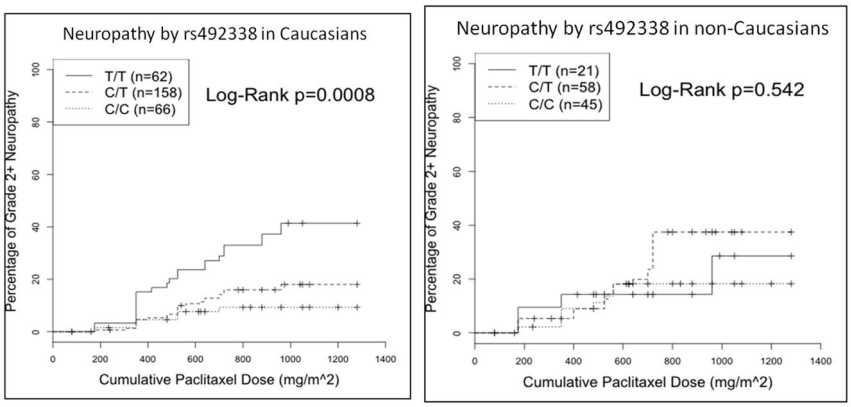

Fig 2.

Incidence of grade 2+ neuropathy by ABCG1 (rs492338) genotype in Caucasian (n=285) (Fig 2A, left) and non-Caucasian (n=124) (Fig 2B, right) patients. The increase in neuropathy risk for patients carrying rs492338 discovered in Caucasians (p=0.0008) could not be replicated in non-Caucasians (p=0.542), suggesting that if the original association is true it may be race-specific.

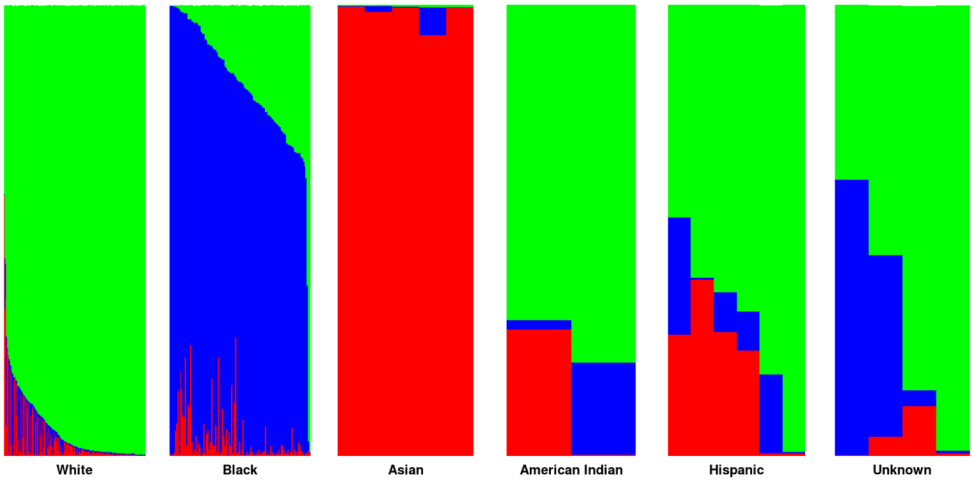

Fig 3.

Stacked bar plots displaying the percentage of Caucasian (green), African (blue), and Asian (red) ancestry, visualized in ascending order of Caucasian genetics stratified by self-reported race. Race was verified by other documentation within the medical record for all patients whose genetic ancestry was <80% concordant with their self-reported race. The self-reported white patients are genetically predominantly Caucasian; however, some patients have substantial African and/or Asian genetic ancestry. Similarly, the self-reported black patients exist on a continuum between almost entirely genetically African and almost entirely genetically Caucasian. Interestingly, Asians are the most genetically homogeneous of the racial groups.

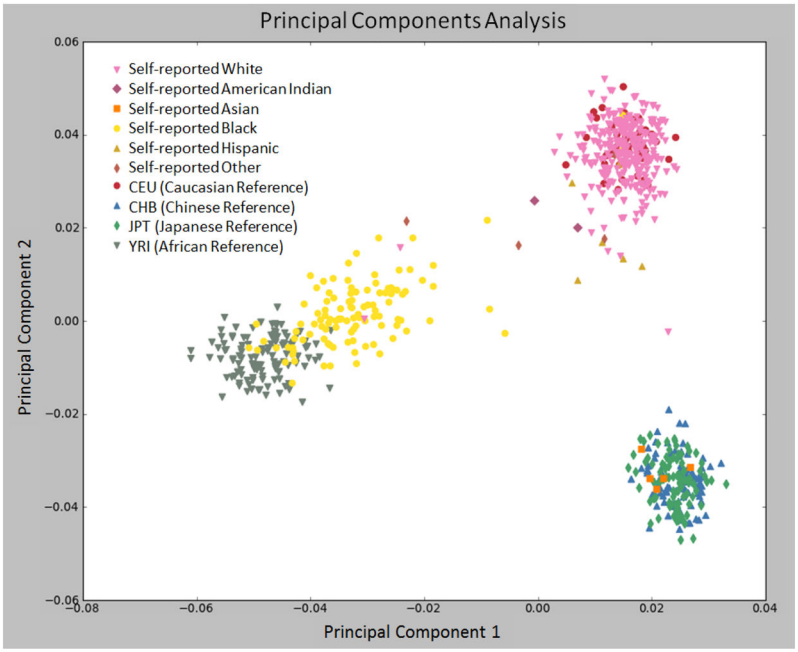

Fig 4.

Plot of first two principal components for 412 breast cancer patients and 359 reference samples (YRI=118, JPT=91, CHB=90, CEU=59). The reference samples tightly cluster by race due to genetic homogeneity. The patient samples tend to cluster near the corresponding reference samples but demonstrate substantially greater genetic heterogeneity, particularly for the self-reported black patients who represent a continuum between the African and Caucasian reference populations.

Table 1

Characteristics of Caucasian (n=288) and non-Caucasian (n=124) 9830 Cohort

		Primary	Replication
Self-reported Race	Caucasian	288	0
	African-American	0	107 (86%)
	Other	0	17 (14%)
Age (Years)	Median	52	45
	Range	24–84	22–68
Grade 2+ Neuropathy	Yes	49 (17%)	28 (23%)
	No	239 (83%)	97 (78%)
Diabetes Diagnosis	Yes	24 (8%)	24 (19%)
	No	264 (92%)	100 (81%)
Paclitaxel Schedule & Dose	80–90 mg/m^2 Weekly	91 (32%)	40 (32%)
	175 mg/m^2 Every 2 weeks	163 (57%)	73 (59%)
	175 mg/m^2 Every 3 weeks	34 (12%)	11 (9%)
Supplemental Neuropathy Therapy	Glutamine	84 (29%)	28 (23%)
	Gabapentin	7 (2%)	8 (6%)
	Amitriptyline	8 (3%)	1 (1%)
	Vitamin B6	5 (2%)	2 (2%)
	Vitamin B Complex	1 (<1%)	1 (1%)
	None	183 (64%)	85 (69%)
Cumulative Paclitaxel (mg/m^2)	Median	700	700
	Range	80–1280	80–1280
Paclitaxel Cycles	Median	4	4
	Range	1–16	1–16
Treatment Prior to Paclitaxel	AC (Doxorubicin/Cyclophosphamide)	216 (75%)	100 (81%)
	AC + Bevacizumab	2 (1%)	0
	A (Doxorubicin)	2 (1%)	0
	AC + Docetaxel	0	1 (1%)
Treatment Concurrent to Paclitaxel	Trastuzumab	46 (16%)	25 (20%)
	Bevacizumab	4 (1%)	5 (4%)
	Carboplatin	1 (<1%)	1 (1%)
	Carboplatin + Bevacizumab	1 (<1%)	2 (2%)
	Trastuzumab + Lapatinib	4 (1%)	0
	Trastuzumab + Cyclophosphamide	1 (<1%)	0
Treatment Setting[a]	Neoadjuvant	137 (48%)	52 (42%)
	Adjuvant	153 (53%)	73 (59%)

Counts and percentages (in parentheses) are presented for categorical data. Medians and ranges are presented for quantitative data.

[a]Two patients in the primary cohort and one in the replication cohort were treated with paclitaxel neoadjuvantly and adjuvantly

Table 2

Variants with strongest association with grade 2+ peripheral neuropathy occurrence in Caucasian cohort

Rank	Gene Variant	rsID	Fisher's Exact P-Value Conditioned on CYP2C8*3
1	ABCG1 (intronic)	rs492338	0.0008*
2	CYP4A11 (3′UTR)	rs11211402	0.0010
3	CYP4B1_14422C>T(R173W)	rs4646487	0.0015
4	GSTA5 (intronic)	rs4715354	0.0018
5	ABCG1 (intronic)	rs3788007	0.0033
6	CBR1 (intronic)	rs998383	0.0037
7	ABCC1_94714T>C(V275V)	rs246221	0.0039
8	GSTA1 (5′ UTR)	rs4715332	0.0049
9	SLC16A1_15385T>A(D490E)	rs1049434	0.0056
10	CYP17A1_195G>T(S65S)	rs6163	0.0066

* Surpassed exploratory α=0.001

Table 3

Neuropathy Occurrence by Genotype for intronic ABCG1 SNP (rs492338) in Caucasian Patients.

Gene Variant	Genotype	Neuropathy Incidence	Odds Ratio Vs. Homozygous Wild-Type
ABCG1 rs492338 [Intronic]	C/C	6/66 (9.1%)	-
	C/T	23/157 (14.6%)	OR= 1.70, 95% CI: (0.63- 5.37) p=0.38
	T/T	20/62 (32.2%)	OR= 4.70, 95% CI: (1.64- 15.57) p=0.002