Reproducibility of pharmacogenetics findings for paclitaxel in a heterogeneous population of patients with lung cancer

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Abstract

Pharmacogenetics studies have identified several allelic variants with the potential to reduce toxicity and improve treatment outcome. The present study was designed to determine if such findings are reproducible in a heterogenous population of patients with lung cancer undergoing therapy with paclitaxel. We designed a prospective multi-institutional study that recruited $n = 103$ patients receiving paclitaxel therapy with a 5-year follow up. All patients were genotyped using the Drug Metabolizing Enzymes and Transporters (DMET) platform, which ascertains 1931 genotypes in 235 genes. Progression-free survival (PFS) of paclitaxel therapy and clinically-significant paclitaxel toxicities were classified and compared according to genotype. Initial screening revealed eleven variants that are associated with PFS. Of these, seven variants in *ABCB11* (rs4148768), *ABCC3* (rs1051640), *ABCG1* (rs1541290), *CYP8B1* (rs735320), *NR3C1* (rs6169), *FMO6P* (rs7889839), and *GSTM3* (rs7483) were associated with paclitaxel PFS in a multivariate analysis accounting for clinical covariates. Multivariate analysis revealed four SNPs in *VKORC1* (rs2884737), *SLC22A14* (rs4679028), *GSTA2* (rs6577), and *DCK* (rs4643786) were associated with paclitaxel toxicities. With the exception of a variant in *VKORC1*, the present study did not find the same genetic outcome associations of other published research on pharmacogenetics variants that affect paclitaxel outcomes. This finding suggests that prior pharmacogenomics research findings may not be reproduced in the most frequently-diagnosed malignancy, lung cancer.

Introduction

Heritable variants in genes that affect drug absorption, distribution, metabolism, transport, and activation (ADME-A) may partly explain inter-individual differences in susceptibility to drug inefficacy or toxicity in lung cancer.[1–6] Such variants also play a role in the exposure to nicotine and nicotine dependence[7] and changes in drug metabolism that are induced by smoking. [8, 9]

Paclitaxel is approved as first-line therapy for patients with lung cancer. Hepatic uptake of paclitaxel is primarily regulated by the transporter OATP1B3 (encoded by *SLCO1B3*),[10] metabolized by several enzymes (e.g., CYP2C8 and CYP3A4),[11] and effluxed by ABCB1 (P-glycoprotein) and the bile acid transporters, ABCB4, and ABCB11.[12–14] Although controversial, several studies suggest some polymorphisms in these ADME genes are related to paclitaxel outcomes and toxicity.[15] Two studies have examined paclitaxel disposition and paclitaxel-induced neutropenia in a Dutch population (*N* = 279) using the DMET array–a platform that tests 1931 variants in 235 pharmacogenes.[2, 4] Yet, these studies did not find allelic variation in these genes was related to paclitaxel pharmacokinetics or toxicity, except for *ABCB11*.

The present study was designed to explore associations between ADME genes and paclitaxel outcome in patients undergoing various treatments in a multi-institutional setting, specifically focusing on whether previous pharmacogenomics findings would be validated in a clinically representative and non-uniform cohort taking taxanes as single agents and in combination with other therapies.

Results

Patients and treatment

Patient characteristics including age, race, gender, smoking history, and various chemotherapy treatment combinations are shown in Table 1. Most patients (*N* = 103) received either paclitaxel in combination with carboplatin (59%, *N* = 61), or paclitaxel and carboplatin in combination with other agents (33%, *N* = 34), with few receiving either paclitaxel alone (5.8%, *N* = 6), or paclitaxel combined with cisplatin (1.9%, *N* = 2). Clinical outcome measures included toxicities and progression-free survival (PFS). Given that hematological toxicity and neuropathy (*N* = 14) were the most frequently observed clinically significant toxicities, we chose to evaluate these outcomes. Other ≥ grade 3 toxicities were too infrequent (*N* ≤ 2) to evaluate associations with genotype. Overall, a total of *n* = 16 patients stopped paclitaxel therapy due to toxicity.

Genotype versus progression-free survival (PFS)

There were 72 variants for which the univariate Cox regression identified as *P*<0.05 whereas 44 variants were found potentially important after an exact trend test was applied S1 Table. Only eleven variants were considered for further consideration, having P<0.01 after combining into two categories (see last column in S1 Table). We also analyzed a set of potential covariates (race, histology/disease type, metastatic status, second hand tobacco, number of prior therapies, age, and cigarette pack-years, Table 1. Initial Cox proportional hazards regression results indicated that metastatic status was weakly associated with PFS and was excluded from further analyses (N = 94; OR (95% CI): 2.27 (0.711, 7.25)). Both stepwise and backward selection methods indicated that the number of prior therapies (0 vs. 1 vs. 2+; N = 73, 17, 13, respectively) was associated with PFS such that patients with one prior therapy are at less, or the same, risk of progression (HR (95%CI): 0.845 (0.281, 2.54)) as patients with no prior therapy; patients with two or more prior therapies are at more risk of progression (HR (95%CI):

Table 1. Patient demographics, baseline disease characteristics, and treatment (safety analysis population).

	Carboplatin + Paclitaxel (N = 61)	Carboplatin + Paclitaxel and other agents (N = 34)	Cisplatin + Paclitaxel (N = 2)	Paclitaxel (N = 6)	Overall Total (N = 103)
Age, y					
Median	66.4	63.5	59.2	70.6	66.3
Minimum-Maximum	49.9–85.3	34.8–91.3	57.4–60.9	58.5–83.6	34.8–91.3
Sex, N (%)					
Male	43 (70)	14 (41)	1 (50)	2 (33)	60 (58)
Female	18 (30)	20 (59)	1 (50)	4 (67)	43 (42)
Race, N (%)					
White	36 (59)	24 (71)	2 (100)	6 (100)	68 (66)
Black	18 (30)	4 (12)	0 (0)	0 (0)	22 (21)
Asian	3 (4.9)	4 (12)	0 (0)	0 (0)	7 (6.8)
Hawaiian or Pacific Islander	1 (1.6)	0 (0)	0 (0)	0 (0)	1 (0.97)
Other	1 (1.6)	0 (0)	0 (0)	0 (0)	1 (0.97)
Not Specified	2 (3.3)	2 (5.9)	0 (0)	0 (0)	4 (3.9)
Smoking classification, N (%); N missing = 1					
Never smoked	10 (16)	8 (24)	0 (0)	0 (0)	18 (17)
Current smoker	6 (10)	2 (5.9)	0 (0)	0 (0)	8 (7.8)
Former smoker	44 (72)	24 (71)	2 (100)	6 (100)	76 (74)
Non-metastatic vs metastatic, N (%)					
Non-metastatic	37 (61)	10 (29)	0 (0)	0 (0)	47 (46)
Metastatic	22 (36)	21 (62)	2 (100)	2 (33)	47 (46)
N/A (Small Cell)	2 (3.3)	3 (8.8)	0 (0)	4 (67)	9 (8.7)
Histology, N (%)					
Adenocarcinoma	31 (51)	22 (65)	1 (50)	2 (33)	56 (54)
Bronchoalaveolar carcinoma	2 (3.3)	1 (2.9)	0 (0)	0 (0)	3 (2.9)
Squamous cell carcinoma	19 (31)	6 (18)	1 (50)	0 (0)	26 (25)
Small cell	2 (3.3)	3 (8.8)	0 (0)	4 (67)	9 (8.7)
Large cell carcinoma	1 (1.6)	1 (2.9)	0 (0)	0 (0)	2 (1.9)
Unclassified	6 (9.8)	1 (2.9)	0 (0)	0 (0)	7 (6.8)
No. prior systemic therapy regimens, N (%)					
0	48 (79)	22 (65)	2 (100)	1 (17)	73 (71)
1	9 (15)	7 (21)	0 (0)	1 (17)	17 (17)
2	2 (3.3)	4 (12)	0 (0)	1 (17)	7 (6.8)
3	0 (0)	0 (0)	0 (0)	1 (17)	1 (0.97)
4	0 (0)	1 (2.9)	0 (0)	2 (33)	3 (2.9)
5	2 (3.3)	0 (0)	0 (0)	0 (0)	2 (1.9)
Other therapy in combination with paclitaxel, N (%)					
Bevacizumab		17 (50)			
YM 155		8 (23.5)			
Cetuximab		2 (5.9)			
Irinotecan		2 (5.9)			
Gemcitabine		1 (2.9)			
Radiation		1 (2.9)			
Etoposide		1 (2.9)			
Bevacizumab + SSIP (i.e., antimesothelin)		1 (2.9)			
Cetuximab + Bevacizumab		1 (2.9)			
Pack-years smoked, N missing = 4					

(Continued)

Table 1. (Continued)

	Carboplatin + Paclitaxel (N = 61)	Carboplatin + Paclitaxel and other agents (N = 34)	Cisplatin + Paclitaxel (N = 2)	Paclitaxel (N = 6)	Overall Total (N = 103)
Median	36.0 (N = 59)	20.5 (N = 32)	30.8	56.3	32.8 (N = 99)
Upper, lower quartile	15.0, 58.5	1.0, 45.6	Insuf. Data	Insuf. Data	8.0, 57.0
Second-hand smoke, N (%), N missing = 1					
Yes	52 (87)	27 (79)	2 (100)	5 (83)	86 (84)
No	8 (13)	7 (21)	0 (0)	1 (17)	16 (16)

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2.43 (1.03, 5.72)) than those with no prior therapy Table 2. All other clinical and demographic parameters (i.e., race, histology, disease type, second hand smoke, age, and cigarette pack-years) were found to not be associated with PFS in univariate analyses and were not considered further. Thus, despite the heterogeneity of the patients included in the study, only the number of prior therapies would be worthy of consideration in a multivariable analysis of factors associated with PFS and in the interpretation of results

Initial screening of the 12 SNPs using both backward and stepwise selection processes resulted in 7 SNPs in the final Cox model (both backward and stepwise selection processes agreed; see multivariable results including the 7 SNPs as well as number of prior therapies in Table 2. Remarkably, 5 of 7 potentially important genes identified in this study encode multiple enzymes and transporters involved in bile acid synthesis, canalicular transport of bile, and sterol clearance: *ABCB11* (Bile Salt Export Pump; BSEP), *ABCC3* (Canalicular Multispecific Organic Anion Transporter 2; CMOAT2), *ABCG1* (ATP-Binding Cassette Transporter G1), *CYP8B1* (Sterol 12-Alpha-Hydroxylase), and *NR3C1* (Glucocorticoid Receptor; GR). Other genes include a pseudogene, *FMO6P* (Flavin Containing Monooxygenase 6 Pseudogene), and the detoxification of electrophilic compounds, *GSTM3* (Glutathione S-Transferase, Mu-3). Median PFS and their 95% confidence intervals are provided in Table 3 and Kaplan-Meier plots are included in Fig 1.

Genotype versus toxicity

Univariate screening of the 1931 SNPs indicated that only 46 had P<0.05 (S2 Table; both 2 x 2 and 2 x 3 cross-tabs) but only one of the seven 2 x 2 tables had P<0.01 (rs4679028; P = 0.004; OR (95% CI): 9.43 (1.68, 53.5)). Of the remaining 39 2 x 3 cross-tabs only 27 had sufficient observations for testing for a trend; the balance (12) were further analyzed after combining groups (see below). Of the 27 2 x 3 cross-tabs with sufficient data, only 5 had P<0.01

Table 2. Proportional hazards analysis of PFS (N = 99).

Variable Type	Parameter	Gene	Allele	Parameter Estimate	SE	Pr > ChiSq	HR	95% Hazard Ratio Confidence Limits	
SNP	rs4148768	*ABCB11*	C>T	1.673	0.539	0.002	5.33	1.85	15.3
	rs1051640	*ABCC3*	A>G	1.385	0.490	0.005	3.99	1.53	10.4
	rs1541290	*ABCG1*	G>A	-0.955	0.461	0.038	0.385	0.156	0.949
	rs735320	*CYP8B1*	G>A	1.797	0.480	0.0002	6.03	2.36	15.5
	rs7889839	*FMO6P*	A>G	1.244	0.438	0.005	3.47	1.47	8.19
	rs7483	*GSTM3*	G>A	-1.463	0.564	0.009	0.232	0.077	0.699
	rs6196	*NR3C1*	T>C	1.076	0.468	0.021	2.93	1.17	7.33
Covar.	Prior therapies: 0 vs. 1			-0.168	0.562	0.76	0.845	0.281	2.54
	Prior therapies: 0 vs. 2+			0.889	0.436	0.041	2.43	1.03	5.72

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Table 3. Median progression-free survival.

Gene	Variant	Stratum 1 (months)	Stratum 2 (months)
ABCB11	rs4148768	7.83 (5.00, NR*)	7.36 (1.31, 7.36)
ABCC3	rs1051640	7.83 (6.28, NR)	3.81 (2.99, 4.83)
ABCG1	rs1541290	4.83 (3.03, 11.08)	7.83 (6.28, NR)
CYP8B1	rs735320	7.83 (6.54, NR)	5.00 (2.01, NR)
FMO6P	rs7889839	7.36 (6.28, NR)	5.00 (2.14, 12.85)
GSTM3	rs7483	5.00 (3.03, 6.54)	12.85 (11.08, NR)
NR3C1	rs6196	7.83 (6.28, NR)	3.03 (1.61, 7.36)

* Not reached (NR)

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(rs4643786, rs910795, rs6811453, rs6577, rs1056836); Somers' D indicates that the associations are most likely weak. Of the remaining 12 SNPs, only two had P<0.01 (rs3093105 (N = 85), rs2884737).

None of the covariates evaluated were considered in the multiple logistic regression analysis as they were found to be weakly associated with toxicity in the univariate analyses (race, histology/disease type, metastatic status, second hand tobacco, number of prior therapies, age, and cigarette pack-years (S3 Table). An initial analysis indicated that the variant corresponding to rs3093105 was not an important predictor of toxicity, so it was dropped from further consideration in analyses. Two similar, but competing, models were identified. In both models, variants in the vitamin K epoxide reductase complex (*VKORC1*; rs2884737) and the organic-cation transporter-like 4 (*OCTL4*, *SLC22A14*; rs4679028) were included. The odds ratios (OR; 95% CI) were 0.092 (0.002, 0.807) and 9.77 (1.65, 63.8), respectively for VKORC1 and SLC22A14 in Model 1, while they were 0.097 (0.002, 0.909) and 5.60 (0.992, 33.1) in Model 2; Table 4). Two other variants only appeared in one model each. Variants in the glutathione S-transferase alpha-2 (*GSTA2*; rs6577) were only found in model 1 (OR (95%CI) = 6.03 (1.42, 28.9) and those in the deoxycytidine kinase (*DCK*; rs4643786) were found in model 2 OR (95% CI) = 0.111 (0.009, 0.899), respectively). Model 1 correctly predicted 72/86 (83.7%) of those without toxicity and 11/16 (68.8%) of those with toxicity, and Model 2 correctly predicted 82/87 (94.3%) of those without toxicity and 8/16 (50.0%) of those with toxicity. The former model provided a more balanced set of classification probabilities, and is considered preferable for that reason. Different associations were found when hematological toxicities were considered as a group, and neutropenia and thrombocytopenia were considered as individual categories (S4 Table).

Conclusions

The present study identified 7 genetic variants that were associated with paclitaxel PFS and 3 variants associated with major paclitaxel-induced dose-limiting toxicities in patients with lung cancer treated with paclitaxel. Findings included few genes that have been previously associated with paclitaxel outcomes and/or lung cancer progression. Other genes represent novel associations as are summarized below. The present data suggest that pharmacogenomics associations demonstrated in previous studies are not replicated in a clinically representative cohort of patients with lung cancer who have undergone paclitaxel therapy.

A previous study by de Graan *et al.* discovered that a set of four linked polymorphisms in *VKORC1* was related to low paclitaxel clearance in a cohort of individuals with a variety of cancers.[2] In the present study, the *VKORC1* SNP associated with a higher probability of toxicity, rs2884737 (T/T), is in strong linkage disequilibrium with the same allele (T/T) in rs9934438
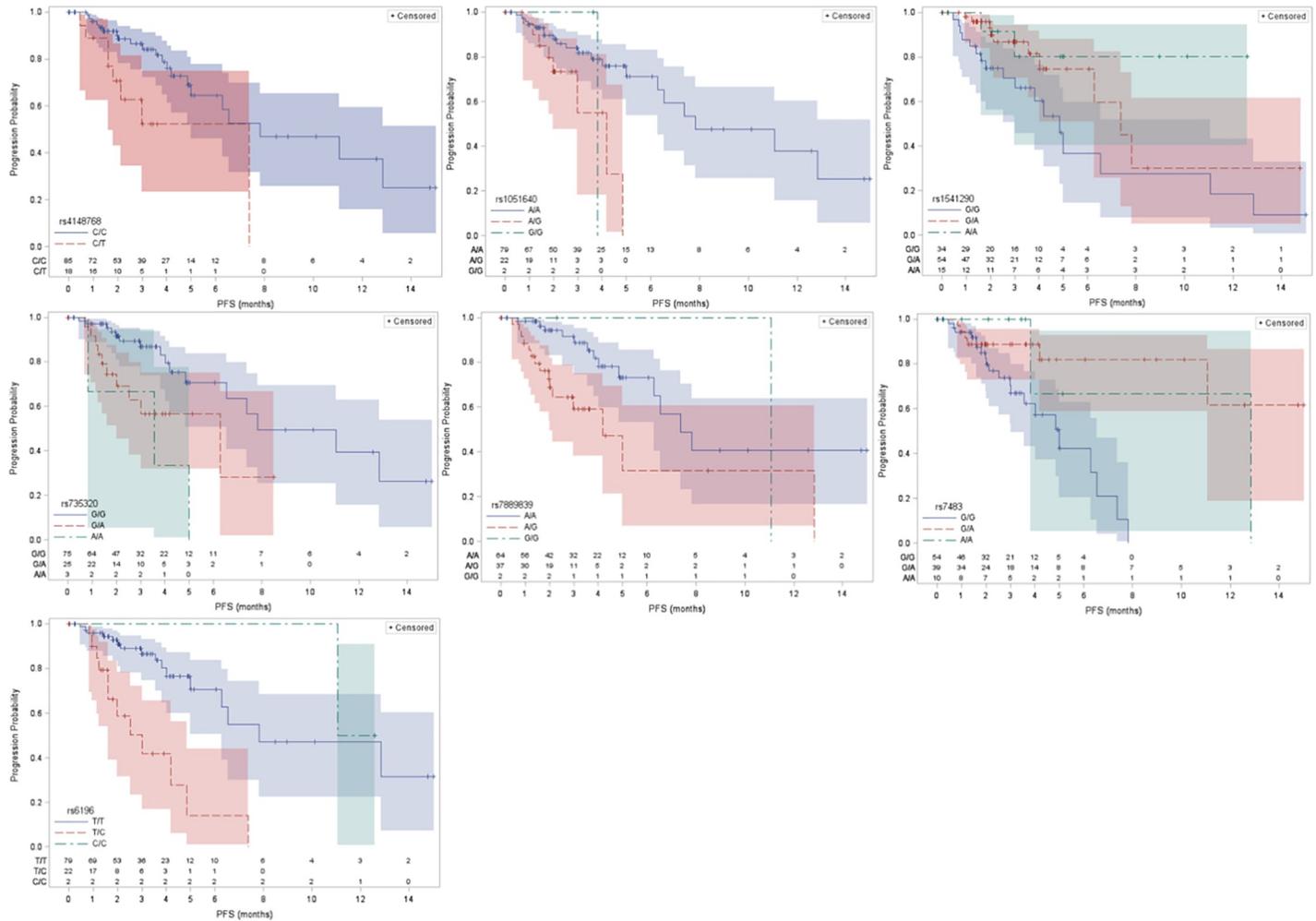
Fig 1. Kaplan-Meier plots of potentially important associations following Cox regression analyses. Paclitaxel PFS was related to genetic variants in seven genes: A) *ABCB11* rs4148768, B) *ABCC3* rs1051640, C) *ABCG1* rs1541290, *CYP8B1* rs735320, *FMO6P* rs7889839, *GSTM3* rs7483, and *NR3C1* rs6196.

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that was associated with low clearance S1 Fig. Taken together with the findings of de Graan et al., our data suggest that this haploblock is associated with low clearance, and therefore a

Table 4. Logistic regression models of toxicity.

Gene	Genotype with Higher Risk	Parameter	Parameter Estimate	SE	OR	95% Odds Ratio Confidence Limits		P (Exact)
Model 1 (N = 102)								
		Intercept	-2.288	0.491				
VKORC1	T/T	rs2884737	-2.49	1.171	0.092	0.002	0.807	0.024
GSTA2	A/C, C/C	rs6577	1.864	0.673	6.03	1.42	28.9	0.012
SLC22A14	A/A	rs4679028	2.425	0.817	9.77	1.65	63.8	0.010
Model 2 (N = 103)								
		Intercept	0.592	0.949				
VKORC1	T/T	rs2884737	-2.414	1.235	0.097	0.002	0.909	0.037
DCK	C/C	rs4643786	-2.394	0.975	0.111	0.009	0.899	0.038
SLC22A14	A/A	rs4679028	1.845	0.781	5.60	0.992	33.1	0.051

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high probability of paclitaxel toxicity. However, the mechanism underlying the relationship between these SNPs and clearance/toxicity is currently unclear since, to our knowledge, VKORC1 activity regulates the oxidation state of vitamin K and clotting factors, which appears to have little relationship with drug metabolism or activity.

Four of seven (57.1%) variants identified in association with PFS were associated with bile synthesizing and transporting proteins (*ABCB11*, *ABCC3*, *ABCG1*, and *CYP8B1*). Bile acid enzymes and transporters have been previously associated with paclitaxel and docetaxel neutropenia,[4, 16] and, as explained above, some of these genes have previously been associated with paclitaxel outcomes for other reasons. For instance, ABCB11 transfection confers a low level of paclitaxel resistance in ovarian carcinoma cells, and is likely involved in hepatobiliary paclitaxel elimination,[12] and paclitaxel is an inhibitor of the BSEP.[17] However, *ABCB11* rs2287622 identified in Nieuweboer *et al.* (4 or 31) is not in linkage with the presently associated polymorphism, rs4148768. We propose that bile disposition is responsible for changing the expression of various drug metabolizing enzymes and transporters that are controlled by bile-responsive nuclear receptors,[18] and this possibility has been very poorly explored in the literature. For example, cholic acid induces *ABCC3* expression, which provides a hepatoprotective effect during cholestasis,[19] but the promotion of ABCC3 expression would also be expected to have significant consequences on paclitaxel and platinum disposition.[20–28]

Other findings are potentially related to the combination of multiple therapeutics. ABCC3 was identified as one of the most up-regulated genes in in chemotherapy-resistant lung cancer [28] and taxane-resistant breast cancer[22]. In lung cancer, carboplatin is responsible for increasing the expression of MRP3,[23] and acquired paclitaxel resistance during carboplatin cotherapy often appears to be a function of MRP3.[21] Many cisplatin-treated cells also upregulate MRP3,[20] and platinum resistance is associated with MRP3 in lung cancer.[26, 27] The polymorphism identified in the present study (rs1051640; E1503E) has also been observed in relation to cisplatin-induced ototoxicity;[24, 25] however, no study has yet characterized the functional effects of E1503E to our knowledge. DCK typically catalyzes the rate-limiting step of the formation of deoxynucleoside triphosphates, and the present SNP (rs4643786, 948T>C) is related to lower DCK expression in lymphocytes.[29] Consistent with this finding, 948C carriers with AML receiving topoisomerase inhibitors had superior response than those who carried 948TT [30]. DCK also phosphorylates gemcitabine, promoting its incorporation into DNA. Paclitaxel increases expression of dCK in NSCLC cell lines.[31] Our observations indicate that paclitaxel caused greater toxicity in patients carrying the low-DCK expressing genotype. This polymorphism should be studied further in this context.

Still other findings could be related to tumor progression. ABCG1 effluxes intracellular cholesterol to high-density lipoprotein particles that undergo reverse cholesterol transport from the periphery to the liver. Macrophages with ABCG1 expression undertake a tumor promoting phenotype, M2, and have been associated with tumor growth and antiapoptosis.[32] ABCG1 expression in macrophages is also associated with poor survival outcomes in patients with lung cancer.[33, 34] Accordingly, a polymorphism in *ABCG1* that was related to higher *ABCG1* expression (rs225388) was associated with poor survival in patients with lung cancer. [35] Another SNP (rs492338) was strongly associated with paclitaxel-induced neuropathy,[36] but this SNP was not associated with outcome in our study. Interestingly, the currently associated SNP (rs1541290 G/G) was previously related to low docetaxel clearance; which counterintuitively suggests that those carrying low clearance alleles had shorter PFS.[37] Therefore, it is unclear whether this polymorphism is predictive or prognostic, and could be both.

A polymorphism in the glucocorticoid receptor (*GR*; *NR3C1*) was also associated with poor PFS. Basal expression of CYP2C8 is regulated by the glucocorticoid receptor, and dexamethasone induces CYP2C8.[38, 39] Glucocorticoids reduce paclitaxel efficacy because GCs reduce

paclitaxel-induced apoptosis, and blocking GR restores paclitaxel sensitivity.[40–43] High GR expression is related to LC cell survival[44] and corticosteroid co-therapy typically results in therapeutic resistance in lung cancer.[45] The N766N (rs6196 T>C) polymorphism in the glucocorticoid receptor is present in exon 9α, which comprises the ligand binding,[46] AP-1/NF-κB/dimerization/nuclear translocation domains of the *GR* and was associated with corticosteroid resistance in Crohn's disease [47] although this finding is controversial.[48] Therefore, the glucocorticoid receptor could both affect paclitaxel metabolism and promote growth and prevent death signaling in lung cancers, and the mechanism behind the association of this polymorphism and poor paclitaxel PFS remains unclear.

Other findings include variants in GSTA2, GSTM3, FMO6P, and SLC22A14. GSTM3 is broadly distributed in the airway epithelium and smooth muscle of the lung and is expressed at higher levels in smokers than in ex-smokers.[49] Several GSTs have also been related to outcomes and clearance of paclitaxel.[2, 4] SNPs in GSTM3 and GSTA2 have not yet been explored in relation to paclitaxel outcomes in the literature.

With the exception of variants in *VKORC1*, most previously-studied genetic variants that are often related to paclitaxel pharmacokinetics and outcome were not reproducible in this study.[2, 4, 10–15] We suggest that such a finding would be expected since pharmacogenetics research is typically conducted retrospectively on clinical trials that select rather homogeneous populations of individuals in a controlled setting. By contrast, paclitaxel is most-often combined with a wide variety of agents in heterogenous populations with numerous different diseases in the community setting, Thus, prior pharmacogenomics findings are not reproducible in a prospectively recruited, clinically representative, and non-uniform cohort taking taxanes as single agents or in combination with other therapies. Such a finding is unfortunate for those undergoing paclitaxel therapy for the most frequently diagnosed malignancy, lung cancer. This study is based on a heterogeneous population and because of the limited number of patients which would be present in each population subgroup, it was not practical to further explore the associations of SNPs and outcomes in the subgroups. Except possibly for the number of previous systemic treatments, with the marginal association with PFS of the few patients with 2 or more prior treatments vs. those without any prior treatments, this was not likely to result in substantially different findings given that the various subgroups themselves were not found to differ in prognosis. However, it would be worthwhile if future evaluations on larger, possibly more homogeneous populations of patients could be undertaken to more definitively confirm the findings of the present study.

Materials and methods

Patients and treatment

A total of 546 patients with histologic diagnosis of primary lung carcinoma were enrolled in this study (NCT#00923884) between 2009 and 2012. Patients were treated at the National Cancer Institute (N = 441) and the Washington D.C. Veteran's Affairs Medical Center (N = 105), and the present analysis focuses on those treated with paclitaxel. Of these 546 patients, 103 patients who received paclitaxel, either alone (N = 6) or in combination (N = 97), were successfully genotyped and had available smoking history, progression-free survival (PFS), and toxicity data. The study enrolled individuals who were over the age of 18 with non-small cell or small cell lung cancer who received any treatment (surgical resection, chemotherapy, radiation, or molecularly targeted therapy), had ECOG performance status of 0–3, and normal or impaired organ function. Those with a current diagnosis of or a prior history of other cancers were also enrolled. The study was approved by the Institutional Review Board at the National Cancer Institute (Bethesda) and the Veteran's Affairs Medical Center (Washington D.C.). All

patients provided written informed consent, and data for the present study are included on https://www.ncbi.nlm.nih.gov/gap.

Assessments

All patients were followed for disease recurrence and survival for up to a 5-year period following enrollment. PFS was calculated by subtracting the date of initiation of a particular treatment from the date of progression on that treatment (censored if the patient was alive and remained on treatment as of the last follow-up). For the purposes of this analysis, only grade 3 or greater toxicities possibly, probably, or definitely related to the therapy were assessed. All patients received a questionnaire ascertaining cigarette pack years and secondhand tobacco exposure.

Sample handling

A whole blood sample was obtained from each patient in an EDTA (lavender) top tube. DNA was extracted via ethanol precipitation, and samples were stored at -80˚C until genotyping was conducted. Prior to genotyping, all samples underwent gDNA quantitation using the human RNase P TaqMan Copy Number Reference Assay (Thermo Fisher Scientific, Waltham, MA, USA) per the manufacturer's instructions. Samples were diluted to 5ng/uL (150ng total) for analysis by the Drug Metabolizing Enzymes and Transporters platform (DMET; Affymetrix, Santa Clara, CA), which was also conducted using the manufacturer's instructions. The DMET array ascertains 1931 allelic variants in 235 pharmacogenes. Of these 21% SNPs had at least one missing value, which is typically attributable to poor sample integrity or low DNA concentration; albeit, many other reasons are possible. All genotype and phenotype data used in this study are posted on dbGAP: http://www.ncbi.nlm.nih.gov/projects/gapprev/gap/cgi-bin/preview1.cgi?GAP_phs_code=PJfddSlJgReHqFfc

Statistical considerations

Univariate genotype relationships with progression-free survival (PFS) were initially performed using a univariate proportional hazards (Cox) regression analysis and the Chi-square test to calculate the P-value as a screen. We next performed actuarial analysis on the PFS data and used both a log-rank based trend test and log-rank test to calculate two-tailed P-values. SNPs for which $P<0.05$ were noted for both Cox and actuarial analyses and were then further analyzed via Kaplan-Meier plots. If there were two strata, then a log-rank test was performed to compare stratum (exact tests were used if $N<6$ for either stratum). If there were three strata, and at least one stratum had $N<6$, then an exact trend test and log-rank test was performed. For those tests where $P<0.05$ Kaplan-Meier plots were produced and examined to determine whether adjacent stratum could be combined (e.g., G/A+A/A, but not G/G+A/A). If any of the strata were combined, then another log-rank test was performed. Finally, a multivariable Cox proportional hazards analysis including the SNPs identified by the univariate analyses, as well as any of the clinical or demographic factors identified to be potentially associated with PFS on univariate analyses, was performed to demonstrate the joint impact of the SNPs and any clinical or demographic factors on PFS.

Univariate genotype relationships with toxicity were first screened using Fisher's exact test (2 x 2 tables) or Mehta's modification to Fisher's Exact test (2 x 3 tables), as appropriate [50]. Second, when applicable, if the two-tailed P-value from the first test was below 0.05, an exact Cochran-Armitage trend test was conducted. Finally, if a 2 x 3 table had small sample sizes ($N<6$) in any of the levels, adjacent levels were combined (e.g., 1+2, or 2+3) and then a Fisher's

exact test was conducted on the newly-created 2 x 2 table. If the last P-value was ≤ 0.010, then an odds ratio or Somers' D was calculated (and 95% confidence intervals).

To further interpret the association between SNPs and toxicity, we also analyzed the SNPS along with a set of potential covariates (race, histology/disease type, metastatic status, second hand tobacco, number of prior therapies, age, and cigarette pack-years) in a multiple logistic regression analysis, which consisted of both backward and stepwise selection processes.

Given that this study is subject to a high number of comparisons, a P-value <0.010 was considered potentially important whereas $P \leq 0.001$ with sample sizes >30 in each group was considered to afford the most interpretive weight.

Supporting information

S1 Fig. The polymorphism identified in the present study, rs2884737, is in strong linkage with the haplotype block identified by de Graan et al.[2].
(PDF)

S1 Table. Results of univariate screening of variants vs PFS. [1]P-value calculated by the Chi-square test.
(PDF)

S2 Table. Results of univariate screening of variants vs toxicity. [1]Mehta's modification to Fisher's Exact test were conducted where appropriate. [2]Combined categories with small sample sizes.
(PDF)

S3 Table. Results of multiple logistic regression analysis of covariates.
(PDF)

S4 Table. Results of univariate screening of variants when neutropenia and thrombocytopenia were considered as individual categories. [1]Mehta's modification to Fisher's Exact test were conducted where appropriate. [2]Combined categories with small sample sizes.
(PDF)

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