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Lung Cancer

Association Between *CASP8* and *CASP10* Polymorphisms and Toxicity Outcomes With Platinum-Based Chemotherapy in Chinese Patients With Non-Small Cell Lung Cancer

Ji Qian,[a,i] Hui-Qi Qu,[b] Lixin Yang,[c] Ming Yin,[i,j] Qiming Wang,[i] Shaohua Gu,[a] Qihan Wu,[d] Xueying Zhao,[a] Wenting Wu,[a,k] Junjie Wu,[a,c] Xiaoming Tan,[f,g] Wenqing Chen,[h] Haijian Wang,[a] Jiucun Wang,[a] Weiwei Fan,[a] Hongyan Chen,[a] Baohui Han,[e] Daru Lu,[a] Qingyi Wei,[i] Li Jin[a]

[a]State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Fudan Taizhou Institute of Health Sciences, Fudan University, Shanghai, China; [b]Division of Epidemiology, Human Genetics and Environmental Sciences, Brownsville Regional Campus, The University of Texas School of Public Health, Brownsville, Texas, USA; [c]Department of Cardiothoracic Surgery and Pneumology, Changhai Hospital of Shanghai, Second Military Medical University, Shanghai, China; [d]School of Life Science, East China Normal University, Shanghai, China; [e]Department of Respiratory Disease, Shanghai Chest Hospital, Shanghai Jiaotong University, Shanghai, China; [f]Department of Respiratory Disease, Changzheng Hospital, Second Military Medical University, Shanghai, China; [g]Department of Respiratory Disease, Renji Hospital, Shanghai Jiaotong University, Shanghai, China; [h]Department of Respiratory Disease, Jilin Province Cancer Hospital, Changchun, China; [i]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA; [j]Department of Internal Medicine, Geisinger Medical Center, Philadelphia, Pennsylvania, USA; [k]Beyster Center for Genomics of Psychiatric Diseases, Department of Psychiatry, University of California, San Diego, La Jolla, California, USA

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Abstract

Caspase-8 and caspase-10 play crucial roles in both cancer development and chemotherapy efficacy. In this study, we aimed to comprehensively assess single nucleotide polymorphisms (SNPs) of the caspase-8 (*CASP8*) and caspase-10 (*CASP10*) genes in relation to toxicity outcomes with first-line platinum-based chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). We genotyped 13 tag SNPs of *CASP8* and *CASP10* in 663 patients with advanced NSCLC treated with platinum-based chemotherapy regimens. Associations between SNPs and chemotherapy toxicity outcomes were identified in a discovery set of 279 patients and then validated in an independent set of 384 patients. In both the discovery and validation sets, variant homozygotes of *CASP8* rs12990906 and heterozygotes of *CASP8* rs3769827 and *CASP10* rs11674246 and rs3731714 had a significantly lower risk for severe toxicity overall. However, only the association with the rs12990906 variant was replicated in the validation set for hematological toxicity risk. In a stratified analysis, we found that some other SNPs, including

Correspondence: Daru Lu, Ph.D., State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Science, Fudan University, Handan Road, Shanghai 200433, China. Telephone: 86-21-65642799; Fax: 86-21-65642799; e-mail: drlu@fudan.edu.cn and Li Jin, State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Science, Fudan University, Handan Road, Shanghai 200433, China. Telephone: 86-21-55664474; Fax: 86-21-55665277; e-mail: lijin.fudan@gmail.com Received November 29, 2011; accepted for publication April 15, 2012; first published online in ***The Oncologist** Express* on July 27, 2012. 1083-7159/2012/$20.00/0 http://dx.doi.org/10.1634/theoncologist.2011-0419

rs3769821, rs3769825, rs7608692, and rs12613347, were significantly associated with severe toxicity risk in some subgroups, such as in nonsmoking patients, patients with adenocarcinoma, and patients treated with cisplatin combinations. Consistent results were also found in haplotype analyses. Our results provide novel evidence that polymorphisms in *CASP8* and *CASP10* may modulate toxicity outcomes in patients with advanced NSCLC treated with platinum-based chemotherapy. If validated, the findings will facilitate the genotype-based selection of platinum-based chemotherapy regimens. ***The Oncologist*** 2012;17:1551–1561

Introduction

Lung cancer is the leading cause of cancer death worldwide, and the incidence of lung cancer continues to increase in China [1, 2]. Non-small cell lung cancer (NSCLC), including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, is the major histological type of lung cancer [3], accounting for ~80% of lung cancer deaths [4], and most cases are diagnosed at advanced stages (III or IV) and cannot be surgically resected.

Platinum (cisplatin or carboplatin) double-agent chemotherapy is the most common first-line treatment for patients with advanced NSCLC at present, and the efficacies of different combinations have been demonstrated to be similar in series of trials in unselected patients, with response rates of 30%–40% [5–7]. Standard treatment using platinum compounds induces both intrastrand and interstrand DNA adducts that result in bulky distortion of DNA and activates apoptosis signaling pathways, leading to cancer cell death [8, 9]. Nonetheless, the cytotoxic effects of platinum drugs may produce severe adverse effects, by damaging normal cells, which may hinder further treatment and impact outcomes. Differences in toxicities experienced and responses in patients who have received the same chemotherapy agent or regimen are commonly observed [10, 11], and these are likely to be a result of polymorphic genetic variation in genes involved in drug metabolism and DNA repair and apoptosis [5, 12]. Recently, numerous clinical studies have elucidated that genes involved in drug transportation, drug metabolism, DNA repair, and apoptosis may modulate platinum-based chemotherapeutic efficacy and drug-related toxicity outcomes [13, 14]. Therefore, the identification of genetic markers may enable us to predict the toxicity outcome with platinum-based chemotherapy and select optimal regimens for personalized therapy.

Caspase proteins are the central components of the apoptotic response [15, 16] and thus are widely recognized to play a crucial role in cancer development and host response to cancer chemotherapy. In the caspase family, caspase-8 and caspase-10 are the initiators and form the death-inducing signaling complex to activate effector caspases (e.g., caspase-3, caspase-6, and caspase-7) by mediating the death signal receptor and/or other signals, including the cytotoxicity of therapeutic regimens [17, 18]. Indeed, several prior basic and clinical studies have suggested that caspases (e.g., caspase-8 and caspase-10) are involved in p53-dependent and p53-independent transcriptional responses in cisplatin-induced apoptosis and are related to the clinical outcomes (including toxicity) of lung cancer patients treated with chemotherapy [19–21]. In addition to previous studies that demonstrated that caspases may play an important role in the etiology of multiple malignancies [22–25], it has also been documented that the gene-expression signatures (including expression levels of the genes encoding caspase-8 [*CASP8*] and caspase-10 [*CASP10*]) may be related to the survival rate of patients with stage I NSCLC [26]. In cancer treatment, apoptosis is also the main mechanism of therapeutic effects involving caspases [27]. Down-regulation or inhibition of caspase activity often results in resistance of human cancers to chemotherapeutics [28, 29]. Our previous study indicated that three functional candidate single nucleotide polymorphisms (SNPs) (rs3834129, promoter, −652 6 N ins>del; rs13006529, Ile522Leu, A>T; and rs3900115, Ser59Ser, A>G) of *CASP8* and *CASP10* may be significantly associated with drug response and severe toxicity in subgroups of NSCLC patients (manuscript under review). We believe that it is possible that genetic variants of *CASP8* and *CASP10* may modulate cell death and affect cancer development and treatment effects or drug-related toxicity.

Currently, there is still a lack of comprehensive studies on the association between *CASP8* and *CASP10* polymorphisms and chemotherapy toxicity. To expand our previous study, we genotyped 13 tag SNPs at the *CASP8* and C*ASP10* loci in 663 Chinese patients with advanced NSCLC. To minimize type I errors, we performed a two-stage analysis in which the significant associations identified in the first discovery set were further validated in an independent validation set.

Materials and Methods

Study Design and Patient Recruitment

In total, 663 Han Chinese patients with newly histopathologically diagnosed stage IIIA–IV NSCLC in Shanghai in March 2005 to January 2010 were included in this analysis. Of these 663 patients, 279 patients from two participating Hospitals (Zhongshan and Changhai) were used as the discovery set, and an additional 384 patients from another hospital (Shanghai Chest Hospital) were used as the validation set. The study protocol was approved by the Ethical Review Committee of Fudan University and the participating hospitals, and written informed consent was obtained from each individual. The recruitment criteria and clinical data collection were described in our previous report elsewhere [10]. Patient blood samples were collected in ethylenediaminetetraacetic acid tubes and stored at −80°C for later DNA extraction. The research assistants who performed the genotyping assays were blinded to the case–control status and the clinical investigators were blinded to the genotype status of the patients when they scored chemotherapy toxicity, including overall toxicity, gastrointestinal toxicities (nausea and vomiting), and hematologic toxicities (leukocytopenia, neutropenia, amemia, and thrombocytope-

nia) according to the National Cancer Institution Common Toxicity Criteria version 3.0 [30]. The incidence of grade 3 or 4 toxicity was assessed twice a week during chemotherapy.

Chemotherapy Regimens

All patients enrolled in this study were inoperable and had received first-line platinum-based chemotherapy (definitive chemoradiotherapy was excluded), that is, cisplatin (75 mg/m^2) or carboplatin (area under the concentration–time curve of 5), administered on day 1 every 3 weeks, in combination with navelbine (25 mg/m^2) on days 1 and 8 every 3 weeks, gemcitabine (1,250 mg/m^2) on days 1 and 8 every 3 weeks, paclitaxel (175 mg/m^2) on day 1 every 3 weeks, or docetaxel (75 mg/m^2) on day 1 every 3 weeks. All chemotherapeutic drugs were administered i.v., and each patient was treated for two to six cycles.

SNP Selection and Genotyping

Common tag SNPs of two apoptotic initiator genes (*CASP8* and *CASP10*) were included in the present study on the basis of biological interactions between these two caspases in the extrinsic apoptosis pathway. The genotyping information of the Han Chinese population was acquired from the HapMap phase II study [31], and tag SNPs with a minor allele frequency (MAF) $\geq$0.05 and r^2 value $<$0.8 were selected using Haploview software ()[32]. Genomic DNA was extracted using the QIAamp DNA Maxi Kit (Qiagen GmbH, Hilden, Germany). All selected SNPs were genotyped using a customized iSelect HD BeadChip (Illumina, Inc., San Diego, CA). Quality control criteria included a genotyping call rate by SNP $>$0.95 and a GenCall score $>$0.2. Each of 13 tag SNPs passed the quality filters. Concordance among the genotyping replicates was $>$99.9%. Genotyping information is shown in supplemental online Table S1. GenomeStudioV2010.1 (Illumina, Inc., San Diego, CA) and GeneMapper version 4.0 (Applied Biosystems, Foster City, CA) were used to analyze the BeadChip data and generate genotyping reports.

Statistical Analysis

Two-phase screening was performed to investigate associations between *CASP8* and *CASP10* SNPs and the incidence of severe chemotherapy toxicity for all patients and for subgroups by treatment regimen (platinum plus a DNA-damaging agent and platinum plus a microtubule-targeting agent). Because of the relatively small sample size for each regimen, a dominant model was assumed for the genotypic association test for each SNP. Toxicity outcomes were dichotomized by the presence or absence of (a) any grade 3 or 4 toxicity, (b) any grade 3 or 4 hematologic toxicity (leukocytopenia, neutropenia, anemia, and thrombocytopenia), and (c) any grade 3 or 4 gastrointestinal toxicity (nausea and vomiting). Hardy–Weinberg equilibrium (HWE) was checked using the χ^2 goodness-of-fit test. The association between each genetic variant or haplotype and grade 3 or 4 toxicity was estimated by the odds ratio (OR) and its 95% confidence interval (CI), using unconditional logistic regression with adjustment for age (age at diagnosis in cases), sex, performance status, stage, histological type, and treatment regimen. Pairwise linkage disequilibrium (LD) among SNPs was examined using D′ and r^2 values, and haplotype blocks were defined using the four-gamete rule with Haploview Software. Individual haplotype frequency was estimated based on the Bayesian algorithm using the PHASE 2.0 program (version 2.0.2) (University of Washington, Seattle, WA) [33]. The haplotype–toxicity association was tested for each LD block using unconditional logistic regression with the same adjustment as mentioned above.

As shown in Figure 1, in the first discovery phase, 13 SNPs were examined for their associations with toxicity outcomes and different levels of interaction with treatment regimen for all 279 and 224 subgroup patients who received a platinum plus a DNA-damaging agent (i.e., paclitaxel) or a platinum plus a microtubule-targeting agent (i.e. gemcitabine), respectively. In the second validation phase, SNPs that had p-values $<$ .10 by the trend test or genotype test for associations with toxicity and p-values $<$ .20 for an interaction with regimen were subjected to confirmation for 384 and 131 subgroup patients who received paclitaxel and gemcitabine, respectively. Finally, SNPs that had p-values $<$ .10 for association with toxicity outcomes and p-values $<$ .20 for an interaction with regimen in patients in the validation set were further subjected to the combined analyses for all 663 and 355 patients.

Reported p-values are two sided for phase I and one sided for phase II. A p-value $<$ .05 was defined as statistically significant, whereas a p-value $<$ .1 was considered marginal. For multiple statistical testing, the Bonferroni correction was used. Unless otherwise specified, all statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC).

Results

All 663 patients had stage III or IV NSCLC and received two to six cycles of first-line platinum-based chemotherapy. The main patient characteristics and toxicity events are summarized in Table 1 for both the discovery and validation sets. Each of the 13 SNPs met HWE ($p > .05$). The SNP call rate and sample call rate were $>$95% and the GenCall score was $>$0.2. The observed allele frequency was consistent with that previously reported in the HapMap except for three SNPs (two in *CASP8* and one in *CASP10*) that did not meet the quality filter of a MAF $\geq$0.05, and these were excluded from further analyses (supplemental online Table S1). No significant associations between polymorphisms and patient characteristics were observed (data not shown).

Chemotherapy Toxicity by Clinical Characteristics of NSCLC Patients

We stratified patients by age, sex, performance status, stage, smoking status, histopathologic type, and chemotherapy regimen and assessed whether or not clinical variables contributed to chemotherapy toxicity (gastrointestinal toxicity, hematologic toxicity, overall toxicity) using unconditional logistic regression (Table 1). Smoking status and sex were found to be associated with toxicity outcomes. For example, compared with men, women had higher incidences of gastrointestinal toxicity and overall toxicity ($p < .001$ and $p = .016$, respec-

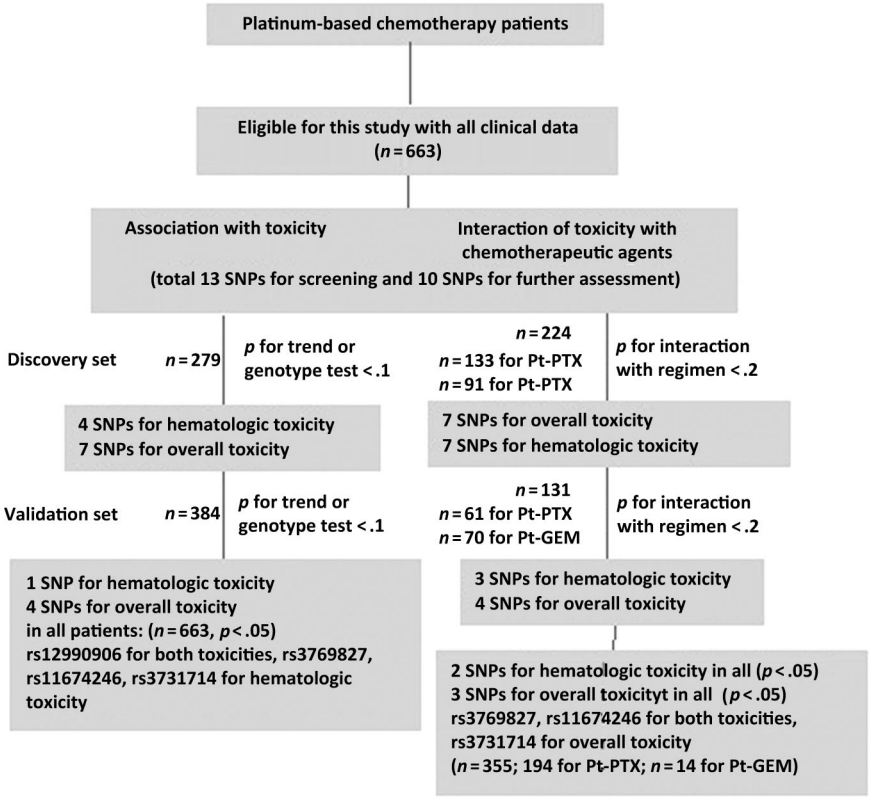

Figure 1. Patients and strategy, including selection of eligible cases and a two-phase screening of SNPs associated with toxicity of platinum-based chemotherapy.

Abbreviations: GEM, gemcitabine; Pt, platinum; PTX, paclitaxel; SNP, single nucleotide polymorphism.

tively), and compared with nonsmokers, ever-smokers had a higher incidence of gastrointestinal toxicity ($p < .001$). Also, patients who were treated with different chemotherapy regimens had varied toxicity outcomes ($p_{trend} = .013$ for hematologic toxicity and $p_{trend} = .001$ for overall toxicity during platinum-based therapy).

Association Between *CASP8* and *CASP10* Polymorphisms and Grade 3 or 4 Toxicity

Analysis of Discovery Set

Associations between genetic variants and chemotherapeutic toxicities were assessed using unconditional logistic regression with adjustment for age, sex, performance status, tumor–node–metastasis (TNM) score, histopathologic type, and chemotherapy regimen. In the discovery phase, four SNPs were found to be associated with hematologic toxicity and seven SNPs were associated with overall toxicity ($p < .10$ by the trend test or genotype test) (Fig. 1, Table 2, and supplemental online Table S2), but none of these SNPs was associated with gastrointestinal toxicity.

Analysis of Validation Set

The associations for one SNP with hematologic toxicity and four SNPs with overall toxicity were reproduced using the aforementioned criteria ($p < .10$ by the trend test or genotype test) (Fig. 1, Table 2, and supplemental online Table S2) in the remaining 384 patients in the validation set (Table 1). All these five SNPs were associated with the risk for grade 3 or 4 toxicity in the 663 patients overall ($p < .01$).

In All 663 Patients

For the *CASP8* rs12990906 SNP, patients with the variant allele had a lower incidence of severe hematologic toxicity (incidence rate, 24 of 140 versus 60 of 187; adjusted OR, 0.45; 95% CI, 0.26–0.78; $p = .004$, $p_{trend} = .004$). A consistently lower risk for severe toxicity overall was also observed in homozygous variant carriers of rs12990906 (26.4%; $p = .002$), compared with homozygous carriers of the common allele (37.9%), and the toxicity risk was also lower with higher numbers of minor alleles ($p = .002$ by the trend test). Heterozygotes of the *CASP10* rs11674246 SNP also exhibited a lower incidence of toxicity overall (incidence rate, 50 of 215 versus 142 of 394; adjusted OR, 0.52; 95% CI, 0.35–0.77; $p = .001$; OR, 0.54; 95% CI, 0.37–0.78; $p = .001$ under the dominant model; $p_{trend} = .004$), and similar results were observed for the *CASP8* rs3769827 and *CASP10* rs3731714 SNPs (incidence rate, 57 of 226 versus 138 of 384; $p = .004$ for heterozygotes of rs3769827; incidence rate, 46 of 199 versus 146 of 416; $p = .002$ for heterozygotes of rs3731714), and the association remained significant after Bonferroni correction (i.e., $< \alpha = 0.05/10 = 0.005$).

Table 1. Patient demographics and their association with chemotherapy toxicity outcomes ($n = 663$)

Patient characteristic	All *n* (%)	Discovery set *n* (%)	Validation set *n* (%)	Overall toxicity event (*p*)	Hematologic toxicity event (*p*)	Gastrointestinal toxicity event (*p*)
Median age, yrs, $n = 663$	58	59	58			
Range	26–80	26–80	28–80			
≤58	334 (50.4)	139 (49.8)	195 (50.8)	90 (reference)	75 (reference)	23 (reference)
>58	329 (49.6)	140 (50.2)	189 (49.2)	110 (.098)	91 (.132)	35 (.096)
Gender						
Male	465 (70.1)	192 (68.8)	273 (71.1)	126 (reference)	110 (reference)	25 (reference)
Female	198 (29.9)	87 (31.2)	111 (28.9)	74 (.016)	56 (.216)	33 (<.001)
Performance status score, $n = 660$						
0–1	606 (91.8)	236 (88.4)	361 (94.3)	186 (reference)	155 (reference)	52 (reference)
2	54 (8.2)	32 (11.6)	22 (5.7)	14 (.647)	11 (.479)	6 (.478)
Smoking status, $n = 663$						
Nonsmokers	277 (41.8)	119 (42.7)	158 (41.1)	93 (reference)	74 (reference)	36 (reference)
Ever-smokers	386 (58.2)	160 (57.4)	226 (58.9)	106 (.102)	91 (.347)	22 (<.001)
TNM stage, $n = 661$						
IIIA	49 (7.4)	22 (7.9)	27 (7.0)	16 ($p_{trend} = .924$)	13 ($p_{trend} = .925$)	5 ($p_{trend} = .934$)
IIIB	189 (28.6)	72 (26.0)	117 (30.5)	57 (.692)	46 (.868)	16 (.748)
IV	423 (64.0)	183 (66.1)	240 (62.5)	127 (.945)	107 (.748)	37 (.903)
Histological type, $n = 658$						
Adenocarcinoma	430 (65.4)	188 (67.4)	242 (63.9)	131 ($p_{trend} = .515$)	111 ($p_{trend} = .942$)	36 ($p_{trend} = .622$)
Squamous cell	141 (21.4)	71 (25.5)	70 (18.5)	38 (.424)	32 (.870)	11 (.360)
Adenosquamocarcinoma	13 (2.0)	1 (0.4)	12 (3.2)	5 (.253)	3 (.595)	2 (.391)
Other[a]	74 (11.3)	19 (6.8)	55 (14.5)	26 (.552)	20 (.883)	9 (.640)
Chemotherapy regimen						
Platinum plus vinorelbine	215 (32.4)	16 (5.7)	199 (51.8)	73 ($p_{trend} = .007$)	62 ($p_{trend} = .013$)	22 ($p_{trend} = .212$)
Platinum plus gemcitabine	152 (22.9)	91 (32.6)	61 (15.9)	49 (.407)	40 (.143)	16 (.670)
Platinum plus paclitaxel	203 (30.6)	133 (47.7)	70 (18.2)	44 (.695)	37 (.307)	5 (.674)
Platinum plus docetaxel	54 (8.1)	24 (8.6)	30 (7.8)	23 (.308)	20 (.998)	5 (.067)
Or						
Cisplatin combination	375 (56.5)	150 (53.8)	225 (58.6)	117 (reference)	95 (reference)	38 (reference)
Carboplatin combination	199 (30.0)	98 (35.1)	101 (26.3)	49 (.057)	43 (.292)	7 (.006)
Other platinum combination	89 (13.4)	31 (11.1)	58 (15.1)	34 (.182)	28 (.217)	13 (.228)
Toxicity outcomes						
Grade 3 or 4 toxicity, $n = 637$	200 (31.4)	277 63 (22.7)	360 137 (38.1)			
Grade 3 or 4 hematologic toxicity, $n = 646$	166 (25.7)	276 48 (17.3)	370 118 (31.8)			
Leukocytopenia, $n = 651$	102 (15.7)	275 17 (6.2)	376 85 (22.6)			
Agranulocytosis, $n = 620$	84 (13.5)	268 33 (12.3)	352 51 (14.5)			
Anemia, $n = 628$	16 (2.5)	268 2 (0.75)	360 14 (3.9)			
Thrombocytopenia, $n = 631$	26 (4.1)	267 6 (2.2)	364 20 (5.5)			
Grade 3 or 4 gastrointestinal toxicity						
Nausea/vomiting, $n = 642$	58 (9.0)	276 24 (8.7)	366 34 (9.2)			

[a]Other carcinomas include mixed cell, neuroendocrine carcinoma, and undifferentiated carcinoma.
Abbreviations: CI, confidence interval; OR, odds ratio; TNM, tumor–node–metastasis.

Although carriers of the *CASP8* rs3769827 *AG* and rs3769825 *AG* genotypes seemed to have a significantly lower risk for severe gastrointestinal toxicity in all 663 patients ($p = .028$ and $p = .013$, respectively) (supplemental online Table S2), but they did not show this consistently in both the discovery and validation phases. A similar situation occurred for the *CASP8* rs3769825 variant regarding overall toxicity and the *CASP8* rs3769827, *CASP10* rs11674246,

Table 2. Association between *CASP8* and *CASP10* polymorphisms and grade 3 or 4 chemotherapy toxicity

		Patients		Discovery set		Patients		Validation set		Patients		All	
Toxicity	**Gene/variable**	***n***	**Event**	**Adjusted OR[a] (95% CI)**	***p*[a]**	***n***	**Event**	**Adjusted OR[a] (95% CI)**	***p*[a]**	***n***	**Event**	**Adjusted OR[a] (95% CI)**	***p*[a]**
Hematologic toxicity	*CASP8* rs12990906												
	AA	88	21	1.00 (reference)	.022[b]	99	39	1.00 (reference)	.067[b]	187	60	1.00 (reference)	.004[b,c]
	AG	126	23	0.84 (0.42–1.69)	.631	195	59	0.66 (0.39–1.10)	.108	321	82	0.73 (0.49–1.10)	.132
	GG	63	4	0.25 (0.08–0.77)	.016	77	20	0.56 (0.29–1.08)	.084	140	24	0.45 (0.26–0.78)	.004[c]
	AG + GG	189	27	0.61 (0.32–1.19)	.148	272	79	0.63 (0.39–1.02)	.062	461	106	0.64 (0.44–0.94)	.023
	AA + AG	214	44	0.27 (0.09–0.80)	.018	294	98	0.73 (0.41–1.30)	.291	508	142	0.55 (0.34–0.89)	.015
Overall toxicity	*CASP8* rs3769827												
	AA	159	41	1.00 (reference)	.081[b]	224	97	1.00 (reference)	.007[b]	383	138	1.00 (reference)	.001[b,c]
	AG	107	21	0.68 (0.36–1.26)	.215	119	36	0.55 (0.34–0.89)	.014	226	57	0.58 (0.40–0.84)	.004[c]
	GG	11	1	0.23 (0.03–1.94)	.176	17	4	0.40 (0.12–1.27)	.120	28	5	0.37 (0.14–1.01)	.052
	AA + AG	118	22	0.62 (0.34–1.15)	.129	136	40	0.53 (0.33–0.84)	.007	254	62	0.55 (0.38–0.80)	.001[c]
	CASP8 rs12990906												
	AA	88	26	1.00 (reference)	.018[b]	94	43	1.00 (reference)	.042[b]	182	69	1.00 (reference)	.002[b,c]
	AG	127	31	0.93 (0.49–1.78)	.830	189	71	0.70 (0.42–1.17)	.172	316	102	0.79 (0.53–1.16)	.226
	GG	62	6	0.27 (0.10–0.73)	.010	77	23	0.52 (0.27–0.99)	.045	110	29	0.43 (0.26–0.73)	.002[c]
	AG + GG	189	37	0.67 (0.36–1.23)	.193	266	94	0.64 (0.40–1.05)	.076	426	131	0.66 (0.46–0.96)	.031
	AA + AG	215	57	0.28 (0.11–0.72)	.008	283	114	0.65 (0.37–1.14)	.130	498	171	0.50 (0.32–0.80)	.003[c]
	CASP10 rs11674246												
	GG	160	42	1.00 (reference)	.116[b]	234	100	1.00 (reference)	.020[b]	394	142	1.00 (reference)	.004[b,c]
	GA	105	18	0.54 (0.28–1.04)	.065	110	32	0.53 (0.32–0.87)	.013	215	50	0.52 (0.35–0.77)	.001[c]
	AA	12	3	0.74 (0.18–3.07)	.673	16	5	0.60 (0.20–1.83)	.372	28	8	0.68 (0.29–1.63)	.390
	GA + AA	117	21	0.56 (0.30–1.05)	.069	126	37	0.54 (0.34–0.87)	.011	243	58	0.54 (0.37–0.78)	.001[c]
	CASP10- rs3731714												
	GG	172	44	1.00 (reference)	.171[b]	244	102	1.00 (reference)	.051[b]	416	146	1.00 (reference)	.016[b]
	GA	97	16	0.52 (0.27–1.02)	.056	102	30	0.55 (0.33–0.91)	.021	199	46	0.53 (0.35–0.78)	.002[c]
	AA	8	3	1.13 (0.25–5.26)	.872	14	5	0.77 (0.25–2.40)	.655	22	8	0.98 (0.40–2.43)	.966
	GA + AA	105	19	0.57 (0.30–1.07)	.081	116	35	0.57 (0.35–0.93)	.024	221	54	0.57 (0.39–0.83)	.003[c]

[a]Data were calculated using unconditional logistic regression, adjusted by gender, age at diagnosis, performance status, and type of treatment regimen.
[b]p_{trend}.
[c]Significance remained after Bonferroni correction.
Abbreviations: CI, confidence interval; OR, odds ratio.

and *CASP10* rs3731714 variants regarding hematologic toxicity.

No significant associations ($p < .01$) were found between *CASP8* and *CASP10* gene polymorphisms and the risk for severe leukocytopenia or thrombocytopenia (data not shown).

Stratified Analysis of Association Between *CASP8* and *CASP10* Polymorphisms and Grade 3 or 4 Toxicity

Paclitaxel (203 patients) and gemcitabine (152 patients) were commonly used drugs in the platinum-based regimens of our study among different centers (Table 1). Thus, we looked into differences in toxicity outcomes between these two regimens by genotype.

For overall toxicity, seven of the 10 SNPs met the criteria of $p < .20$ for an interaction with these two regimens (Fig. 1 and supplemental online Table S3) in the 224 patients in the discovery set with the paclitaxel (133 patients) and gemcitabine (91 patients) regimens. In the validation set, four SNPs reproducibly had a p-value $< .20$, and in all 355 patients only minor allele carriers of *CASP8* rs3769827 still showed a statistically significant protective effect for gemcitabine (incidence rate, 17.6%; OR, 0.29; 95% CI, 0.13–0.69; $p = .005$ under a dominant model) (Table 3). The association was mar-

Table 3. Stratified analysis of association between *CASP8* and *CASP10* polymorphisms and grade 3 or 4 chemotherapy toxicity

		Patients		Overall toxicity				Patients		Hematologic toxicity	
Gene/variable	**Subgroup**	***n***	**Event**	**Adjusted OR[a] (95% CI)**	***p*[a]**	**Gene/variables**	**Subgroups**	***n***	**Event**	**Adjusted OR[a] (95% CI)**	***p*[a]**
CASP8	Chemotherapy				*CASP8*	Chemotherapy					
rs3769827	Regimen	51	9	0.29 (0.13–0.69)	.005[b]	rs3769827	Regimen	52	7	0.31 (0.12–0.78)	.013
AG + GG	Pt plus gemcitabine				*AG + GG*	Pt plus gemcitabine					
CASP8	Histological type				*CASP10*	Chemotherapy					
rs3769825		51	8	0.26 (0.09–0.71)	.009	rs11674246	Regimen	51	8	0.39 (0.16–0.96)	.039
AG + GG	Squamous cell				*GA + AA*	Pt plus gemcitabine					
CASP8					*CASP10*	Histological type					
rs3769827	Smoking status	106	26	0.40 (0.22–0.71)	.002[b]	rs11674246		159	30	0.53 (0.32–0.86)	.010
AG + GG	Nonsmoker				*GA + AA*	Adenocarcinoma					
CASP8					*CASP10*						
rs3769821	Smoking status	123	53	2.19 (1.27–3.76)	.005[b]	rs11674246	Gender	172	31	0.53 (0.33–0.86)	.010
AG + GG	Nonsmoker				*GA + AA*	Male					
CASP10					*CASP8*						
rs11674246	Smoking status	106	26	0.42 (0.24–0.74)	.003[b]	rs7608692	TNM	206	40	0.50 (0.31–0.79)	.003[b]
GA + AA	Nonsmoker				*GA + AA*	TNM IV					
CASP10					*CASP10*						
rs3731714	Smoking status	99	25	0.45 (0.25–0.80)	.007	rs12613347	TNM	238	51	0.55 (0.35–0.86)	.009
GA + AA	Nonsmoker				*GA + AA*	TNM IV					
CASP8					*CASP8*	Chemotherapy					
rs3769825	TNM	79	18	0.42 (0.22–0.81)	.010	rs7608692	Regimen	95	22	0.53 (0.32–0.86)	.010
AG + GG	TNM III				*GA + AA*	Cisplatin combination					
CASP10					*CASP10*	Chemotherapy					
rs3731714	TNM	74	15	0.37 (0.19–0.73)	.004[b]	rs12613347	Regimen	217	45	0.53 (0.32–0.86)	.010
GA + AA	TNM III				*GA + AA*	Cisplatin combination					

[a]Stratified tests were estimated by unconditional logistic regression and adjusted for gender, age at diagnosis, performance status, with the wild-type homozygotes as reference group.
[b]Significance remained after Bonferroni correction.
Abbreviations: CI, confidence interval; OR, odds ratio; Pt., platinum; TNM, tumor–node–metastasis.

ginally significant after Bonferroni correction (i.e., $<\alpha = 0.05/10 = 0.005$).

For hematologic toxicity, three of seven SNPs remained significant according the criteria $p < .20$ for interaction after the two phases, and carriers of the minor alleles *CASP8* rs3769827 and *CASP10* rs11674246 had a lower risk for hematologic toxicity with gemcitabine in all 355 patients (incidence rate, 13.5%; $p = .013$ and incidence rate, 15.7%; $p = 0$ .039, respectively).

However, none of the four SNPs discovered in the first phase met our criteria of a p-value $< .20$ for gastrointestinal toxicity in the end (supplemental online Table S3).

We further performed stratified analyses for chemotherapy toxicity outcomes by other clinical characteristics. The results showed several evident associations between *CASP8* and *CASP10* polymorphisms and toxicity by sex, smoking status, TNM stage, and histopathologic type (Table 3). For example, minor allele carriers for both the rs11674246 and rs3731714 SNPs had a significant protective effect against overall toxicity (adjusted OR, 0.42; 95% CI, 0.24–0.74; $p = .003$ and adjusted OR, 0.45; 95% CI, 0.25–0.80; $p = .007$) in nonsmoking patients, and the same effect against hematological toxicity was found in patients with adenocarcinoma (adjusted OR, 0.53; 95% CI, 0.32–0.86; $p = .010$ for rs3731714). For rs12613347, minor allele carriers also had a protective effect against hematological toxicity in patients treated with cisplatin combinations (adjusted OR, 0.53; 95% CI, 0.32–0.86; $p = .010$).

Haplotype Analysis

The reconstructed LD plot of the *CASP8* and *CASP10* SNPs in 663 patients is shown in supplemental online Figure 1. *CASP8*

Table 4. CASP8 and 10 haplotypes and grade 3 or 4 chemotherapy toxicity

	Patients		Overall toxicity		Patients		Hematologic toxicity		Patients		Gastrointestinal toxicity	
Genes/haplotypes	*n*	Event	Adjusted OR[a] (95% CI)	p^a	*n*	Event	Adjusted OR[a] (95% CI)	p^a	*n*	Event	Adjusted OR[a] (95% CI)	p^a
CASP10												
G/G/G	558	204	1.00 (reference)	.002[b]	569	172	1.00 (reference)	.004[b]	567	57	1.00 (reference)	.646
G/G/A	445	130	0.71 (0.54–0.94)	.016	453	105	0.69 (0.51–0.92)	.010	452	38	0.84 (0.54–1.32)	.455
A/A/G	243	62	0.59 (0.42–0.83)	.003[b]	246	52	0.61 (0.43–0.88)	.008	243	20	0.78 (0.45–1.35)	.370
A/G/G	28	4	0.28 (0.09–0.85)	.024	28	3	0.27 (0.08–0.93)	.037	28	1	0.40 (0.05–3.05)	.376
CASP8-1												
A/G	627	227	1.00 (reference)	.001[b]	639	190	1.00 (reference)	.004[b]	636	66	1.00 (reference)	.093
A/A	365	106	0.73 (0.57–0.93)	.028	372	84	0.74 (0.57–0.95)	.015	370	33	0.86 (0.55–1.36)	.523
G/G	282	67	0.71 (0.55–0.93)	<.001[b]	285	58	0.79 (0.60–1.04)	.004[b]	284	17	0.53 (0.30–0.94)	.029
CASP8-2												
A/A/A/G	585	159	1.00 (reference)	.023	592	129	1.00 (reference)	.024	592	47	1.00 (reference)	.613
A/G/A/A	381	133	1.44 (1.08–1.93)	.012	391	110	1.41 (1.04–1.90)	.027	384	39	1.34 (0.85–2.13)	.211
G/A/C/A	270	96	1.53 (1.12–2.11)	.009	275	84	1.61 (1.16–2.25)	.004[b]	274	26	1.29 (0.77–2.16)	.341

[a]Data were calculated using unconditional logistic regression and adjusted for gender, age at diagnosis, performance status, and type of treatment regimen accordingly, with the majority haplotype as the reference group (*CASP10*, Block 1; *CASP8*-1, Block 2; *CASP8-2*, Block 3 in supplemental online Fig. S1).
[b]Significance remained after Bonferroni correction.
Abbreviations: CI, confidence interval; OR, odds ratio.

and *CASP10* have three blocks defined by the four-gamete rule in the Haploview Software, which were deduced using PHASE 2.1 software. Consistent with the results of the single SNP analysis, we found many evident correlations between haplotypes and severe overall toxicity in all three blocks of *CASP8* and *CASP10,* especially for block 1 of *CASP8* ($p_{trend} = 6.86 \times 10^{-4}$). Haplotypes *AA* and *GG* had a protective effect for severe toxicity overall, compared with the most common haplotype *AG* (OR, 0.73; 95% CI, 0.57–0.93; $p = .028$ and OR, 0.71; 95% CI, 0.55–0.93; $p = 2.74 \times 10^{-4}$, respectively). Some significant associations were also found between haplotypes of these three blocks and hematological toxicity (Table 4).

Discussion

Molecular and cellular studies have provided ample evidence for the roles of the caspase genes in several diseases [34–36]. It is also known that the actions of most cancer chemotherapy regimens are mainly dependent on caspase-mediated apoptosis [27], because the activities of caspase-8 and caspase-10 were found to be required for full induction of cell death during treatment and basal levels of caspase-8 were correlated with treatment sensitivity [37]. However, pharmacogenetic studies on associations between *CASP8* and *CASP10* polymorphisms and chemotherapy-related cytotoxicity have not been reported. On the basis of these preliminary data, in the current study, we used the tagging SNP approach to comprehensively assess the genetic effects of these two genes on chemotherapy-related toxicity outcomes in lung cancer patients.

Overall, we did find some significant associations between *CASP8* and C*ASP10* tag SNPs and the risk for severe toxicity in NSCLC patients treated with first-line platinum-based chemotherapy. For example, *CASP8* rs12990906 was shown to be associated with hematologic and overall toxicities; variants rs3769827, rs11674246, and rs3731714 were associated only with overall toxicity. All associations with these SNPs observed in the discovery set were confirmed in an independent dataset. Furthermore, these SNPs were consistent in their association with a significantly lower risk for toxicity with platinum-based chemotherapy, possibly through the regulation of hematopoietic cell proliferation and survival during chemotherapy.

Previous studies indicated that caspases are not only involved in drug-induced cancer cell death but also play an important role in the development and apoptosis of normal hematological cells [38]. It was reported that caspase-3 is essential in the regulation of normal B-cell homeostasis in a caspase-3 knockout mouse model [39], that caspase-8 regulates T-cell activation and proliferation [40], and that caspase-10 is involved in lymphocyte proliferation [41]. Therefore, it is biologically plausible that SNPs in several genes involved in caspase-related apoptosis pathways could affect the risk for drug- induced toxicity in NSCLC patients treated with first-line platinum-based treatment.

In the present study, we observed that the *CASP8* rs12990906 SNP had a significant influence on both hematologic and overall chemotherapy toxicity outcomes with platinum-based treatment, acting in an allele dose–dependent manner. The lower risk associated with rs12990906 was particularly pronounced for overall toxicity (incidence rate, 26.4% with the *GG* genotype and 37.9% with the *AA* genotype; $p = .002$), which could be driven by hematologic toxicity. Be-

cause significant SNPs found in our study are mainly tag SNPs, further research is needed to determine the functionality of these SNPs that may be in LD with the identified tag SNPs or molecular mechanisms underlying the identified associations. Nevertheless, several lines of evidence support our findings. Sun et al. [25] reported that T lymphocytes with the deletion variant *CASP8* rs3834129, which is in the same LD block as our rs3769827 SNP, had lower caspase-8 activity and activation-induced cell death upon stimulation with cancer cell antigens, leading to lower susceptibility for several cancers. Some SNP–toxicity associations were only observed in heterozygotes (e.g., *CASP8* rs11674246), which may be a result of the lower incidence rate of severe toxicity and the relatively small number of homozygous variants (especially in subgroups), if no genetic reason for the selection bias could account for the heterozygotes [42]. However, there may be several other possibilities that could explain this finding, such as loss of heterozygosity in the investigated gene region and the possibility that heterozygotes function in an unknown mode [43].

Our present study also suggested that some genetic variants of *CASP8* and *CASP10* might differentially affect hematologic and overall toxicity outcomes based on the chemotherapeutic agent administered, including rs3769827 and rs11674246 in the subgroup of patients treated with platinum plus gemcitabine therapy and *CASP8* rs7608692 and *CASP10* rs12613347 in the subgroup of patients treated with carboplatin. On the other hand, the effects of these SNPs were also apparent in patients with adenocarcinoma and patients in other subgroups, such as males, nonsmokers, and those with stages III or IV disease. However, most of the observed associations were not significant after Bonferroni correction.

Our findings indicate that the predictive role of biomarkers for drug- related toxicity seems to be largely identified by our subgroups, including the specific chemotherapy regimen used, a nonsmoking status, and adenocarcinoma histology. In addition, we found that only the *CASP8* rs3769827 variant was weakly associated with response to platinum-based chemotherapy ($p = .036$). Therefore, genetic factors associated with toxicity are likely to be different from those associated with response and overall survival and progression-free survival outcomes, as also suggested by Shiraishi et al. [5]. Thus, larger studies are needed to validate these subgroup findings, and the underlying mechanism also warrants further investigation.

Residing in tandem order on chromosome 2q33, *CASP8* and *CASP10* form three LD blocks according to our data, and a consistent correlation was found in block 2 (*CASP8–1*), which contains rs3769827 and rs7608692. The haplotype *GG* showed a striking protective effect for severe toxicity overall, suggesting that the lower risk effect of *GG* was probably driven by rs3769827, especially by its minor allele *G*. A similar situation was observed for *CASP8* rs12990906 and *CASP10* rs11674246 and rs3731714, which are located in block 3 (*CASP8–2*) and block 1 (*CASP10*), respectively. Although the biologic significance of these tag SNPs and their interactions with chemotherapy agents are unknown at present, it is possible that they may be in strong LD with functionally important but untyped SNPs regulating function and expression of the genes and proteins in these blocks. For instance, a putative microRNA binding site of rs1045487 is completely linked with our SNP rs1045494 (LD $r^2 = 1.00$), according to the SNPINFO Web site [44] (supplemental online Table S4). For *CASP10* rs11674246, located in the putative promoter region, this SNP may affect transcription factor binding activity and *CASP10* expression at a transcription factor binding site according to prediction using the TFSEARCH [44] and SNPINFO Web sites.

The present study adds to the existing literature on genetic susceptibility to platinum-based chemotherapy toxicity, but a few limitations need to be addressed. First, although our sample size of 663 patients may be one of the largest groups reported to date, the numbers of patients in some subgroups, such as treatment arms and histopathology subtypes ($n < 100$), were still small. This may result in lower statistical power to detect toxicity associations with mild-effect or low-penetrance SNPs and thus could result in both false-negative and false-positive findings [46]. This problem can only be resolved by studies with larger sample sizes in the future. Additional independent prospective replication is also required to validate our findings. Second, all patients in our present study were treated in three medical centers in Shanghai, which could potentially result in some bias caused by the heterogeneity of the patient populations. However, we used the exact same criteria for patient recruitment and clinical data collection, which may have limited potential confounding effects and reduced the heterogeneity to some extent. In addition, we observed no significant differences in age, sex, or family history in NSCLC patients enrolled from different centers in the study. Third, although we used two independent datasets to validate our findings, this study was performed in a Chinese population, and therefore the results may not be true for other ethnicities. For example, the allele frequencies of all SNPs investigated herein are known to be different among ethnic populations (e.g., rs10192461, whose MAF = 0.00 is lower here than in the HapMap data, MAF = 0.222). Therefore, examination of these tag SNPs in other NSCLC patient populations will help elucidate possible interethnic differences in chemotherapeutic toxicity outcome. We plan to confirm the current findings in our ongoing prospective expansion studies with more stringent conditions and with much larger sample sizes.

Conclusion

In conclusion, the present hospital-based pharmacogenetic study showed some associations between platinum-based chemotherapy toxicity and variants of *CASP8* and *CASP10* in Chinese NSCLC patients and confirmed that patients homozygous for the *CASP8* rs12990906 variant had a significantly lower risk for developing toxicity with platinum-based chemotherapy. Our results support the idea that genetic variations in *CASP8* and *CASP10* may act as potential predictive biomarkers for platinum-based chemotherapy toxicity in Chinese NSCLC patients. In patients with risk alleles, a cisplatin-based rather than carboplatin-based regimen might be able to reduce severe toxicities. Administration of G-SCF to advanced patients may prevent those patients from experiencing severe he-

matological toxicities. To date, no genomewide association study (GWAS) has addressed toxicity outcomes with platinum-based chemotherapy in NSCLC patients, although there was a GWAS on the survival outcome of NSCLC patients receiving platinum-based chemotherapy [47] and a GWAS on the efficacy of platinum-based chemotherapy in small cell lung cancer patients [448] that did not identify any genetic association with SNPs from the *CASP8* and *CASP10* loci. Therefore, further studies or pooled analyses are warranted to validate our findings on the effects on toxicity of *CASP8* and CASP*10* for potential clinical application.

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Author Contributions

Conception/Design: Ji Qian, Daru Lu, Shaohua Gu, Haijian Wang, Jiucun Wang, Baohui Han, Jin Li, Junjie Wu

Provision of study material or patients: Lixin Yang, Wenqing Chen, Wenting Wu, Xiaoming Tan, Baohui Han

Collection and/or assembly of data: Lixin Yang, Wenqing Chen, Qihan Wu, Xueying Zhao, Wenting Wu, Xiaoming Tan, Baohui Han, Weiwei Fan, Hongyan Chen, Junjie Wu

Data analysis and interpretation: Qingyi Wei, Ji Qian, Hui-Qi Qu, Ming Yin, Xueying Zhao, Xiaoming Tan

Manuscript writing: Qingyi Wei, Ji Qian, Hui-Qi Qu, Ming Yin, Qiming Wang, Junjie Wu

Final approval of manuscript: Daru Lu, Jin Li, Junjie Wu

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