Genetic variation in platinating agent and taxane pathway genes as predictors of outcome and toxicity in advanced non-small-cell lung cancer

Aim: Lung carcinoma is the most common malignancy and the leading cause of cancer deaths worldwide. Although clinical factors including age, performance status and stage influence the likelihood of benefit from and tolerability of chemotherapy, the genetic profile of individual patients may be an independent predictor of response and toxicity. The present study aimed to identify pharmacogenetic markers associated with clinical response and toxicity in patients with advanced non-small cell lung cancer (NSCLC) treated primarily with carboplatin and paclitaxel.

Materials & methods: Genomic DNA samples from 90 adult male patients diagnosed with stage IIIB/IV NSCLC were genotyped for SNPs in candidate genes of relevance to platinating agents and paclitaxel and analyzed for association with survival and toxicities in univariate and multivariate models. **Results:** After adjusting for performance status and stage, SNPs in the drug transporters ABCB1 and ABCC1, as well as within NQO1 were associated with progression-free survival. With respect to hematological and nonhematological toxicities, SNPs in drug transporters (ABCB1 and ABCG2) were associated with thrombocytopenia, nausea and neutropenia, whereas SNPs in the DNA repair pathway genes *ERCC4* and *XPC* were significantly associated with neutropenia and sensory neuropathy, respectively. **Conclusion:** Our study evaluated and identified SNPs in key candidate genes in platinating agent and taxane pathways associated with outcome and toxicity in advanced NSCLC. If validated in large prospective studies, these findings might provide opportunities to personalize therapeutic strategies.

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Background

Lung carcinoma is the most common malignancy worldwide, and the leading cause of cancer deaths. According to the most recent estimates, the global incidence of lung cancer is more than 1.6 million cases/year, resulting in more than 1.3 million deaths/year (>18% of all cancer deaths) [1]. In the USA, the incidence of lung cancer was estimated to be 228,000 cases in 2013, leading to nearly 160,000 deaths (27.5% of all cancer deaths) [2]. Histologically, the majority (approximately 80%) of lung cancers are non-small-

cell carcinomas (NSCLCs). The 5-year relative survival of patients with advanced NSCLC remains dismal at approximately 4% [3]. Indeed, the median survival of unselected patients diagnosed with metastatic NSCLC is only 4 months [4].

Platinating agent-based combination chemotherapy is the standard treatment for patients with advanced NSCLC who have adequate organ function and performance status, and whose tumor does not have a driver mutation amenable to treatment with a specific inhibitor. However, combination

chemotherapy achieves objective response in only approximately 26% of patients, improves survival modestly, and is associated with diverse side effects [5]. Interpatient variation in response and toxicity, which cannot be predicted for individual patients, precludes the selection and tailoring of chemotherapy that might improve outcomes and minimize adverse events. Although clinical factors including age, performance status and stage influence the likelihood of benefit from and tolerability of chemotherapy, the genetic profile of individual patients may contribute significantly to the marked variation in response and toxicity. Adverse effects associated with platinating agent-based combination chemotherapy include gastrointestinal toxicity, myelosuppression, nephrotoxicity, peripheral neuropathy and ototoxicity [6].

Platinating drugs such as cisplatin and carboplatin act by forming platinum–DNA adducts, which lead to cell cycle arrest and apoptosis. Several genes are involved in the carboplatin drug pathway (platinating agents pathway PharmGKB: [Supplementary Figure 1](#); see online at www.futuremedicine.com/doi/suppl/10.2217/pgs.14.107). Intracellular levels of carboplatin are regulated by drug transporters SLC31A1 (CTR1), ABCC2 (MRP2), ATP7A and ATP7B [7], as well as drug-metabolizing enzymes including MPO, SOD1, GSTM1, NQO1, GSTP1 and MT, which are implicated in the development of cellular resistance to these drugs [8–11]. Genes of pharmacodynamic significance include *HMGB1*, which is involved in recognition and cellular response to platinum–DNA adducts and DNA repair genes including mismatch repair genes *MSH6* and *MLH1*, and nucleotide excision repair genes *XRCC1*, *ERCC1*, *ERCC2* and *XPA* [12,13]. Genetic variation in these genes of importance to pharmacokinetic and pharmacodynamic pathways of platinating agents may thus contribute to interpatient variation in response and tolerability [14].

Taxanes are routinely given in combination with platinating agents; genes involved in efflux (ABCC1, ABCC2, ABCG2 and ABCB1) and metabolism of taxane (CYP3A4 and CYP2C8) are critical for its therapeutic efficacy (taxane pathway PharmGKB: [Supplementary Figure 1](#)). Although the role of genetic variation in taxane response is unclear at this time, some studies have found no significant association between SNPs and treatment outcome [15], whereas others have found significant associations between *ABCB1* SNPs and response to paclitaxel [16], and gastrointestinal toxicity in patients treated with taxane and platinum combination therapy [17].

In the present study, we evaluated SNPs in genes of relevance to the pharmacokinetic/pharmacodynamic pathways of platinating agents and taxanes in patients

diagnosed with advanced NSCLC and treated primarily with carboplatin-based doublet chemotherapy, and determined the association of individual SNPs with outcomes and toxicity. We identified specific SNPs that were predictive of progression-free survival (PFS) and multiple adverse effects, after adjusting for known clinical prognostic factors in multivariate models. Results of our proof-of-concept study provides evidence that in real world clinical settings the association of genetics with clinical outcome is evaluable and although validation in larger cohorts is required, genetic information can be utilized to develop to more effective treatment strategies.

Materials & methods

Patients

A total of 635 patients diagnosed with stage IIIB or IV NSCLC between December 1998 and December 2008 were identified by the institutional tumor registry ([Figure 1](#)). Of these, 90 patients met the following eligibility criteria: histological or cytological confirmation of NSCLC, presence of measurable disease, performance status Eastern Cooperative Oncology Group (ECOG) 0–2, no neoadjuvant or concurrent radiation therapy or surgery, no second malignancies, availability of adequate diagnostic tumor tissue, treatment with first-line platinating-agent based doublet chemotherapy and complete follow-up at the Minneapolis VA Health Care System (MN, USA). The study was approved by the institutional human subjects committee.

Treatment schedules, dose & toxicity assessments

Of the 90 patients identified, 87 received carboplatin, two received cisplatin, and two received cisplatin and carboplatin as first-line chemotherapy. Patients were evaluated every 2–3 cycles using the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. We assessed the following hematologic toxicities: anemia, neutropenia, febrile neutropenia and thrombocytopenia. Nonhematologic toxicities assessed included nausea, vomiting, diarrhea, mucositis, sensory and motor neuropathy, renal dysfunction (increase in creatinine), and liver dysfunction (abnormal liver function tests). Grading of toxicities was performed using the National Cancer Institute Common Toxicity Criteria Version 4.0.

Clinical evaluation and blood tests were performed prior to the first cycle of chemotherapy and before each subsequent cycle. Baseline CT scans were obtained before the start of treatment, and repeated every 2–3 cycles to evaluate treatment response. Patients had open access to the oncology clinic for reporting any side effects or concerns during treatment.

Figure 1. Patient selection and study strategy.

NSCLC: Non-small-cell lung carcinoma; MAF: Minor allele frequency; XRT: Radiotherapy.

SNP analysis

Genomic DNA was isolated from formalin-fixed paraffin-embedded specimens ($n = 86$ NSCLC patients) using Qiagen DNA isolation from formalin-fixed paraffin-embedded tissues kit (Qiagen, Valencia, CA, USA) as per manufacturer's instructions. Genes of importance to platinating agents as well as potentially significant SNPs within them were selected from PharmGKB (Supplementary Figure 1) [18] and literature search. Overall, 63 SNPs within 29 genes in 86 subjects with detailed clinical information were genotyped using the Sequenome platform at the University of Minnesota Genomics Center (MN, USA). Six samples were removed from further statistical analyses owing to low call rates (<90%). There was no significant difference in age, performance status and survival between patients excluded versus included in study ($p > 0.05$). Quality control of the SNP data resulted in one SNP being removed with a call rate <90%, with no SNPs removed owing to deviations from Hardy–Weinberg equilibrium.

Statistical methods

All analyses were restricted to subjects with self-reported race of white or 'unknown'. Association of each clinical

feature with clinical outcomes of PFS and overall survival (OS) was assessed using Cox-proportion hazards models. Associations of each SNP genotype with clinical outcomes were examined using Cox-proportion hazards models, where the SNP genotyped was coded under a dominant, codominant or additive genetic model. Models were fit with and without adjustment for clinical features associated with clinical outcomes. For analysis of PFS, we included covariates of stage and performance status. For OS, we included performance status as a covariate. For analyses with clinical features, stage was treated as a categorical variable and performance status was treated as a continuous variable. Age at diagnosis was categorized into four levels based on the 25, 50 and 75% percentiles of the distribution. All statistical analyses were completed using R (version 2.15.3). Adjustment for multiple testing was carried out using Bonferroni correction.

Results

Demographic features & clinical characteristics

Demographic and clinical features of the 90 evaluable patients at diagnosis are shown in Table 1. The median age was 66 years (range: 45–81). All the patients were

males, a reflection of the patient population at a VA Medical Center. The majority (93%) of patients were white; 81% of patients had stage IV disease. Adenocarcinoma was the most common histological subtype (42% of patients) of NSCLC, followed by squamous cell (20%) and large cell (16%) carcinoma. The median performance status (ECOG scale) was 1 (range: 0–2).

Treatment, response & outcomes

Chemotherapy regimens administered, including the number of cycles and average dose intensities of each agent, are shown in Table 2. The overall response rate was 19%, with a median duration of response of 182 days, median PFS of 148 days and median OS of 288 days. No patient achieved a complete response. Hematological and nonhematological toxicities selected for further analysis are shown in Table 3. No patients developed treatment-related abnormalities in liver function tests, or experienced CTCAE grade 5 (fatal) toxicity.

Pharmacogenetics

We first examined the association of known clinical prognostic factors (covariates) with clinical outcomes. Stage ($p = 0.02$) and performance status ($p = 0.03$) were predictive of PFS, and performance status ($p = 0.02$) was associated with OS. Therefore, we adjusted for these covariates when analyzing the association between SNPs and outcomes. In the analysis of toxicity, we adjusted for the total number of cycles received, since the cumulative dose of chemotherapeutic agents received would be expected to increase the risk of toxicities such as peripheral neuropathy. For genetic association testing, 86 patients had both clinical and genotype data. Of these 86 patients we included patients with white ancestry ($n = 80$) for further analysis. Of 58 SNPs with a minimum allele frequency of >0.05 , one SNP was excluded due to call rate of <0.90 . Although number of chemotherapy cycles were associated with OS, we decided not to adjust for them as genetic factors might be influencing number of cycle and if we adjust for cycles, we might mask some of the effects due to patient genetics (as it might be ‘surrogate’ for length of survival [depends on time; changes over time]).

PFS was associated with SNPs in drug efflux transporters *ABCC1* (intronic SNPs: rs246240 and rs2238476) and *ABCB1* (coding synonymous SNP, rs1128503); a quinone reductase *NQO1* (missense SNP, rs1800566); *TMEM63A* (rs10158985); and other genes such as *KCNC1* (rs17718902) and *CCDC127* (rs9312960; summarized in Table 4). SNPs within *ABCB1* (rs1045642; $p = 0.04$), *ABCC1* (rs2238476; $p = 0.03$), *ABCG2* (rs17731538; $p = 0.04$) and *TMEM63A* (rs10158985; $p = 0.03$) were also associated with OS.

We also evaluated association of SNPs with major hematological and nonhematological toxicities. Toxicities were not associated with the initial performance status ($p > 0.05$) but were associated with age and number of treatment cycles. After adjusting for age and number of treatment cycles, we found associations of *ABCB1* SNP with thrombocytopenia (rs2235015; $p = 0.04$), and *ABCG2* (rs2231142; $p = 0.045$) and *ATP7B* (rs1801244; $p = 0.027$) SNPs with nausea (Table 5), and *ABCG2* SNP (rs13120400, $p = 0.027$) with sensory neuropathy, respectively. Interestingly, SNPs in the DNA repair pathway genes *ERCC4* (rs744154; $p = 0.04$) and *XPC* (rs2228001; $p = 0.045$) demonstrated associations with neutropenia and sensory neuropathy, respectively (Table 5). Additionally, we observed SNPs in *TP53* (rs165895; $p = 0.02$), *CYP2C8* (rs11572080; $p = 0.017$) and *CCDC127* (rs9312960; $p = 0.019$) to be associated with diarrhea. rs447978, an intronic SNP in transcription fac-

Table 1. Patient demographics and clinical characteristics (n = 90).			
	n (%)	p-value (PFS)	p-value (OS)
Age at diagnosis (years)			
Median	66	0.95	0.25
Range	45–81		
Race			
White	84 (93.3)		
African–American	1 (1.1)		
American Indian/Alaska Native	2 (2.2)		
Unknown	3 (3.3)		
Stage			
IIIB	17 (19)	0.02	0.48
IV	73 (81)		
Histology			
Adenocarcinoma	40 (44.4)		
Adenocarcinoma, mucinous	2 (2.2)		
Squamous cell	18 (20)		
Large cell	12 (13.3)		
Large cell neuroendocrine	2 (2.2)		
Non-small-cell carcinoma, NOS	16 (17.8)		
Brain metastasis	12 (13.3)		
Performance status (ECOG)			
Median	1	0.03	0.02
Range	0–2		
ECOG: Eastern Cooperative Oncology Group; NOS: Not otherwise specified; OS: Overall survival; PFS: Progression-free survival.			

Table 2. Chemotherapy regimens, dose intensity, response and survival.

Regimen	Number of patients	Number of cycles, mean (range)	Dose intensity (mg/m ² /week) [†] , mean ± SE		Response rate, n (%)	Median duration of response (days)	Median PFS (days)	Median OS (days)
			Platinum [‡]	Second drug				
C + T	77	3.3 (1–6)	1.8 ± 0.3	60.0 ± 10.0	14 (18)	163	148	292
C + G	9	3.2 (1–5)	1.4 ± 0.4	455.1 ± 169.1	3 (33)	185	211	465
C + E	2	3 (1–3)	1.9 ± 0.5	84.9 ± 19.0	0 (0)	0	NE	NE
Cis + E	2	1.5 (1–2)	21.8 ± 4.2	75.0 ± 0.0	0 (0)	0	NE	NE
Total	90	3.2 (1–6)	–	–	17 (19)	182	148	288

None of the patients had a complete response.

The median PFS and OS were calculated from the Kaplan–Meier estimates.

[†]The target dose intensities for each agent were: C AUC 2/week; Cis, 27 mg/m²/week; T, 67 mg/m²/week; G, 667 mg/m²/week and E, 100 mg/m²/week (with C) and 80 mg/m²/week (with Cis).

[‡]Dose of C is expressed as AUC.

AUC: Area under the concentration–time curve; C: Carboplatin; Cis: Cisplatin; E: Etoposide; G: Gemcitabine; NE: Not evaluable; OS: Overall survival; PFS: Progression-free survival; SE: Standard error; T: Paclitaxel.

tor *GTF2E1*, was associated with both neutropenia ($p = 0.024$) and nausea ($p = 0.027$). After adjustment for multiple testing (Bonferroni) the above indicated associations were not significant and the results need to be validated in a larger study.

Discussion

This comprehensive analysis identified pharmacogenomics variants of clinical significance within key genes ($n = 29$) in the platinating agents pathway and taxane pathway in NSCLC patients treated with a combination of carboplatin and paclitaxel.

We found significant associations of drug transporter (*ABCC1* and *ABCB1*) SNPs with PFS. Although we did not observe significant association of *ABCB1* rs1045642 with outcome/toxicity, *ABCB1* SNPs rs1128503 (occurring in partial linkage disequilibrium with rs1045642) and rs2235015 were associated with PFS and thrombocytopenia, respectively. The functional consequence of the three most commonly studied SNPs (rs1128503, rs1045642 and rs2032582 also occurring in linkage disequilibrium) in *ABCB1* is still not completely understood. Association of these SNPs with mRNA/protein expression have been shown in some studies but not all the studies. rs1045642, a synonymous SNP (3435C>T; Ile1145Ile) has also been indicated to influence protein conformation and substrate specificity [19]. Previous studies have identified association of *ABCB1* SNPs (especially rs1045642) with survival in osteosarcoma patients treated with cisplatin-containing chemotherapy [20]. In esophageal cancer patients treated with platinating agents, presence of the T allele for rs1045642 is associated with significantly longer survival and reduced risk of recurrence [21]. Together, these findings suggest that drug

transporter SNPs that might influence outcomes in several malignancies by altering intracellular drug concentrations.

NQO1 belongs to the quinone dehydrogenase family of proteins and has been implicated in metabolizing platinum agents. A missense SNP rs1800566 (*NQO1**2: Pro187Ser) is in the active site of the enzyme and has been associated with reduced NQO1 activity. The NQO1 isoform with Ser (*NQO1**2) undergoes rapid degradation [22,23]. A recent study reported association of the *NQO1**2 SNP with shorter OS in NSCLC patients treated with adjuvant radiation therapy with or without platinating agent based chemotherapy [24]. Our results are in concordance with these previous observations, with presence of *NQO1**2 associated with poor PFS (Table 4).

Toxicity due to platinating agent and taxane-based chemotherapy remains a major challenge faced by clinicians and patients. There are no well-established biomarkers that can predict such toxicities in individual patients. In our efforts to identify SNPs predictive of major hematological and nonhematological toxicities, we observed that a missense SNP in *CYP2C8*, a drug-metabolizing enzyme involved in the metabolism of taxanes, was significantly associated with higher incidence of diarrhea (OR: 4.396; $p = 0.017$). Previous results in breast cancer patients receiving paclitaxel have shown significant association of *CYP2C8**3 (which denotes two highly linked SNPs rs11572080 and rs10509681) with better response and a trend towards greater risk of peripheral neuropathy [25]. *CYP2C8**3 is associated with lower paclitaxel- α hydroxylation activity, and hence carriers of this SNP have lower clearance of paclitaxel, which can contribute to better response but also greater risk of toxicity to normal cells [26,27].

Table 3. Hematological and nonhematological toxicities.

Toxicity and grade ^a	Chemotherapy regimen			
	C + T, n (%)	C + G, n (%)	C + E, n (%)	Cis + E, n (%)
Neutropenia				
1–2	10 (12)	5 (56)	0 (0)	0 (0)
3–4	19 (25)	2 (22)	1 (50)	0 (0)
Febrile neutropenia				
1–2	0 (0)	0 (0)	0 (0)	0 (0)
3–4	3 (4)	0 (0)	0 (0)	1 (50)
Anemia				
1–2	72 (94)	7 (78)	2 (100)	0 (0)
3–4	2 (3)	2 (22)	0 (0)	1 (50)
Thrombocytopenia				
1–2	18 (23)	4 (44)	0 (0)	1 (50)
3–4	3 (4)	2 (22)	0 (0)	1 (50)
Nausea				
1–2	28 (36)	3 (33)	0 (0)	2 (100)
3–4	5 (7)	0 (0)	0 (0)	0 (0)
Vomiting				
1–2	4 (5)	1 (11)	0 (0)	0 (0)
3–4	4 (5)	0 (0)	0 (0)	0 (0)
Diarrhea				
1–2	15 (20)	2 (22)	0 (0)	0 (0)
3–4	7 (10)	0 (0)	1 (50)	1 (50)
Mucositis				
1–2	1 (1)	1 (11)	0 (0)	0 (0)
3–4	0 (0)	0 (0)	0 (0)	0 (0)
Sensory neuropathy				
1–2	24 (31)	3 (33)	0 (0)	0 (0)
3–4	5 (7)	0 (0)	0 (0)	0 (0)
Motor neuropathy				
1–2	0 (0)	0 (0)	0 (0)	0 (0)
3–4	2 (3)	0 (0)	0 (0)	0 (0)
Increased creatinine				
1–2	2 (3)	1 (11)	0 (0)	0 (0)
3–4	0 (0)	0 (0)	0 (0)	0 (0)

^aToxicities were graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. None of the patients developed abnormal liver function tests or experienced grade 5 toxicity. The number of patients who received each chemotherapy regimen was: C + T = 77, C + G = 9, C + E = 2 and Cis + E = 2.

C: Carboplatin; Cis: Cisplatin; E: Etoposide; G: Gemcitabine; T: Paclitaxel.

In addition to drug-metabolizing enzymes, several SNPs in drug transporters (*ABCG2* and *ABCB1*) were associated with nausea and thrombocytopenia, respectively. These drug transporters have been implicated in efflux of either platinating agents or taxanes. The *ABCG2* missense SNP rs2231142 results in a

Gln141Lys change; which has been associated with lower expression [28] as well as with reduced drug efflux capacity of *ABCG2* [29]. Cell lines that are resistant to cisplatin have been shown to express higher levels of the copper transporters *ATP7A* and *ATP7B* [6]. *ATP7A* has been implicated in sequestering both

Table 4. Association of SNPs with progression-free survival after adjusting for covariates (performance status and stage).

SNP	Gene	Major/ minor allele	MAF	p-value, additive model (dominant model)	Hazard ratio, additive model (dominant model)	95% CI, additive model (dominant model)
rs1800566 (missense)	<i>NQO1</i>	C/T	0.22	0.021 (0.037)	1.615 (1.689)	0.089 to 0.870 (0.040 to 1.009)
rs10158985 (intron)	<i>TMEM63A</i>	C/A	0.25	0.032 (0.025)	0.655 (0.592)	-0.821 to -0.025 (-0.987 to -0.062)
rs246240 (intron)	<i>ABCC1</i>	A/G	0.13	0.037 (0.072)	0.640 (0.617)	0.409 to 1.001 (-1.026 to 0.062)
rs2238476 (intron)	<i>ABCC1</i>	C/T	0.06	0.046 (0.037)	1.972 (2.323)	-0.894 to 0.001 (0.120 to 1.565)
rs1128503 (synonymous)	<i>ABCB1</i>	C/T	0.46	0.066 (0.019)	0.730 (0.541)	-0.652 to 0.022 (-1.112 to -0.117)
rs17718902 (intron)	<i>KCNC1</i>	A/G	0.39	0.069 (0.035)	1.354 (1.717)	-0.017 to 0.623 (0.032 to 1.048)
rs9312960	<i>CCDC127</i>			0.059 (0.042)	1.378 (1.620)	-0.003 to 0.645 (0.019 to 0.945)

Data are presented as: values from additive model, three group analysis (values from dominant model, variant carrier vs noncarrier).
MAF: Minimum allele frequency.

carboplatin and cisplatin in vesicles. Since SNPs in these transporters have not been evaluated for potential clinical impact, we performed an exploratory evaluation of SNPs in *ATP7A* and *ATP7B* and found that rs1801244 (valine to leucine change) in *ATP7B* is associated with nausea. The functional effect of the valine to leucine change resulting from this SNP remains to be determined.

Since platinating agents such as cisplatin and carboplatin form platinum–DNA adducts that are repaired by the nucleotide excision repair pathway, interpatient variation in DNA repair mechanism due to presence of SNPs in DNA damage/repair pathway genes may influence treatment outcome. Although none of the SNPs in the DNA repair pathway genes were associated with PFS or OS, significant correlations were observed with toxicities. A 3′-UTR SNP (rs3212986) and a coding SNP in *ERCC1* (rs11615; Asn118Asn) have been associated with reduced *ERCC1* mRNA/protein-expression levels, and lower *ERCC1* levels have been associated with better outcomes in NSCLC patients receiving platinating agent based chemotherapy [30,31]. Nevertheless, while multiple studies have evaluated these two *ERCC1* SNPs, results have been quite variable and remain inconclusive. A recent meta-analysis of 39 previously published studies on these two *ERCC1* SNPs in lung cancer patients demonstrated that the rs11615 SNP might be a potential biomarker for risk of developing lung cancer as well as a prognostic marker in

NSCLC patients treated with platinating agents [32]. In our dataset we did not observe significant association of these SNPs with toxicity of survival. We did observe a marginally increased risk of neutropenia with *ERCC4* intronic SNP and reduced risk of sensory neuropathy with *XPC* missense SNP rs2228001 (C>A; Gln939Lys). Though not extensively studied, rs2228001 has been shown to have a trend towards higher risk of ototoxicity in osteosarcoma patients treated with cisplatin [33].

Finally, in addition to candidate genes in drug pathways, we tested selected genes that have been identified by genome-wide association study (GWAS). GWAS analysis in colorectal cancer patients treated with 5-fluorouracil with or without oxaliplatin identified SNPs in *KCNC1* (rs17718902), *GTF2E1* (rs447978), *CCDC127* (rs9312960) or *TMEM63A* (rs10158985) to be associated with nausea, vomiting and drug-induced neuropathy [34]. In our study, an intronic SNP (rs447978) in the transcription factor *GTF2E1* was associated with reduced risk of nausea and neutropenia and an intronic SNP in *TMEM63* (rs10158985) was associated with neutropenia. Furthermore, SNPs in *KCL3* and *CCDC127* were associated with increased risk of sensory neuropathy and diarrhea, respectively. Although the functional relevance of these genes/SNPs is not known, future studies on functional characterization as well as validation of these SNPs are needed to confirm the results of the GWAS analysis.

Table 5. Association of SNPs with toxicity after adjusting for covariates (age and total number of chemotherapy cycles).

Toxicity	Gene	SNP	SNP function or location	p-value, log additive model (dominant model)	Odds ratio, log additive model (dominant model)	95% CI, log-additive model (dominant model)
Nausea	<i>ABCG2</i>	rs2231142	Missense	0.045 (0.045)	3.938 (4.052)	0.033 to 2.708 (0.031 to 2.768)
	<i>GTF2E1</i>	rs447978	Intron	0.024 (0.004)	0.409 (0.223)	-1.672 to -0.117 (-2.520 to -0.488)
	<i>ATP7B</i>	rs1801244	Missense	0.078 (0.027)	1.927 (4.626)	-0.073 to 1.384 (0.178 to 2.885)
Neutropenia	<i>GTF2E1</i>	rs447978	Intron	0.027 (0.039)	0.444 (0.357)	-1.531 to -0.093 (-2.01 to -0.053)
	<i>ERCC4</i>	rs744154	Intron	0.04 (0.01)	2.176 (3.697)	0.035 to 1.52 (0.308 to 2.307)
	<i>TMEM63A</i>	rs10158985		0.031 (0.09)	2.557 (2.307)	0.087 to 1.79 (-0.158 to 1.83)
Sensory neuropathy –		rs1347851 [†]		0.016 (0.023)	0.235 (0.225)	-2.625 to -0.27 (-2.773 to -0.208)
	<i>ABCG2</i>	rs13120400	Intron	0.027 (0.055)	0.271 (0.295)	-2.467 to -0.146 (-2.465 to 0.024)
	<i>KLC3</i>	rs13181		0.073 (0.021)	2.27 (5.893)	-0.076 to 1.715 (0.273 to 3.274)
	<i>XPC</i>	rs2228001	Missense	0.096 (0.045)	0.456 (0.296)	-1.71 to 0.14 (-2.406 to -0.03)
Diarrhea	<i>CCDC127</i>	rs9312960_	5'-UTR	0.019 (0.09)	2.429 (2.434)	0.149 to 1.626 (-0.138 to 1.918)
	<i>CYP2C8</i>	rs11572080	Missense	0.017 (0.017)	4.396 (4.396)	0.26 to 2.702 (0.26 to 2.702)
	<i>TP53</i>	rs1625895	Intron	0.02 (0.023)	3.004 (3.633)	0.174 to 2.026 (0.179 to 2.401)
Thrombocytopenia	<i>ABCB1</i>	rs2235015		0.074 (0.04)	2.605 (3.467)	-0.093 to 2.004 (0.058 to 2.428)
		rs17098912 [†]		0.072 (0.027)	2.212 (3.115)	-0.071 to 1.659 (0.131 to 2.142)

Data are presented as: values from additive model, three group analysis (values from dominant model, variant carrier vs noncarrier).

[†]SNPs not in a gene, these were selected from genome-wide association study.

Conclusion

In conclusion, we identified SNPs within key candidate drug pathway genes that are independently predictive of PFS and/or major hematological and non-hematological toxicities in patients with advanced NSCLC treated with platinating agent based chemotherapy. There is a paucity of predictive markers that can be used to guide clinical decisions for such patients. Our results confirm some previously reported associations, but more importantly identify several new candidates that warrant testing in prospective studies, that could contribute towards

the development of personalized medicine. Indeed, a recent Phase II trial demonstrated that response rates are higher when chemotherapy is selected based on SNPs in *ERCC1* and *RRM1* in patients with advanced NSCLC [35], suggesting that incorporation of pharmacogenomic biomarkers into clinical decision-making has considerable potential for improving therapeutic outcomes.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Lung carcinoma is the most common malignancy and the leading cause of cancer deaths worldwide.
- The genetic make up of individual patients in addition to other clinical factors can influence the likelihood of benefit from and tolerability of chemotherapy.
- We evaluated SNPs in candidate genes of relevance to platinating agents and paclitaxel for association with survival and toxicity in non-small-cell lung cancer patients.
- After adjusting for covariates, SNPs in drug transporters and *NQO1* were associated with progression-free survival and SNPs in drug transporters as well as in the DNA repair pathway genes *ERCC4* and *XPC* were associated with toxicity.
- Comprehensive evaluation of the genetic variants in conjunction with known prognostic factors may help optimize therapeutic decisions to maximize benefit and minimize toxicity in non-small-cell lung cancer patients.

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