Genetic Variants of the MDM2 Gene Are Predictive of Treatment-Related Toxicities and Overall Survival in Patients With Advanced NSCLC

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Abstract

We investigated the association of 5 tagging single nucleotide polymorphisms (SNPs) of MDM2 with chemotherapy-related toxicities and clinical outcomes in 663 patients with advanced non–small-cell lung cancer. We identified 2 SNPs (rs1470383 and rs1690924) with significant associations with chemotherapy-related toxicities. One SNP rs1470383 also influenced the overall survival of patients without overall toxicity or hematologic toxicity.

Introduction: Platinum agents can cause the formation of DNA adducts and induce apoptosis to eliminate tumor cells. The aim of the present study was to investigate the influence of genetic variants of MDM2 on chemotherapy-related toxicities and clinical outcomes in patients with advanced non–small-cell lung cancer (NSCLC). **Materials and Methods:** We recruited 663 patients with advanced NSCLC who had been treated with first-line platinum-based chemotherapy. Five tagging single nucleotide polymorphisms (SNPs) in MDM2 were genotyped in these patients. The associations of these SNPs with clinical toxicities and outcomes were evaluated using logistic regression and Cox regression analyses. **Results:** Two SNPs (rs1470383 and rs1690924) showed significant associations with chemotherapy-related toxicities (ie, overall, hematologic, and gastrointestinal toxicity). Compared with the wild genotype AA carriers, patients with the GG genotype of rs1470383 had an increased risk of overall toxicity (odds ratio [OR], 3.28; 95% confidence interval [CI], 1.34-8.02; $P = .009$) and hematologic toxicity (OR, 4.10; 95% CI, 1.73-9.71; $P = .001$). Likewise, patients with the AG genotype of rs1690924 showed more sensitivity to gastrointestinal toxicity than did those with the wild-type homozygote GG (OR, 2.32; 95% CI, 1.30-4.14; $P = .004$). Stratified survival analysis revealed significant associations between rs1470383 genotypes and overall survival in patients without overall or hematologic toxicity ($P = .007$ and $P = .0009$, respectively). **Conclusion:** The results of our study suggest that SNPs in MDM2 might be used to predict the toxicities of platinum-based chemotherapy and overall survival in patients with advanced NSCLC. Additional validations of the association are warranted.

Clinical Lung Cancer, Vol. 16, No. 5, e37-53

Keywords: Non–small-cell lung cancer, Platinum-based chemotherapy, Polymorphisms, Progression-free survival, Toxicity

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Submitted: Dec 15, 2014; Revised: Feb 9, 2015; Accepted: Feb 10, 2015; Epub: Feb 19, 2015

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1525-7304/$ - see frontmatter
http://dx.doi.org/10.1016/j.cllc.2015.02.001

Introduction

Lung cancer is the leading cause of cancer death in the world, and non–small-cell lung cancer (NSCLC) accounts for nearly 80% to 85% of all lung cancer cases.[1] Platinum-based doublet regimens are widely used as the first-line treatment of patients with advanced NSCLC and lead to improved survival outcomes.[2] However, a number of patients who received platinum-based treatment developed high grade toxicities, which might decrease treatment efficiency or cause therapy withholding.[3] Identification of the inherited variants associated with platinum-based therapy toxicities and outcomes would be useful in making suitable individualized treatment plans.

Platinum drugs might inhibit tumor growth mainly by the formation of platinum-DNA adducts and intrastrand/interstrand crosslinks, which result in cell cycle arrest and apoptosis.[3] The p53 gene plays a central role in cell cycle control, DNA repair, and apoptosis initiation.[4] In many cellular processes, MDM2 acts as a key negative regulator of p53 through directing binding, ubiquitination, and degradation of p53.[5] Many cancer cells display high levels of MDM2 expression, resulting in rapid cancer progression and a lack of response to therapy in a subset of human cancers.[6,7] However, in NSCLC, overexpression of MDM2 was associated with favorable survival outcomes.[8-10] Such disparity might be resulted from the relative importance of p53-dependent and p53-independent functions of MDM2 in different cancers.[7] In NSCLC, MDM2 might play a role in p53-independent antitumor activity (eg, as a regulator of cell proliferation).

Single nucleotide polymorphisms (SNPs) have been shown to influence gene functions and clinical phenotypes.[11] Currently, 2 functional SNPs, SNP309 (rs2279744)[12] and its antagonist SNP285 (rs117039649),[13] have been reported to enhance and decrease MDM2 gene expression, respectively. SNP309 has divergent minor allele frequency among different populations. Also, SNP285 is only observed in whites but not in Asians. The associations between these functional SNPs and cancer risk have been evaluated in different types of cancers.[14-16] However, until now, few studies have investigated the effect of MDM2 SNPs on chemotherapy toxicity.[17,18] In the present study, we selected 5 tagging SNPs in MDM2 to evaluate their associations with chemotherapy toxicity and clinical outcomes in 663 patients with advanced NSCLC who had received first-line platinum-based chemotherapy.

Materials and Methods

Study Population

In the present study, we recruited patients with histologically confirmed advanced NSCLC from 3 hospitals in Shanghai, China, from March 2005 to January 2010 (Shanghai Chest Hospital, Shanghai Zhongshan Hospital, and Shanghai Changhai Hospital). The criteria for patient recruitment have been detailed previously.[19] In brief, our study enrolled 663 eligible patients who had been diagnosed with stage IIIA-IV NSCLC and had been given first-line platinum-based chemotherapy without any previous surgery, radiotherapy, or concurrent chemoradiotherapy. All chemotherapeutic drugs were administered intravenously, and all patients were treated for 2 to 6 cycles. The chemotherapy toxicities were assessed twice a week. The grade of toxicity was recorded using the National Cancer Institute Common Toxicity Criteria, version 3.0, including overall toxicity, gastrointestinal toxicity (nausea/vomiting), and hematologic toxicity (including leukocytopenia, agranulocytosis, anemia, and thrombocytopenia). The toxicity outcome was dichotomized by the presence or absence of any grade 3 or 4 overall toxicity, any grade 3 or 4 gastrointestinal toxicity, and any grade 3 or 4 hematologic toxicity.

The demographic and other clinical data (ie, sex, age, smoking history, family history of cancer, clinical stage, and tumor histologic type) were collected at entry to the study. Survival were collected from several sources, including follow-up telephone interviews, the Social Security Death Index, and inpatient and outpatient clinical medical records. The ethical review committee of Fudan University and the hospitals approved the study protocol. All participants provided written informed consents.

SNP Selection and Genotyping

TagSNPs were selected according to the HapMap phase II data with 44 unrelated individuals from Tokyo, Japan (JPT) and 45 unrelated Han Chinese individuals from Beijing, China (CHB). There are 16 SNPs within 2 kb up- and downstream of the MDM2 gene with a minor allele frequency (MAF) > 0.05. As shown in Supplemental Figure 1 (available in the online version), 6 tagging SNPs were selected using SNPinfo (available at: http://snpinfo.niehs.nih.gov/snpinfo/snptag.htm) with a minimum pairwise linkage disequilibrium (LD) r2 threshold of 0.8. However, 5 of them could pass the genotyping quality control.

Blood samples were obtained at entry to the study and stored in ethylenediaminetetraacetic acid tubes at −80°C. The QIAamp DNA Maxi Kit (Qiagen GmbH) was used to extract genomic DNA. We used iSelect HD BeadChip (Illumina) to genotype the selected SNPs in MDM2 with the following quality control criteria: genotyping call rate of SNP ≥ 0.95, MAF ≥ 0.01, and GenCall score ≥ 0.2. GenomeStudio, version 2010.1, and GeneMap software were used to analyze the data and prepare the reports. Concordance between replicates was $> 99.9\%$.

Statistical Analysis

The Hardy-Weinberg equilibrium was tested using Pearson's χ^2 test. The associations between genotypes and toxicity outcomes were assessed using the χ^2 test and logistic regression analyses with or without adjustment for other significant demographic and clinical variables. Sex and chemotherapy regimens were significant related factors for gastrointestinal toxicity and hematologic toxicity, respectively. For overall toxicity, both patient sex and chemotherapy regimen were significantly influential factors and were included as adjusted covariates. Progression-free survival (PFS) was calculated from the date of the patient started chemotherapy to the date of disease progression or death (whichever occurred first) or the last follow-up visit. Overall survival (OS) was calculated from the date of the first chemotherapy session to the date of death from any cause or the last follow-up visit. The median PFS and OS were estimated using the Kaplan-Meier method, and their differences by genotypes were tested using the log-rank test. Univariate and multivariate Cox regression analyses were used to estimate the hazard ratio (HR) and 95% confidence

Table 1 Distribution of Clinical Characteristics Stratified by Chemotherapy Toxicity

Characteristic	GI Toxicity[a]			Hematologic Toxicity[b]			Overall Toxicity		
	No	Yes	*P* Value	No	Yes	*P* Value	No	Yes	*P* Value
Total patients	587 (91.0)	58 (9.0)		482 (74.4)	166 (25.6)		437 (68.6)	200 (31.4)	
Age (years)			.805			.344			.306
Median	58	59.5		58	60		58	60	
Range	26-80	34-78		26-80	28-78		26-80	28-78	
Sex			<.0001[c]			.216			.015[c]
Male	424 (72.2)	25 (43.1)		344 (71.4)	110 (66.3)		317 (72.5)	126 (63.0)	
Female	163 (27.8)	33 (56.9)		138 (28.6)	56 (33.7)		120 (27.5)	74 (37.0)	
ECOG PS			.476			.478			.646
0-1	540 (92.3)	52 (89.7)		439 (91.7)	155 (93.4)		400 (92.0)	186 (93.0)	
2	45 (7.7)	6 (10.3)		40 (8.3)	11 (6.6)		35 (8.0)	14 (7.0)	
Smoking status[d]			.001[c]			.347			.101
Never smokers	234 (39.9)	36 (62.1)		196 (40.7)	74 (44.9)		174 (39.8)	93 (47.0)	
Ever smokers	352 (60.1)	22 (37.9)		286 (59.3)	91 (55.1)		263 (60.2)	106 (53.0)	
TNM stage			.933			.925			.924
IIIA	43 (7.4)	5 (8.6)		35 (7.3)	13 (7.8)		31 (7.1)	16 (8.0)	
IIIB	168 (28.7)	16 (27.6)		140 (29.2)	46 (27.7)		124 (28.5)	57 (28.5)	
IV	374 (63.9)	37 (63.8)		305 (63.5)	107 (64.5)		280 (64.4)	127 (63.5)	
Histologic type			.462			.942			.509
Adenocarcinoma	385 (65.8)	36 (62.1)		314 (65.4)	111 (66.9)		287 (66.0)	131 (65.5)	
Squamous cell carcinoma	123 (21.0)	11 (19.0)		103 (21.5)	32 (19.3)		96 (22.0)	38 (19.0)	
Adenosquamous carcinoma	10 (1.7)	2 (3.5)		9 (1.9)	3 (1.8)		6 (1.4)	5 (2.5)	
Other[e]	67 (11.5)	9 (15.5)		54 (11.3)	20 (12.1)		46 (10.6)	26 (13.0)	
Platinum chemotherapy regimen			.189			.012[c]			.006[c]
Navelbine	182 (31.0)	22 (37.9)		143 (29.7)	62 (37.4)		125 (28.6)	73 (36.5)	
Gemcitabine	133 (22.7)	16 (27.6)		111 (23.0)	40 (24.1)		99 (22.7)	49 (24.5)	
Paclitaxel	191 (32.5)	10 (17.3)		164 (34.0)	37 (22.3)		156 (35.7)	44 (22.0)	
Docetaxel	48 (8.2)	5 (8.6)		33 (6.9)	20 (12.0)		31 (7.1)	23 (11.5)	
Other combinations	33 (5.6)	5 (8.6)		31 (6.4)	7 (4.2)		26 (5.9)	11 (5.5)	

Table 1 Continued

Characteristic	GI Toxicity[a]			Hematologic Toxicity[b]			Overall Toxicity		
	No	Yes	*P* Value	No	Yes	*P* Value	No	Yes	*P* Value
Objective response			.078			.109			.046[c]
CR	NA	NA		NA	NA		NA	NA	
PR	101 (17.3)	15 (26.8)		81 (17.0)	37 (22.6)		70 (16.1)	45 (22.7)	
SD/PD	484 (82.7)	41 (73.2)		397 (83.0)	127 (77.4)		365 (83.9)	153 (77.3)	

Data presented as n (%), unless otherwise noted.
Abbreviations: CR = complete response; ECOG PS = Eastern Cooperative Oncology Group performance status; GI = gastrointestinal; NA = not applicable; PD = progressive disease; PR = partial response; SD = stable disease.
[a]Grade 3 or 4 GI toxicity, including nausea and vomiting.
[b]Grade 3 or 4 hematologic toxicity, including neutropenia, anemia, and thrombocytopenia.
[c]Statistically significant.
[d]Those who had smoked < 1 cigarette daily and < 1 year in their lifetime were defined as never smokers.
[e]Including mixed cell and undifferentiated carcinoma.

interval (CI) with and without adjustment for age and sex, together with the other clinical variables that had a significant association with OS or PFS (Supplemental Table 2; available in the online version).

Stratified analyses were performed by age, sex, smoking status, TNM stage, histologic type, and chemotherapy regimen. To evaluate the joint effects of SNPs in MDM2, we also performed haplotype analysis. Haploview software was used to define the blocks according to the algorithm of confidence bound on D prime of each pair of SNPs by standard Haploview parameters.[20] The expectation–maximization algorithm implemented in SAS, the genetic module, was used to infer the haplotypes and their frequencies. The false-positive report probability (FPRP) was calculated to adjust for false-positive findings.[21] For all significant results in the study, we calculated the FPRP values using 3 factors: prior probability of a true association of the tested genetic variant with a disease (2 prior probabilities were assumed to be 0.1 and 0.01), the observed *P* value, and the statistical power to detect the odds ratio of the alternative hypothesis at the given *P* value (the prior effect size was assumed to be 2.5 and 1.5 for SNPs for platinum toxicity and OS, respectively). An association was considered noteworthy when its FPRP value was < 0.2.

All tests of statistical significance were two sided. If not specifically mentioned, all statistical analyses were performed using Statistical Analysis Systems software, version 9.2 (SAS Institute Inc., Cary, NC).

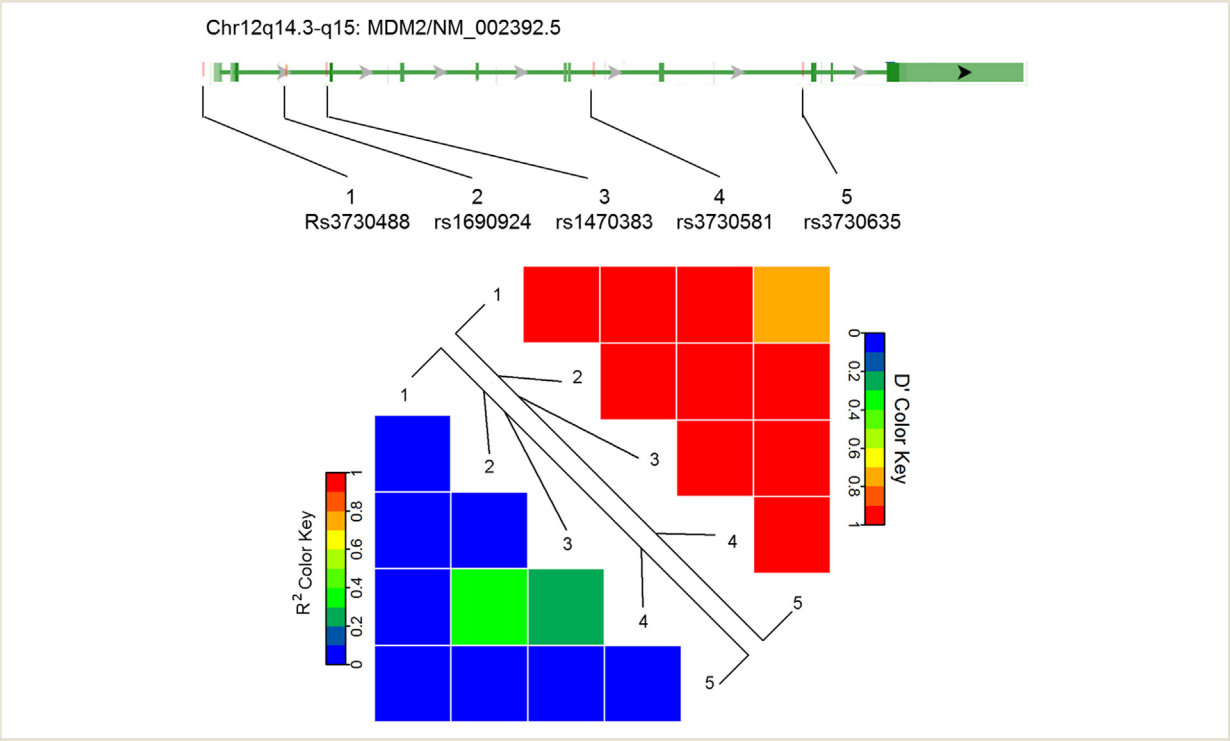
Results

All the patients in the present study had histologically confirmed stage IIIA-IV NSCLC. The main demographic and clinical characteristics have been previously described.[19] Of 648 patients, 166 had grade 3 or 4 hematologic toxicity; 58 of 645 patients had grade 3 or 4 gastrointestinal toxicity, and 200 of 637 patients had overall toxicity (Table 1). Females and never smokers had a significantly greater risk of gastrointestinal toxicity compared with males and smokers ($P < .0001$ and $P = .001$, respectively). The chemotherapy regimens were significantly associated with hematologic toxicity ($P = .012$) and overall toxicity ($P = .006$). In addition, significant differences were seen in the sex distribution of patients with and without overall toxicity ($P = .015$). The patients who experienced overall toxicity had a greater partial response rate (22.0%) than did those without toxicity (partial response rate, 16.1%; $P = .045$). No significant associations were found between chemotherapy toxicities and OS or PFS (Supplemental Table 2; available in the online version). The frequency distributions of the 5 tagging SNPs are summarized in Supplemental Table 1 (available in the online version). All were in Hardy-Weinberg equilibrium ($P > .05$). We also calculated the pairwise LD of these 5 SNPs in our sample. Low pairwise LD ($r2 < 0.5$) was found among them (Figure 1).

Association Between Single Locus and Chemotherapy Toxicity

The significant associations between the SNPs and toxicity outcomes are listed in Table 2. One intron SNP rs1470383 showed significant influence on overall toxicity and hematologic toxicity with

Figure 1 Pairwise Linkage Disequilibrium (Indicated by r^2 and D′) Between the 5 Tagging Single Nucleotide Polymorphisms (SNPs) of MDM2 in 663 Patients With Non–Small-Cell Lung Cancer. These SNPs Have Moderately High D′ (D′ > 0.7) but Relatively Low r2 (r2 < 0.5) Between Each Other



adjustment for sex and chemotherapy regimen. Compared with the wild-type AA homozygotes, patients with the GG genotype tended to have a higher risk of hematologic toxicity (OR, 4.10; 95% CI, 1.73-9.71; $P = .001$) and overall toxicity (OR, 3.28; 95% CI, 1.34-8.02; $P = .009$). These associations were even more significant under a recessive model ($P = .007$ for overall toxicity and $P = .001$ for hematologic toxicity). For rs1690924, the AG heterozygotes had an increased risk of gastrointestinal toxicity (OR, 2.32; 95% CI, 1.30-4.14; $P = .004$) compared with the AA homozygotes.

We also assessed the discriminative accuracy of the prediction model with and without adding these identified SNPs to the significant demographic and clinical features (sex and chemotherapy regimen) by comparing the area under the receiver operating characteristic curve (AUC) (Supplemental Figure 2; available in the online version). When adding rs1470383 in the prediction model of hematologic toxicity, the AUC increased to 0.61 (95% CI, 0.56-0.66) compared with the model that only included the chemotherapy regimen (AUC, 0.59; 95% CI, 0.54-0.64; $P = .103$). For overall toxicity, the AUC increased slightly to 0.61 (95% CI, 0.57-0.66) when adding rs1470383 to the model compared with the AUC for the model that only included the 2 variables of sex and chemotherapy regimen (AUC, 0.60; 95% CI, 0.56-0.65; $P = .283$). For gastrointestinal toxicity, the AUC increased to 0.71 (95% CI, 0.64-0.78) after including rs1690924 in the model compared with the AUC for the model that only included sex and chemotherapy regimen (AUC, 0.65; 95% CI, 0.58-0.71; $P = .002$).

Associations Between SNPs and OS and PFS

One SNP (rs3730635) showed a marginally significant association with lung cancer OS (log-rank, $P = .036$; Table 3, Figure 2A). Patients with the heterozygous AG genotype had longer survival than those with the AA genotype (median OS time, 29.5 vs. 17.8 months; HR, 0.54; 95% CI, 0.29-1.01). No significant association was found for the other SNPs.

Haplotype analysis was also performed for the 3 SNPs (rs1690924, rs1470383, and rs3730581) that were defined in the same block in Haploview. Haplotype "AAG" was significantly associated with favorable survival (HR, 0.66; 95% CI, 0.46-0.95; $P = .025$; Table 3).

Stratified Analysis

We also evaluated the association between SNPs and OS stratified by toxicity (overall toxicity, gastrointestinal toxicity, and hematologic toxicity). In the subpopulation of patients who experienced overall toxicity, those that were heterozygous for rs3740588 experienced a shorter OS than those with the AA genotype (median OS time, 13.3 vs. 18.7 months; $P = .049$; Table 4, Figure 2B, C). For those who experienced severe hematologic toxicity, rs1690924 was shown to significantly influence survival ($P = .043$). However, no significance remained after adjustment for age, sex, TNM stage, and histologic type.

Another 2 SNPs (rs1470383 and rs3730635) were significantly associated with OS in patients without toxicities (Table 4, Figure 2D, E). For SNP rs1470383, the survival of patients without

Table 2 Association of MDM2 SNPs and Chemotherapy Toxicity

	Gastrointestinal Toxicity				Hematologic Toxicity				Overall Toxicity			
SNP	Total	Toxicity Present	OR (95% CI)[a]	*P* Value[a]	Total	Toxicity Present	OR (95% CI)[b]	*P* Value[b]	Total	Toxicity Present	OR (95% CI)[c]	*P* Value[c]
rs3730488												
AA	601	53 (8.8)	1.00 (Ref)	—	602	151 (25.1)	1.00 (Ref)	—	593	184 (31.0)	1.00 (Ref)	.370
AC	42	5 (11.9)	1.35 (0.50-3.65)	.559	44	15 (34.1)	1.52 (0.78-2.93)	.216	42	16 (38.1)	1.35 (0.7-2.61)	.987
CC	1	NA	NA	NA	1	NA	NA	NA	1	NA	NA	NA
Recessive			NA	.989			NA	.982			NA	NA
rs1690924												
AA	333	21 (8.8)	1.00 (Ref)	—	334	87 (26.0)	1.00 (Ref)	—	330	96 (29.1)	1.00 (Ref)	—
AG	260	35 (13.5)	2.32 (1.30-4.14)	.004[d]	260	68 (26.2)	1.04 (0.72-1.52)	.826	254	91 (35.8)	1.42 (1.00-2.04)	.053
GG	52	2 (3.9)	0.58 (0.13-2.57)	.471	54	11 (20.4)	0.76 (0.37-1.55)	.450	53	13 (24.5)	0.83 (0.42-1.64)	.594
Recessive	—	—	0.37 (0.09-1.59)	.181	—	—	0.75 (0.37-1.49)	.407	—	—	0.71 (0.37-1.37)	.305
rs1470383												
AA	448	43 (8.8)	1.00 (Ref)	—	449	110 (24.5)	1.00 (Ref)	—	441	137 (31.1)	1.00 (Ref)	—
AG	175	14 (8)	0.8 (0.42-1.51)	.490	176	43 (24.4)	1.02 (0.68-1.54)	.921	174	50 (28.7)	0.91 (0.61-1.34)	.629
GG	22	1 (4.6)	0.43 (0.06-3.31)	.416	23	13 (56.5)	4.10 (1.73-9.71)	.001[d]	22	13 (59.1)	3.28 (1.34-8.02)	.009[d]
Recessive	—	—	0.45 (0.06-3.5)	.448	—	—	4.08 (1.73-9.58)	.001[d]	—	—	3.37 (1.39-8.18)	.007[d]
rs3730581												
AA	164	13 (8.8)	1.00 (Ref)	—	165	39 (23.6)	1.00 (Ref)	—	117	45 (27.8)	1.00 (Ref)	—
AG	325	32 (9.9)	1.32 (0.67-2.63)	.424	322	83 (25.8)	1.19 (0.76-1.85)	.450	217	102 (32.0)	1.33 (0.87-2.04)	.192
GG	155	13 (8.4)	1.07 (0.48-2.42)	.867	160	43 (26.9)	1.27 (0.76-2.10)	.344	103	52 (33.6)	1.42 (0.87-2.32)	.159
Recessive	—	—	0.89 (0.46-1.71)	.718	—	—	1.13 (0.75-1.7)	.562	—	—	1.18 (0.79-1.74)	.422
rs3730635												
AA	624	57 (8.8)	1.00 (Ref)	—	628	162 (25.8)	1.00 (Ref)	—	617	195 (31.6)	1.00 (Ref)	—
AG	21	1 (4.8)	0.54 (0.07-4.17)	.554	20	4 (20.0)	0.71 (0.23-2.19)	.554	20	5 (25.0)	0.73 (0.26-2.08)	.561

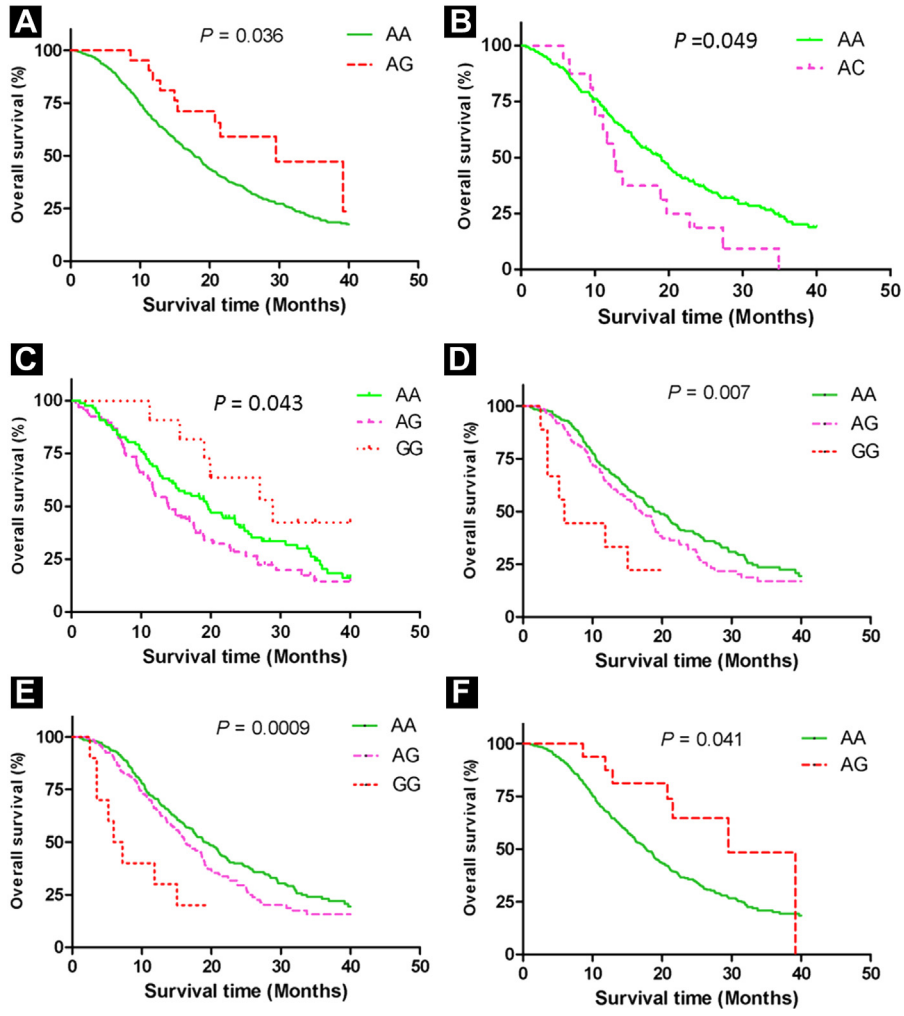
Data presented as n (%), unless otherwise noted.
Abbreviations: CI = confidence interval; NA = not applicable; OR = odds ratio; Ref = reference; SNP = single nucleotide polymorphism.
[a]Adjusted for sex.
[b]Adjusted for chemotherapy regimen.
[c]Adjusted for sex and chemotherapy regimen.
[d]Statistically significant.

Table 3 Association Between MDM2 SNPs and NSCLC Overall Survival and Progression-Free Survival

Genotype	Patients (n)	OS				PFS			
		MST (mo)	Log-Rank *P*	HR (95% CI)[a]	*P* Value[a]	MST (mo)	Log-Rank *P*	HR (95% CI)[b]	*P* Value[b]
rs3730488			.096				.891		
AA	613	18.7	—	1.00 (Ref)	—	6.4	—	1.00 (Ref)	—
AC	48	13.3	—	1.35 (0.98-1.88)	.070	7	—	1.06 (0.75-1.51)	.741
CC	1	13.4	—	2.74 (0.38-19.73)	.316	7.6	—	0.97 (0.14-6.95)	.974
Recessive	—	13.4 versus 18.4	.418	2.66 (0.37-19.12)	.331	7.6 versus 6.5	.897	0.97 (0.13-6.93)	.972
rs1690924			.114				.339		
AA	340	18.7	—	1.00 (Ref)	—	5.5	—	1.00 (Ref)	—
AG	269	16.9	—	1.08 (0.89-1.31)	.418	6.9	—	0.92 (0.76-1.12)	.426
GG	54	22.3	—	0.74 (0.51-1.06)	.095	7	—	0.76 (0.53-1.09)	.137
Recessive	—	22.3 versus 17.8	.056	0.71 (0.50-1.01)	.056	7.0 versus 6.2	.259	0.79 (0.55-1.12)	.183
rs1470383			.382				.778		
AA	460	18.9	—	1.00 (Ref)	—	6.8	—	1.00 (Ref)	—
AG	180	17.2	—	1.18 (0.96-1.44)	.124	5.5	—	1.08 (0.88-1.34)	.456
GG	23	12.4	—	1.20 (0.72-2.00)	.474	4.4	—	1.13 (0.66-1.93)	.663
Recessive	—	12.4 versus 18.6	.536	1.15 (0.70-1.90)	.587	4.4 versus 6.5	.618	1.10 (0.65-1.88)	.725
rs3730581			.766				.252		
AA	167	17.7	—	1.00 (Ref)	—	5.8	—	1.00 (Ref)	—
AG	334	18.8	—	0.99 (0.79-1.24)	.917	6.5	—	1.00 (0.80-1.25)	.999
GG	161	17.2	—	0.92 (0.71-1.19)	.507	6.8	—	0.83 (0.63-1.08)	.156
Recessive	—	17.2 versus 18.6	.478	0.92 (0.75-1.14)	.464	6.8 versus 6.0	.106	0.82 (0.66-1.03)	.823
rs3730635			.036[c]				.444		
AA	642	17.8	—	1.00 (Ref)	—	6.5	—	1.00 (Ref)	—
AG	21	29.5	—	0.54 (0.29-1.01)	.052	6.2	—	0.82 (0.50-1.35)	.428
Haplotype analysis of SNPs (rs1690924, rs1470383, and rs3730581) in same block									
AAA	666	18.6	—	—	—	6	—	1.00 (Ref)	—
GAG	375	18.8	.394	0.93 (0.80-1.09)	.372	7	.098	0.87 (0.75-1.02)	.083
AGG	226	16.5	.372	1.09 (0.91-1.30)	.332	5.4	.975	1.02 (0.85-1.22)	.872
AAG	55	24	.038[c]	0.66 (0.46-0.95)	.025	6.9	.123	0.79 (0.56-1.11)	.167
Other	4	15.8	.204	2.15 (0.80-5.79)	.129	6.3	.448	1.35 (0.50-3.62)	.556

Data presented as n (%), unless otherwise noted.
Abbreviations: CI = confidence interval; HR = hazard ratio; MST = median survival time; NA = not applicable; NSCLC = non–small-cell lung cancer; OR = odds ratio; OS = overall survival; PFS = progression-free survival; Ref = reference; SNP = single nucleotide polymorphism.
[a]Adjusted for age, sex, TNM stage, and histologic type.
[b]Adjusted for age, sex, and performance status.
[c]Statistically significant.

Figure 2 Kaplan-Meier Curves of Overall Survival by Genotype of (A) rs3730635 in All Patients; (B) rs3730488 in Patients With Severe Overall Toxicity; (C) rs1690924 in Patients With Severe Hematologic Toxicity; (D) rs1470383 in Patients Without Severe Overall Toxicity; (E) rs1470383 in Patients Without Severe Hematologic Toxicity; (F) rs3730635 in Patients Without Severe Hematologic Toxicity



overall toxicity but who carried 1 or 2 variant alleles became worse (median OS time, 19.6 vs. 16.9 vs. 5.9 months; HR, 1.39; 95% CI, 1.11-1.75; $P = .004$). Similar results were found for patients without hematologic toxicity (HR, 1.44; 95% CI, 1.17-1.79; $P = .0009$). In addition, improved survival was found for patients without hematologic toxicity but who carried the AG genotype of rs3730635 compared with the survival of those with the AA genotype (median OS time, 17.7 vs. 29.5 months; HR, 0.45; 95% CI, 0.21-0.96; $P = .041$).

We also investigated the association of SNP rs1470383 with hematologic toxicity stratified by different clinical characteristics (ie, age, sex, smoking status, performance status, stage, and histologic type). Female patients, those aged < 58 years, nonsmokers, patients with an Eastern Cooperative Oncology Group performance status of 0 to 1, TNM stage IV, or adenocarcinoma, and those with the GG genotype were more sensitive to hematologic toxicity than were those with the wild-type homozygous AA genotype (Supplemental Table 3; available in the online version).

The FPRPS at different prior probability levels are summarized in Supplemental Table 4 (available in the online version). When the prior probability was assumed to be 0.1 and the prior effect size to be 2.5 and 1.5 for SNPs for platinum toxicities and OS, 4 associations were still considered noteworthy findings, because the probability of false-positive results was < 0.2: the association of rs1470383 with hematologic toxicity and overall toxicity in patients without hematologic or overall toxicity and the association between rs1690924 and gastrointestinal toxicity.

Because no previously reported SNPs were included in the present study, we evaluated the LD between these reported SNPs in MDM2 and the 5 tagSNPs of the present study (Supplemental Figure 3; available in the online version). Their association with cancer risk or survival has been reported for 7 common SNPs.[18,22-29]

Table 4 Association Between MDM2 SNPs and NSCLC Overall Survival Stratified by Chemotherapy Toxicity

SNP	Genotype	Stratification Factor	Patients (n)	MST (mo)	Log-Rank *P*	HR (95% CI)[a]	*P* Value[a]
rs3730488	CC/AC/AA	Overall toxicity					
		Absent	1/26/409	13.4/13.3/18.7	.532	1.19 (0.77-1.82)	.432
		Present	NA/16/184	NA/12.7/19.0	.049[b]	1.55 (0.90-2.66)	.114
		Gastrointestinal toxicity					
		Absent	1/37/548	13.4/13.7/18.7	.291	1.28 (0.89-1.82)	.183
		Present	NA/5/53	NA/12.6/18.8	.134	2.16 (0.80-5.79)	.127
		Hematologic toxicity					
		Absent	1/29/451	13.4/13.4/18.6	.485	1.21 (0.81-1.81)	.359
		Present	NA/15/151	NA/12.6/18.4	.078	1.50 (0.85-1.64)	.160
rs1690924	GG/AG/AA	Overall toxicity					
		Absent	40/163/234	22.3/18.0/17.7	.411	0.91 (0.76-1.09)	.284
		Present	13/91/96	27.0/15.7/19.7	.155	1.01 (0.78-1.31)	.915
		Gastrointestinal toxicity					
		Absent	20/225/312	28.9/17.6/18.6	.120	0.92 (0.80-1.08)	.295
		Present	2/35/21	18.3/15.7/19.9	.960	0.78 (0.41-1.49)	.457
		Hematologic toxicity					
		Absent	43/192/247	22.2/17.8/17.8	.527	0.93 (0.79-1.09)	.365
		Present	11/68/87	28.9/13.9/19.5	.043[b]	1.02 (0.77-1.34)	.905
rs1470383	GG/AG/AA	Overall toxicity					
		Absent	9/124/304	5.9/16.9/19.6	.007[b]	1.39 (1.11-1.75)	.004[b]
		Present	13/50/137	25.8/17.6/18.9	.626	0.90 (0.69-1.19)	.459
		Gastrointestinal toxicity					
		Absent	21/161/405	12.4/17.7/19.0	.478	1.13 (0.94-1.35)	.193
		Present	1/14/43	NA/15.1/18.8	.163	1.09 (0.60-1.98)	.788
		Hematologic toxicity					
		Absent	10/133/339	6.6/16.2/19.3	.0009[b]	1.44 (1.17-1.79)	.0008[b]
		Present	13/43/130	25.8/18.4/16.2	.409	0.83 (0.62-1.11)	.210
rs3730581	GG/AG/AA	Overall toxicity					
		Absent	103/217/117	18.6/18.7/17.7	.920	0.98 (0.83-1.15)	.772
		Present	52/102/45	17.4/19.0/15.0	.556	0.93 (0.73-1.17)	.520
		Gastrointestinal toxicity					
		Absent	142/293/151	18.6/18.8/17.7	.499	0.93 (0.81-1.06)	.274
		Present	13/32/13	15.3/20.0/12.6	.552	0.98 (0.58-1.64)	.923
		Hematologic toxicity					
		Absent	117/239/126	16.1/18.8/17.8	.971	1.01 (0.87-1.18)	.863
		Present	43/83/39	17.8/18.9/15.0	.243	0.87 (0.68-1.12)	.267
rs3730635	AG/AA	Overall toxicity					
		Absent	15/422	29.5/17.8	.070	0.49 (0.23-1.04)	.064
		Present	5/195	15.4/18.0	.777	0.96 (0.30-3.03)	.941
		Gastrointestinal toxicity					
		Absent	20/567	29.5/18.4	.069	0.58 (0.31-1.09)	.091
		Present	1/57	NA/16	NA	NA	NA
		Hematologic toxicity					
		Absent	16/466	29.5/17.7	.041[b]	0.45 (0.21-0.96)	.039[b]
		Present	4/162	15.2/18.4	.870	1.18 (0.37-3.77)	.779

Data presented as number of patients with each genotype, unless otherwise noted.
Abbreviations: CI = confidence interval; HR = hazard ratio; MST = median survival time; NA = not applicable; NSCLC = non–small-cell lung cancer; SNP = single nucleotide polymorphism.
[a]Adjusted for age, sex, TNM stage, and histologic type.
[b]Statistically significant.

Except for 1 indel variant (rs3730485, which was not included in the 1000 genome data), all other 6 reported SNPs had high LD (r2 > 0.8), with 2 tagSNPs in the present study, with rs1690924 tagging 4 SNPs (rs937283, rs937282, rs1144944, and rs1690916), and rs3730581 tagging 2 other SNPs (rs3730536 and rs2279744).

Discussion

In the present study, we evaluated the association of 5 tagging SNPs in the MDM2 gene with chemotherapy toxicity and clinical outcomes (OS and PFS) in patients with advanced NSCLC treated with platinum-based regimens. Our results revealed that multiple SNPs in MDM2 could influence the occurrence of severe chemotherapy toxicity (rs1470383 and rs1690924) and survival outcome (rs3730635) independently. In addition, 2 SNPs (rs1470383 and rs3730635) had significant effects on OS for patients without hematologic or overall toxicity. These results suggest that MDM2 SNPs might have significant prognostic effects in patients with advanced NSCLC treated with first-line platinum-based chemotherapy.

The susceptibility to chemotherapy toxicity varies among patients, making it a major consideration for personalized treatment planning. The identification of related genetic markers would be helpful for screening patients at high risk of chemotherapy toxicity and to develop suitable treatment plans.[30] A series of studies have been performed to identify the genetic variants associated with toxicity susceptibility at different levels. Several genome-wide studies screened the association between SNPs and platinum susceptibility on a genome scale and identified multiple associated loci (eg, SNPs in DAPK3 and METTL6) using the lymphoblastoid cell lines from the International HapMap project.[31-33] However, such studies using cell lines could not replicate the findings from population-based studies, which had reported significant associations between SNPs in multiple genes (eg, drug metabolism genes, DNA repair genes, and apoptosis genes) and the toxicity response.[34-37] This difference might have resulted from different mechanisms underlying the toxicity responses of the 2 levels (cell line and individual).

The p53 pathway plays important roles in chemotherapy response and cancer progression.[38,39] As a key negative regulator of p53, MDM2 is a potential predictive marker for treatment toxicity and survival. The association between the functional SNP T309G (rs2279744) and platinum-based or non−platinum-based chemotherapy responses has been investigated in many cancers.[18,35,40-43] However, the results have been inconsistent regarding the effects of this SNP on gastrointestinal and hematologic toxicities. No significant effects were found in 136 patients with bladder cancer who received combined cisplatin-based systemic chemotherapy and radiotherapy.[35] One recent study observed a significant association between severe hematologic and overall toxicities and SNP309 in 444 patients with advanced NSCLC treated with platinum-based chemotherapy.[18] Although this SNP was not included in our study, one of its high LD SNP rs3730581 (pairwise LD $r^2 = 0.86$), had been investigated but did not show a significant effect on any chemotherapy toxicity. That study also found that the GC heterozygotes of another functional SNP rs937282 had a greater incidence of severe gastrointestinal toxicity than did the homozygotes. This was confirmed by our finding that the heterozygous genotype of its high LD SNP rs1690924 (r2 = 0.91) was significantly associated with an increased risk of gastrointestinal toxicity.

Our study also found that another SNP rs1470383 had a significant association with an increased incidence of severe hematologic toxicity and overall toxicity. Additional stratification analysis revealed that this SNP only influenced the OS of patients without any toxicity. This patient subgroup also had a relatively lower partial response rate, indicating less sensitivity to chemotherapy. Taken together, these results suggest that the effect of this SNP on survival was modified by treatment efficiency. However, this SNP did not have a clear function. In silica prediction using F-SNP (available at: http://compbio.cs.queensu.ca/F-SNP) showed that this SNP could alter the binding activity of transcript factors (eg, GATA-2/3).[44] LD analysis with SNPinfo showed it also had a low LD (r2 = 0.32, D′ = 0.79) with 2 other potential functional SNPs (rs2431655 and rs2259588, located at the transcription factor binding site and exonic splicing enhancer, respectively) in the Asian population. Additional studies are warranted to validate our findings and reveal the underlying mechanism of such associations.

The association between SNP T309G and the survival of patients with NSCLC has been investigated in previous studies. Consistent results were reported on the association between this SNP and worse survival in patients with early-stage NSCLC.[45,46] However, for advanced NSCLC, the prognostic effect of this SNP remains controversial.[18,47-50] Three studies had reported a significant association between SNP T309G and unfavorable survival in patients with advanced NSCLC,[47,48] but 2 other studies did not support the association.[49,50] Based on the results of its high LD SNP rs3730581, our study results were was consistent with those from the latter study. Recently, genome-wide studies were conducted to investigate the association of SNPs with OS in patients with advanced NSCLC; however, no significant association was reported for SNPs in MDM2.[51-55] These results suggested that SNPs in MDM2 might have only limited effects on the survival of patients with advanced NSCLC. The inconsistencies among different studies might have resulted from the heterogeneity among the studies (eg, different tumor cell grade of differentiation, smoking cessation, dietary supplements, or genetic variations).[56]

Never smokers with NSCLC have been defined as a separate population, because they have unique clinical and molecular characteristics.[57] More than one half of the female patients with NSCLCs will be never smokers in the Asian population.[58] One additional finding of that study was that female patients and never smokers tended to have a significantly high risk of gastrointestinal toxicity. We also observed a nonsignificantly greater incidence of hematologic toxicity in these 2 populations than in males and smokers. From the results of previous studies, most of the toxic side effects of chemotherapy could be explained by the sensitivity of normal cells to drug-induced apoptosis.[59] Thus, these tendencies might be because females and never smokers are more sensitive to chemotherapeutic drug-induced apoptosis than are males and smokers, because nicotine in tobacco could inhibit the induced apoptosis.[60]

The present study had several limitations. First, although 2 SNPs in MDM2 were revealed to influence chemotherapy toxicity,

the prediction models performed poorly, even after including the identified SNPs with the other clinical variables. This suggests that the current prediction models have very limited clinical application and additional genetic markers should be identified to improve the prediction accuracy. Second, the 5 tagging SNPs genotyped in the present study were located at introns, which do not have clear biologic functions. Although previous studies have shown that intronic SNPs do have the potential to affect gene function by producing alternative splicing or triggering microRNA arising, directed genotyping of these functional SNPs are warranted. Third, 1 tag SNP failed in genotyping, which influenced the coverage rate. Although the remaining 5 tagging SNPs still represented 93.8% (15 of 16) of the SNPs in MDM2, some real causal SNPs might have been missed. Additional explorations based on 1000 genome database are required in future studies. Finally, the present study only assessed gastrointestinal and hematologic toxicities and did not include the more typical, platinum-associated toxicities, such as nephrotoxicity and ototoxicity, limiting its clinical implications. More comprehensive chemotherapy-related toxicity assessments are needed in future studies.

Conclusion

The present study identified several SNPs in MDM2 that had a significant association with chemotherapy toxicities and clinical outcomes in patients with advanced NSCLC. These SNPs have the potential to be used as genetic markers to predict the toxic response to platinum-based chemotherapy and OS in patients with advanced NSCLC. Additional population studies and functional validations of our findings are warranted.

Clinical Practice Points

- Platinum-based doublet regimens are widely used as first-line treatment of patients with advanced NSCLC and lead to improved survival outcome.
- However, a number of patients who receive platinum-based treatment will develop high-grade toxicities, which might decrease treatment efficiency or cause therapy withholding.
- Identification of the inherited variants associated with platinum-based therapy toxicity and outcomes will be useful in developing suitable individualized treatment plans.
- The association between SNPs in MDMs and OS of lung cancer has been investigated in several studies.
- However, until now, little has been known about the effect of MDM2 SNPs on the chemotherapy toxicities of patients with advanced NSCLC.
- In the present study, we selected 5 tagging SNPs in MDM2 to evaluate their associations with chemotherapy toxicity and clinical outcomes in 663 patients with advanced NSCLC who had received first-line platinum-based chemotherapy.
- The results revealed that 2 SNPs (rs1470383 and rs1690924) had significant associations with chemotherapy-related toxicities.
- One SNP rs1470383 also influenced the OS of patients without overall toxicity or hematologic toxicity.
- These findings could be used as genetic markers to screen susceptible patients who are sensitive to platinum-based chemotherapy.

Acknowledgments

This work was supported in part by the National Basic Research Program (973 program) of China (grant 2011CB503802); Shanghai Science and Technology Research Program (grants 09JC1402200 and 10410709100); National Natural Science Foundation of China (grants 30800622, 81001114, 81172093, and 30890034); the Shanghai Pujiang Program (grant 11PJD005); China Ministry of Health (grant 201002007); and the Shanghai Leading Academic Discipline Project (grant B111).

Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental figures and tables accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.cllc.2015.02.001.

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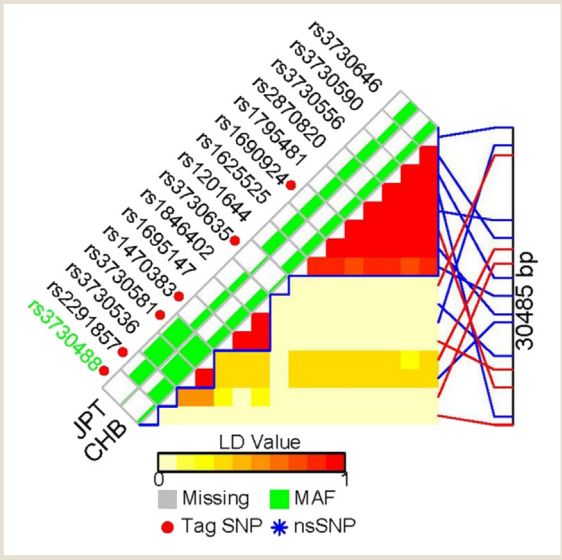
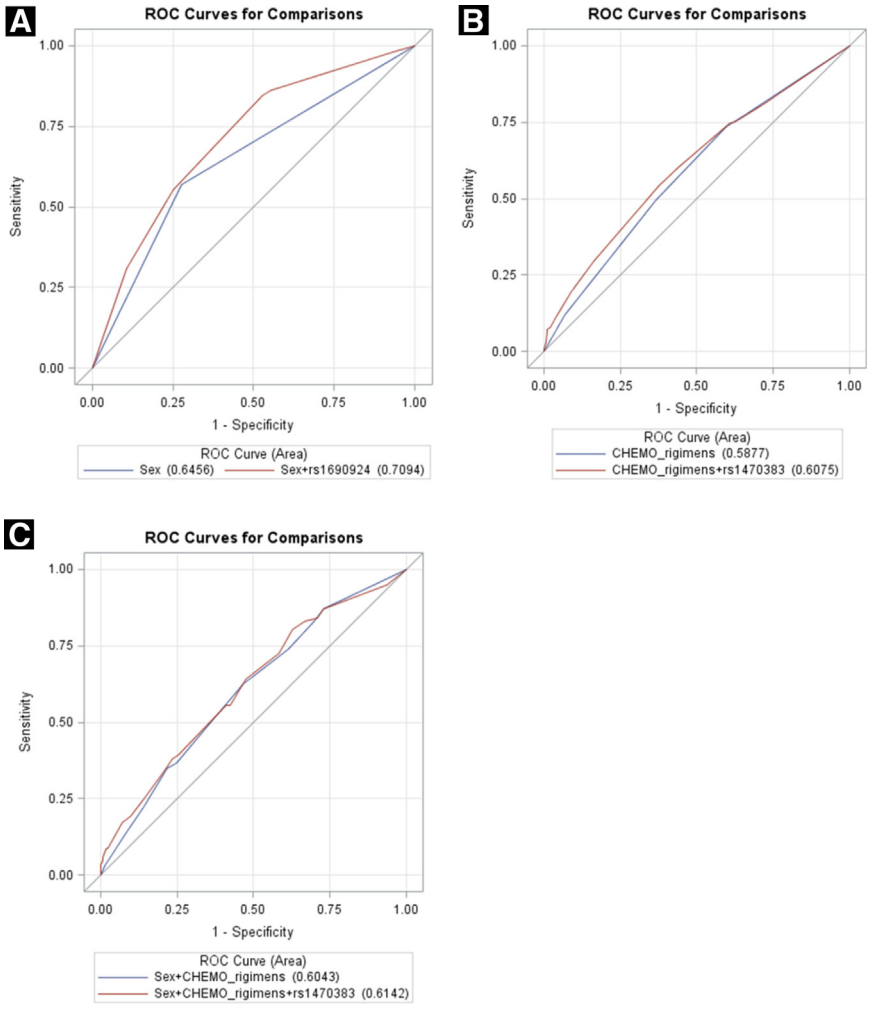
Supplemental Figure 1	TagSNP Selection of MDM2 (Pairwise Linkage Disequilibrium r2 > 0.8) According to Genotyping Data of Han Chinese Individuals From Beijing, China (CHB), and Individuals From Tokyo, Japan (JPT), in HapMap Database Using SNPinfo (Available at: http://snpinfo.niehs.nih.gov/snpinfo/snptag.htm)



Abbreviations: LD = linkage disequilibrium; MAF = minor allele frequency; SNP = single nucleotide polymorphism.

Supplemental Figure 2 Receiver Operating Characteristic (ROC) Curve With and Without Single Nucleotide Polymorphisms in Prediction Models of Chemotherapy-related Toxicity. (A) ROC Curve for Gastrointestinal Toxicity Prediction ($P = .002$). (B) ROC Curve for Hematologic Toxicity Prediction ($P = .103$). (C) ROC Curve for Overall Toxicity Prediction ($P = .283$). A Codominant Model Was Used for All SNPs in ROC Curve Estimation



Supplemental Figure 3 Linkage Disequilibrium (LD) Between 6 Literature Single Nucleotide Polymorphisms (SNPs) and the 5 tagSNPs of Our Study. reported SNPs Labeled Green and the 5 tagSNPs in Black. High LD Seen Between rs1690924 and 4 Literature SNPs (rs937283, rs937282, rs1144944, and rs1690916), rs3730581, and 2 Other Literature SNPs (rs3730536 and rs2279744)

Supplemental Table 1 Summary of Selected MDM2 SNPs in Present Study

SNP	Position	Gene Location	Genotyping Rate (%)	MAF		*P* Value (HWE)
				CHB	Our Study	
rs3730488	69201496	5′ Near gene	99.9	0.06	0.04	.235
rs1690924	69205321	Intron 2	100	0.19	0.28	.693
rs1470383	69207162	Intron 2	100	0.17	0.17	.821
rs2291857[a]	69218038	Intron 3	NA	0.30	NA	NA
rs3730581	69219492	Intron 3	99.9	0.43	0.49	.157
rs3730635	69229123	Intron 4	100	0.05	0.02	.502

Abbreviations: CHB = Han Chinese in Beijing; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; SNPs = single nucleotide polymorphisms.
[a]Failed in design.

Supplemental Table 2 Association of Demographic and Clinical Variables With Overall Survival and Progression-Free Survival in Patients With Advanced NSCLC Treated With First-Line Platinum-Based Chemotherapy

	Overall Survival						Progression-free Survival					
Variable	Patients (n)	Events (n)	MST (mo)	Log-Rank *P*	HR (95% CI)	*P* Value[a]	Patients (n)	Events (n)	MST (mo)	Log-Rank *P*	HR (95% CI)	*P* Value[a]
Sex				.037		.879				.138		.511
Male	465	336 (72.3)	17.2		1.00 (Ref)		465	308 (66.2)	6.8		1.00 (Ref)	
Female	197	130 (66.0)	20		0.98 (0.73-1.32)		197	141 (71.6)	5.8		1.11 (0.82-1.49)	
Age (years)				.107		.227				.445		.446
≤58	333	229 (68.8)	19.4		1.00 (Ref)		333	235 (70.6)	5.9		1.00 (Ref)	
>58	237	237 (72.0)	16.8		1.12 (0.93-1.36)		329	214 (65.0)	7.1		0.93 (0.77-1.12)	
Smoking status				.034		.316				.171		.856
Never smokers	275	183 (66.5)	19.5		1.00 (Ref)		275	192 (69.8)	5.7		1.00 (Ref)	
Ever smokers	386	283 (73.3)	16.9		1.15 (0.88-1.50)		386	256 (66.3)	6.9		0.98 (0.74-1.29)	
ECOG PS				.090		.139				.009		.013
0-1	605	421 (69.6)	18.4		1.00 (Ref)		605	404 (66.8)	6.6		1.00 (Ref)	
2	554	42 (77.8)	17.7		1.27 (0.93-1.75)		54	43 (79.6)	4.4		1.49 (1.09-2.05)	
TNM stage				.042						.419		
IIIA	49	29 (59.2)	27		1.00 (Ref)	—	49	37 (75.5)	9		1.00 (Ref)	—
IIIB	189	136 (71.9)	18.4		1.70 (1.14-2.55)	.01	189	128 (67.7)	6		1.13 (0.78-1.64)	.510
IV	422	300 (71.1)	17.7		1.85 (1.25-2.72)	.002	422	283 (67.1)	6.4		1.18 (0.83-1.68)	.358
Histologic type				.017						.701		
Adenocarcinoma	429	286 (66.7)	19		1.00 (Ref)		429	293 (68.3)	6.4		1.00 (Ref)	—
Squamous cell	141	110 (68.0)	15		1.32 (1.03-1.67)	.026	141	92 (65.2)	6.9		0.98 (0.76-1.26)	.853
Adenosquamous carcinoma	13	11 (84.6)	12.7		1.80 (0.98-3.32)	.059	13	8 (61.5)	6.6		1.19 (0.58-2.43)	.643
Other	77	58 (75.3)	17.4		1.27 (0.95-1.70)	.102	77	54 (70.1)	6.2		0.91 (0.67-1.22)	.515
GI toxicity				.209		.267				.814		
Absent	586	406 (69.3)	18.6		1.00 (Ref)		586	398 (67.9)	6.5		1.00 (Ref)	—
Present	58	43 (74.1)	16.0		1.20 (0.87-1.67)		58	39 (67.2)	6.2		0.98 (0.69-1.38)	.933
Hematologic toxicity				.837		.611				.414		.416
Absent	481	133 (69.2)	18.0		1.00 (Ref)		481	331 (68.8)	6.0		1.00 (Ref)	
Present	166	122 (73.5)	17.8		1.06 (0.86-1.30)		166	107 (64.5)	6.9		0.91 (0.73-1.14)	
Overall toxicity				.831		.735				.456		.417
Absent	436	299 (68.6)	18.6		1.00 (Ref)		436	299 (68.6)	6.3		1.00 (Ref)	
Present	200	145 (72.5)	18.4		1.04 (0.85-1.27)		200	132 (66.0)	6.9		0.92 (0.74-1.13)	

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; GI = gastrointestinal; HR = hazard ratio; MST = median survival time; NSCLC = non–small-cell lung cancer; Ref = reference.
[a]Adjusted for all variables in Supplemental Table 2.

Supplemental Table 3 Stratification Analysis of MDM2 rs1470383 and Hematologic Toxicity by Clinical and Demographic Variables

Variable	Toxicity Present/Absent (n)		OR (95% CI)[a]	*P* Value[a]
	AA + AG	GG		
Age (years)				
≤58	68/254	7/4	6.56 (1.84-23.43)	.004[b]
>58	84/233	6/6	2.53 (0.77-8.27)	.124
Sex				
Male	103/346	7/9	2.67 (0.96-7.49)	.072
Female	94/136	6/1	15.22 (1.74-133.35)	.009[b]
Smoking status				
Never smokers	68/201	6/1	15.74 (1.83-135.49)	.003[b]
Ever smokers	84/186	7/9	2.73 (0.95-7.77)	.061
Performance status				
0-1	142/442	13/9	4.56 (1.88-11.04)	.0008[b]
2	11/43	NA	NA	NA
TNM stage				
IIIA	11/36	2/0	NA	NA
IIIB	45/141	1/2	1.28 (0.11-15.13)	.844
IV	97/308	10/8	4.17 (1.56-11.14)	.004[b]
Histologic type				
Adenocarcinoma	101/312	10/7	4.11 (1.51-11.20)	.001[b]
Squamous cell	31/108	1/1	1.41 (0.08-24.18)	.812
Adenosquamous carcinoma	3/9	0/1	NA	NA
Other	18/56	2/1	10.87 (0.59-200.89)	.198

Abbreviations: CI = confidence interval; NA = not applicable; OR = odds ratio.
[a]With adjustment for chemotherapy regimen.
[b]Statistically significant.

Supplemental Table 4 False-Positive Reporting Probability for SNPs Showing Significant Associations With Clinical Outcomes

SNP	Clinical Outcomes	Genetic Model	HR/OR (95% CI)	*P* Value	Statistical Power	Prior Probability[a]			
						.25	.1	.01	.001
rs1470383	Overall toxicity[b]	Recessive	3.37 (1.39-8.18)	.007	0.037	.079[c]	.204	.738	.966
	Hematologic toxicity[d]	Recessive	4.08 (1.73-9.58)	.001	0.15	.028[c]	.079[c]	.486	.905
	Overall survival[b]								
	Absent overall toxicity	Additive	1.39 (1.11-1.75)	.004	0.74	.020[c]	.058[c]	.404	.872
	Absent hematologic toxicity	Genotypic	1.44 (1.17-1.79)	.0008	0.64	.005[c]	.014[c]	.136[c]	.613
rs1690924	Gastrointestinal toxicity[e]	Genotypic	2.32 (1.30-4.14)	.004	0.60	.022[c]	.062[c]	.421	.880
rs3730635	Overall survival for patients without hematologic toxicity[b]	Additive	0.45 (0.21-0.96)	.039	0.16	.430	.693	.961	.996
Haplotype AAG	Overall survival	Haplotype	0.66 (0.46-0.95)	.025	0.48	.137[c]	.323	.840	.981

Abbreviations: CI = confidence interval; HR = hazard ratio; OR = odds ratio; SNP = single nucleotide polymorphism.
[a]Prior effect sizes were assumed at 2.5 and 1.5 for SNPs for platinum toxicity and overall survival, respectively.
[b]Adjusted for age, sex, TNM stage, and histologic type.
[c]Statistically significant.
[d]Adjusted for chemotherapy regimen.
[e]Adjusted for sex.