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Pol ζ polymorphisms are associated with platinum based chemotherapy response and side effects among non-small cell lung cancer patients

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Lung cancer is the greatest contributor to tumor-derived death. Traditionally, platinum-based chemotherapies are the primary treatment for most patients. However, intrinsic drug resistance and side effects limit the efficacy of platinum-based chemotherapies. Previous studies demonstrated that Pol ζ can modulate cellular sensitivity to chemotherapy. The primary aim of this study was to investigate the potential role of the polymorphism of Pol ζ in platinum-based chemotherapy tolerance and side effects. A total of 663 patients who were newly histologically diagnosed with advanced NSCLC were enrolled. Their treatment response was classified into four categories: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The gastrointestinal and hematological toxicity incidence was assessed twice a week during the entire first line of treatment. Thirteen SNPs of *REV3* and *REV7* were genotyped. The associations between SNPs and the treatment response or toxicity were analyzed with a logistic regression model. We discovered that five SNPs were correlated with the treatment response. Specifically, rs240969 was significantly associated with the treatment response, after a Bonferroni correction, in smokers and a combined cohort (P=0.048 and P=0.0082, respectively) as well as with rs3218573 in smokers (P=0.036). In addition, we discovered that the incidence of grade 3 or 4 gastrointestinal toxicity was significantly higher in patients carrying a G/G genotype of rs240966 or an A allele of rs456865. We also identified that five SNPs, namely rs240966, rs4945880, rs465646, rs2233025 and rs2336030, that were correlated with an increased risk of grade 3 or grade 4 hematologic toxicity. The *REV3* and *REV7* polymorphisms are in a catalytic subunit and an accessory subunit of Pol ζ, respectively, and participate in platinum-chemotherapy tolerance and side effects.

Key words: REV3, REV7, Pol ζ, platinum-based chemotherapies, translesion synthesis, toxicity

Lung cancer is the highest contributor to cancer-related deaths, and non-small cell lung cancer (NSCLC) accounts for nearly 80% of all lung cancer deaths [1]. The incidence rate of lung cancer is rapidly rising due to tobacco use, air pollution, and other cancer-causing factors [2]. Although targeted therapy is very efficient and tremendously improves the progress-free survival (PFS) and overall survival (OS) of lung cancer patients [3-5], however,over 70% of patients lack the positive biomarkers that are considered necessary for platinum-based chemotherapies as the traditional front-line treatment [6, 7]. The efficacy of platinum-based chemotherapies is severely limited by intrinsic drug resistance. In addition, while platinum can kill uncontrollably dividing tumor cells by coupling to DNA and terminating the replication of DNA, normal cells will also be inevitably damaged [8].

Previous studies have shown that DNA repair systems play an essential role in platinum-based chemotherapy tolerance [8-11]. DNA inter- or intra-crosslinking caused by platinum drugs, such as cisplatin, can be removed by several DNA repair pathways [9]. Translesion synthesis (TLS) permits the continuity of the replication fork by allowing replication through those lesions instead of replacing the mutated base or nucleotide around the lesions according to the opposite template [12]. However, the TLS pathway may be a double-edged sword because of its low fidelity, which can lead to platinum chemotherapy tolerance [13-15] and side effects. Pol ζ consists of a catalytic subunit, *REV3,* and an accessory subunit, *REV7*,

Table 1. Genotype distribution of all SNPs

Genotype	n (%)
rs240966	
A/A	31 (4.7)
A/G	222 (33.5)
G/G	410 (61.8)
rs240969 (n=660)	
A/A	84 (12.7)
A/G	303 (45.9)
G/G	273 (41.4)
rs9487643 (n=645)	
A/A	557 (86.4)
A/G	83 (12.9)
G/G	5 (0.8)
rs4945880	
A/A	68 (10.3)
A/G	276 (41.6)
G/G	319 (48.1)
rs3218573	
A/A	536 (80.8)
A/G	119 (17.9)
G/G	8 (1.2)
rs456865 (n=656)	
A/A	227 (34.6)
A/G	307 (46.8)
G/G	122 (18.6)
rs465646 (n=661)	
A/A	437 (66.1)
A/G	195 (29.5)
G/G	29 (4.4)
rs3218606	
A/A	0 (0)
A/G	29 (4.4)
G/G	634 (95.6)
rs17510346 (n=660)	
A/A	0 (0)
A/G	0 (0)
G/G	660 (100)
rs2336030 (n=662)	
A/A	212 (32.0)
A/G	315 (47.6)
G/G	135 (20.4)
rs2233025	
A/A	15 (2.3)
A/G	197 (29.7)
G/G	451 (68.0)
rs2233006 (n=661)	
A/A	138 (20.9)
A/T	317 (48.0)
T/T	206 (31.1)
rs746218	
A/A	17 (2.6)
A/G	177 (26.7)
G/G	469 (70.7)

which are essential for TLS [16]. Pol ζ lacks 3' to 5' exonuclease activity and may extend primers with terminal mismatches [16]. Several studies have reported that Pol ζ can modulate cellular sensitivity to chemotherapy [17-19]. However, very few studies have focused on the association between platinum resistance and polymorphisms in *REV3* and *REV7*.

Platinum-based chemotherapy can cause DNA damage, and TLS might be an efficient rescue pathway for both tumor and normal cells. In this study, we aim to use SNPs to explore the contribution of Pol ζ to side effect toxicity and the prognosis of platinum-based chemotherapy.

Patients and methods

Patients. All patients diagnosed with advanced NSCLC were recruited from the Shanghai Chest Hospital between Mar 2005 and Jan 2010.All were histologically confirmed as having stage IIIA-IV NSCLC and were described in our previous study [20]. All patients consented to participate in the study and to allow their biological samples to be genetically analyzed accordance with the process approved by the Ethical Committee of Shanghai Chest Hospital. See Supplementary for details.

Chemotherapy regiment. All of the patients enrolled in present study accepted platinum-based chemotherapy regimen combined with other medicine. See supplementary for details.

Specimen preparation and genotyping. Over 2 ml of peripheral blood was collected in EDTA-anticoagulant tubes before the patients began their treatment. Genomic DNA was extracted from the blood using the QIAamp DNA MAX Kit (Qiagen, Hilden, Germany) according to the manufacture information.

We chose 9 SNPs of *REV3*, namely rs240966, rs240969, rs9487643, rs4945880, rs3218573, rs456865, rs465646, rs3218606 and rs17510346, while 4 SNPs for *REV7*, namely rs2336030, rs2233025, rs2233006 and rs746218 (Table 1). iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Sequenom, Inc.) was used to genotype the SNPs.

Statistical analysis. The complete response (CR) and partial response (PR) were combined as responders, and stable disease (SD) and progressive disease (PD) were grouped as non-responders. Toxicity outcomes were dichotomized by the presence or absence of grade 3 or 4 toxicity during the first-line of treatment.

Nonparametric χ^2 test was utilized for the test of Hardy–Weinberg equilibrium among the patients with one degree of freedom. The Bonferroni correction was utilized to enhance the reliability of multiple tests. SNPs were further examined by stratified analysis in sub-groups, which were grouped by sex, age, smoking status or regimens. Logistic regression analysis was utilized to study the association between the genotypes and the response to treatment or the severe side effects, and further estimate the odds ratio (OR) and the corresponding 95% confidence interval (95% CI). The Kaplan-Meier method

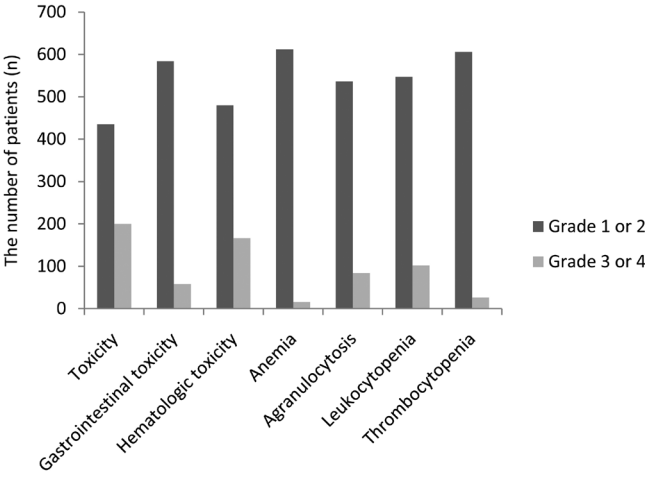

Figure 1. Toxicity outcomes after platinum-based chemotherapy.

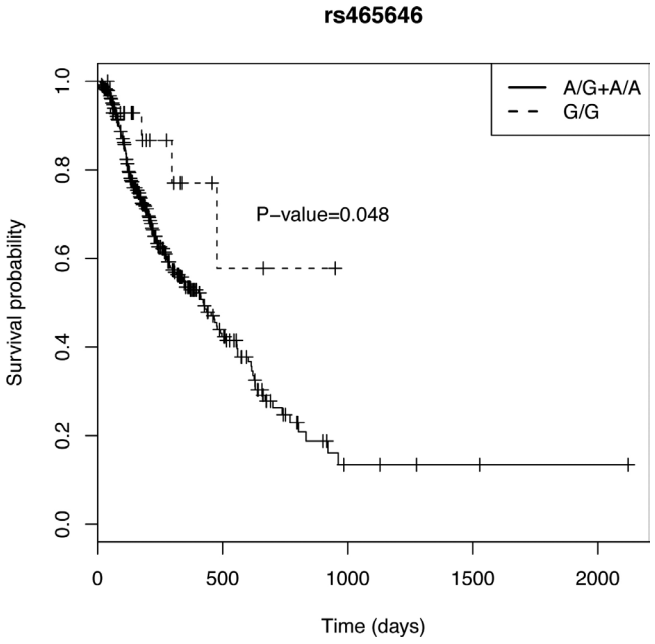

Figure 2. Progression-free survival in advanced NSCLC patients according to rs465646 status

and the log-rank test were utilized to study the progress free survival (PFS) and overall survival (OS) distributions. Cox proportional hazards regression was also utilized to adjust for the patient's gender, age at diagnosis, stage, histological type and smoking status. A value of $P<0.05$ was considered statistically significant. All analyses were performed with R 2.10.0. The program SHEsis [21, 22] was utilized for Haplotype block determination and the association between haplotypes and clinical outcomes.

Results

Patients characteristics. Ultimately, 663 advanced NSCLC patients were included in this study. The study enrolled 464 (70.0%) males and 199 (30.0%) female patients. The average age was 58.3±10.0 years (ranging from 26 to 80 years) for the entire population, with an average age of 59.2±9.97 years (ranging from 26 to 80 years) for males and 56.2 ± 9.75 years for females (ranging from 27 to 76 years). The median age for the entire population was 58.0 years. Forty-nine (7.4%) of the patients were at stage IIIa, 189 (28.5%) were at IIIb, and 423 were (63.8%) at stage IV. Adenocarcinoma was the most common histology type (n = 430, 64.9%), while squamous cell carcinoma accounted for 141 (21.3%), mixed adeno-squamous carcinoma for 13 (2%), and other types of NSCLC counted for 79 (11.9%).

The treatment response was evaluated in 658 patients, among which 120 (18.2%) were identified as responders and 538 (81.8%) showed no response. The incidence of grade 3 or grade 4 gastrointestinal and homological toxicity is showed in Fig. 1.

Genotyping and linkage disequilibrium analysis. A total of thirteen SNPs of two genes were chosen for genotyping. Table 1 shows the genotype distributions of all of the SNPs. Rs17510346 was not a rare mutation distribution in our population and was removed for subsequent analysis. In this study, the genotype distributions of all of the SNPs were consistent with the assumption of a Hardy-Weinberg equilibrium (P>0.05). With a stringent threshold of $r^2>0.66$, the rs240969-rs9487643 and rs240966-rs465646 SNPs were in high disequilibrium in *REV3* ($r^2=0.74$ and $r^2=0.85$, respectively). For *REV7*, we identified rs2336030 and rs2233006 as being linked ($r^2=0.76$).

Association with response to platinum-based chemotherapy. As shown in Table 2, we identified 4 SNPs of *REV3* that were associated with treatment response. Among them, rs240969, rs456865 and rs4945880 were significantly correlated with treatment response in both smokers and the combined cohort, while rs3218573 was only correlated in smokers. After Bonferroni correction, the significance remained for rs240969 (P=0.048 and P=0.0082 for combined cohort and smokers, respectively) and rs3218573 (P=0.036). Patients carrying the G/G genotype of rs240969 or the G allele of rs3218573 were more likely to be responders. We also discovered that patients carrying an A allele of rs2336030, which is located in *REV7*, were more likely to be responders (P=0.037).

Association with side effects of platinum-based chemotherapy. An association between SNPs and side effect outcomes, including gastrointestinal and hematologic toxicity, were analyzed by logistic regression according to the stratification of smoking status.

We identified rs240966 as being significantly correlated with gastrointestinal toxicity in smokers (P=0.040, OR 3.20, 95% CI [1.16-11.32]), while rs456865 (P=0.044, OR 0.48, 95% CI [0.23-0.99]) was correlated with gastrointestinal toxicity in

Table 2. Associations between genotype and treatment effect

Genotype	Smoker (n = 384)				Combined (n = 658)			
	Responder (CR + PR) n (%)	Non-responder (SD + PD) n (%)	OR (95% CI)[a]	P-value	Responder (CR + PR) n (%)	Non-responder (SD + PD) n (%)	OR (95% CI)[a]	P-value
rs240969								
A/A	9 (12.3)	44 (14.2)	0.56 (0.36-0.84)	0.007	12 (10.1)	72 (13.4)	0.65 (0.47-0.90)	0.009
A/G	23 (31.5)	151 (48.9)			45 (37.8)	256 47.8)		
G/G	41 (56.2)	114 (36.9)			62 (52.1)	208 (38.8)		
A/G+A/A	32 (43.8)	195 (63.1)	0.39 (0.22-0.67)	<0.001[b]	57 (47.9)	328 (61.2)	0.55 (0.36-0.83)	0.004[b]
rs456865								
A/A	20 (27.4)	118 (38.4)	0.63 (0.42-0.92)	0.019	32 (26.9)	195 (36.7)	0.72 (0.54-0.96)	0.025
A/G	36 (49.3)	143 (46.6)			60 (50.4)	243 (45.7)		
G/G	17 (23.3)	46 (15.0)			27 (22.7)	94 (17.7)		
A/G+A/A	56 (76.7)	261 85.0)	0.49 (0.25-0.96)	0.035	92 (77.3)	438 (82.3)	0.68 (0.41-1.14)	0.132
rs4945880								
A/A	7 (9.5)	32 (10.3)	0.62 (0.40-0.94)	0.029	9 (7.5)	59 (11.0)	0.72 (0.52-0.99)	0.045
A/G	23 (31.1)	140 (45.2)			45 (37.5)	229 (42.6)		
G/G	44 (59.5)	138 (44.5)			66 (55.0)	250 (46.5)		
A/G+A/A	30 (40.5)	172 (55.5)	0.48 (0.28-0.83)	0.009	54 (45.0)	288 (53.5)	0.68 (0.45-1.02)	0.065
rs3218573								
A/A	52 (70.3)	253 (81.6)	0.42 (0.23-0.75)	0.003[b]	91 (75.8)	442 (82.2)	0.66 (0.43-1.04)	0.064
A/G	20 (27.0)	56 (18.1)			27 (22.5)	90 (16.7)		
G/G	2 (2.7)	1 (0.3)			2 (1.7)	6 (1.1)		
A/G+G/G	22 (29.7)	57 (18.4)	0.44 (0.24-0.83)	0.009	29 (24.2)	96 (17.8)	0.63 (0.39-1.05)	0.068
rs2336030								
A/A	26 (35.1)	88 (28.4)	1.52 (1.03-2.27)	0.037	45 (37.5)	166 (30.9)	1.31 (0.98-1.77)	0.068
A/G	39 (52.7)	152 (49.0)			58 (48.3)	254 (47.3)		
G/G	9 (12.2)	70 (22.6)			17 (14.2)	117 (21.8)		
A/G+A/A	65 (87.8)	240 (77.4)	2.13 (1.03-4.90)	0.054	103 (85.8)	420 (78.2)	1.61 (0.94-2.91)	0.097

Data were calculated by unconditional logistic regression and adjusted for gender, age, smoking, performance status (PS), the type of treatment regimen, the tumor/node/metastasis (TNM) stage and the histological type.
Significance after the Bonferroni correction for multiple tests

non-smokers. The incidence of grade 3 or 4 gastrointestinal toxicity was significantly higher in patients carrying the G/G genotype of rs240966 or the A allele of rs456865. Nevertheless, we did not observe a significant association between the *REV7* polymorphism and gastrointestinal toxicity in this study.

As Table 3 shows, patients carrying the A/A genotype of rs2233025 (P=0.018 and P=0.032 for the combined cohort and nonsmokers, respectively) had a higher incidence of grade 3 or 4 hematologic toxicity in non-smokers and the combined cohort. However, the A allele of rs2336030 (P=0.043), another SNP of *REV7*, was correlated with a higher incidence of grade 3 or 4 hematologic toxicity in smokers. For *REV3*, rs240966 and rs465646 were significantly associated with hematologic toxicity in non-smokers and the combined cohort, while rs4945880 was significantly associated in smokers and the combined cohort.

Survival analysis. Utilizing a multivariable Cox proportional hazards model, we analyzed the relationship between the SNPs and PFS or OS. We identified rs465646 as being significantly associated with PFS (P=0.048, Fig. 2), and the carriers of the G/G genotype showed a longer PFS. None of the SNPs were found to be associated with OS.

Discussion

In this study, the data of 663 patients were analyzed to investigate whether polymorphisms in *REV3* and *REV7*, which encode two subunits of Pol ζ, were associated with better responses and increased toxicity of platinum-based chemotherapy-treated NSCLC. Five SNPs were correlated with the treatment response. Specifically, after Bonferroni correction, rs240969 was significantly correlated to treatment response in smokers and the combined cohort, together with rs3218573 in smokers. We discovered that the incidence of grade 3 or 4 gastrointestinal toxicity was considerably higher in patients carrying a G/G genotype of rs240966 or an A allele of rs456865. In addition, we identified five SNPs, namely rs240966, rs4945880, rs465646, rs2233025 and rs2336030, that were correlated with an increased risk of grade 3 or grade 4 hematologic toxicity. The patients carrying the G/G genotype

Table 3. Stratification analysis between genotype and hematological toxicity by smoking history

	Smoker (n = 386)				Non-smoker (n = 270)				Combined (n = 646)			
Genotype	Any grade 0–2 hematologic toxicity, n (%)	Any grade 3 or 4 hematologic toxicity, n (%)	OR (95% CI)[a]	P-value	Any grade 0–2 hematologic toxicity, n (%)	Any grade 3 or 4 hematologic toxicity, n (%)	OR (95% CI)[a]	P-value	Any grade 0–2 hematologic toxicity, n (%)	Any grade 3 or 4 hematologic toxicity, n (%)	OR (95% CI)[a]	P-value
rs240966												
A/A	13 (4.6)	5 (5.5)	0.95 (0.64-1.44)	0.816	5 (2.6)	8 (10.8)	0.53 (0.34-0.83)	0.005	18 (3.8)	13 (7.8)	0.74 (0.55-0.99)	0.044
A/G	102 (35.9)	31 (34.1)			58 (29.6)	26 (35.1)			160 (33.3)	57 (34.3)		
G/G	169 (59.5)	55 (60.4)			133 (67.9)	40 (54.1)			302 (62.9)	96 (57.8)		
A/G+G/G	271 (95.4)	86 (94.5)	0.79 (0.28-2.52)	0.66	191 (97.4)	66 (89.2)	0.21 (0.06-0.66)	0.009	462 (96.3)	153 (92.2)	0.44 (0.21-0.94)	0.03
rs4945880												
A/A	29 (10.2)	6 (6.6)	1.55 (1.06-2.31)	0.028	24 (12.2)	4 (5.4)	1.36 (0.90-2.09)	0.149	53 (11.0)	10 (6.0)	1.45 (1.09-1.93)	0.011
A/G	127 (44.7)	33 (36.3)			78 (39.8)	30 (40.5)			205 (42.7)	63 (38.0)		
G/G	128 (45.1)	52 (57.1)			94 (48.0)	40 (54.1)			222 (46.3)	93 (56.0)		
A/G+A/A	156 (54.9)	39 (42.9)	1.75 (1.08-2.85)	0.024	102 (52.0)	34 (45.9)	1.29 (0.75-2.21)	0.357	258 (53.8)	73 (44.0)	1.50 (1.05-2.15)	0.025
rs465646												
A/A	182 (64.3)	59 (64.8)	1.09 (0.71-1.64)	0.681	139 (71.3)	43 (58.1)	1.87 (1.19-2.93)	0.006	321 (67.2)	103 (62.0)	1.39 (1.02-1.87)	0.033
A/G	90 (31.8)	27 (29.7)			51 (26.2)	23 (31.1)			141 (29.5)	50 (30.1)		
G/G	11 (3.9)	5 (5.5)			5 (2.6)	8 (10.8)			16 (3.3)	13 (7.8)		
A/G+A/A	272 (96.1)	86 (94.5)	1.49 (0.46-4.26)	0.474	190 (97.4)	66 (89.2)	4.70 (1.50-16.21)	0.009	462 (96.7)	153 (92.2)	2.54 (1.17-5.42)	0.016
rs2233025												
A/A	6 (2.1)	4 (4.4)	0.80 (0.52-1.25)	0.321	1 (0.5)	4 (5.4)	0.81 (0.48-1.37)	0.417	7 (1.5)	8 (4.8)	0.81 (0.58-1.14)	0.217
A/G	83 (29.2)	28 (30.8)			59 (30.1)	19 (25.7)			142 (29.6)	48 (28.9)		
G/G	195 (68.7)	59 (64.8)			136 (69.4)	51 (68.9)			331 (69.0)	110 (66.3)		
A/G+G/G	278 (97.9)	87 (95.6)	0.47 (0.13-1.89)	0.256	195 (99.5)	70 (94.6)	-	0.032	473 (98.5)	158 (95.2)	0.29 (0.10-0.82)	0.018
rs2336030												
A/A	77 (27.1)	35 (38.5)	0.75 (0.53-1.04)	0.091	71 (36.2)	22 (30.1)	1.22 (0.84-1.78)	0.285	148 (30.8)	57 (34.5)	0.93 (0.73-1.20)	0.591
A/G	145 (51.1)	39 (42.9)			87 (44.4)	34 (46.6)			232 (48.3)	74 (44.8)		
G/G	62 (21.8)	17 (18.7)			38 (19.4)	17 (23.3)			100 (20.8)	34 (20.6)		
A/G+A/A	222 (78.2)	74 (81.3)	0.60 (0.36-0.99)	0.043	158 (80.6)	56 (76.7)	1.33 (0.75-2.41)	0.336	380 (79.2)	131 (79.4)	0.85 (0.58-1.24)	0.388

a. Data were calculated by an unconditional logistic regression and adjusted for performance status and the type of treatment regimen.

of rs465646 showed longer PFS. However, further study is needed to confirm this result.

Pol ζ consists of a catalytic subunit, *REV3,* and an accessory subunit, *REV7*. Pol ζ is a B-family polymerase that specializes in TLS. Pol ζ lacks intrinsic 3'-5' exonuclease activity and thus has no proofreading function [23]. When repairing cross-linked agents, which can be caused by platinum-based chemotherapy, Pol ζ is able to induce multiple base substitutions and contribute to spontaneous mutagenesis in mammals [24, 25]. Recent studies have reported that Pol ζ is a major determinant of resistance to platinum-based chemotherapeutic agents [26, 27]. However, to the best of our knowledge, this is the first study identifying the relationship between the polymorphisms in Pol ζ and platinum-based chemotherapy responses or severe toxicity in non-small cell lung cancer patients.

Recently studies have demonstrated that rs465646 is associated with a risk of lung cancer in Chinese patients [28]. This study identified rs465646 not only to be a risk factor but also to play a critical role in chemotherapeutic treatment of lung cancer. This 3'-UTR 460 T>C variant (rs465646) is located in a microRNA binding site. Surface plasmon resonance analysis and luciferase assays demonstrated that the T allele shows a stronger binding affinity for miR-25 and miR-32, leading to a lower expression level of *REV3* [28]. Suppression of *REV3* sensitizes drug-resistant lung tumors to chemotherapy [29]. In addition, we identified rs240969 and rs3218573 as being remarkably associated with the treatment response. An alternative splicing event and an upstream out-of frame ATG of *REV3* have been demonstrated in both human as well as mouse [30, 31]. In addition, the sequence from 253-323 bases upstream of the AUG initiator codon can form a stem-loop hairpin structure in *REV3* mRNA[32, 33]. Such secondary structure might reduce the translational efficiency of *REV3* [33], and the resulting low expression level of *REV3* might further influence the response to chemotherapy [29]. Both of the rs3218573 and rs240969 SNPS are located between exon 28 and exon 29 and may participate in the regulation of alternative splicing. Nevertheless, these require further functional study to confirm their role.

In summary, we discovered that polymorphisms in *REV3* and *REV7* are correlated with platinum-based chemotherapy response and severe toxicity. Our study provides a reference for the future study of platinum-based chemotherapy responses and severe toxicity. Because of the limitations of this study, further in vivo functional studies are needed to elucidate the biological basis of these findings.

Supplementary information is available in the online version of the paper.

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Supplementary Information

Pol ζ polymorphisms are associated with platinum based chemotherapy response and side effects among non-small cell lung cancer patients

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Patients

Patients who accepted at least two cycles of treatment were eligible for present study if they fulfilled the following criteria: age 18 to 80 years old; stage III-IV without radical surgery; no prior history of malignancy except non-melanoma skin cancer, in situ carcinoma of the cervix or "cured" malignant tumors (>5 years disease-free survival); no prior history of chemotherapy; Eastern Cooperative Oncology Group 0-2; with normal liver and kidney function; no uncontrolled infectious diseases; no serious medical or psychological factors that might prevent adherence to the treatment schedule and no active congestive heart failure. All of the patients were unrelated ethnic Han Chinese. All patients consented to participate in the study and to allow their biological samples to be genetically analyzed in accordance with the process approved by the Ethical Committee of the Hospital.

Personal information, including age at diagnosis, gender, smoking status and packs per year, family and personal history of disease, were recorded from the patients' self-reports. A clinical index used in the analysis was gathered from clinical laboratory reports and pathological reports.

The patients' responses to treatment were classified into four categories: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), according to the WHO criteria. The term effect was assessed after two cycles of treatment. The incidence of grade 3 or 4 toxicity was assessed twice a week during the entire first line of treatment, according to the National Cancer Institute Common Toxicity Criteria.

Chemotherapy regiment

All of the patients enrolled in this study were given first-line platinum-based chemotherapy: navelbine: 25 mg/m^2 on days 1 and 8 every 3 wks in combination with cisplatin (NP) 75 mg/m^2 or carboplatin AUC 5 (NC), also administered on day 1, every 3 wks; gemcitabine 1,250 mg/m^2 on days 1 and 8 every 3 wks in combination with cisplatin (GP) 75 mg/m^2 or carboplatin AUC 5 (GC), both administered on day 1, every 3 wks; taxol 175 mg/m^2 on day 1 every 3 wk in combination with cisplatin (TP) 75 mg/m^2 or carboplatin AUC 5 (TC), also administered on day 1, every 3 wks; docetaxel 75 mg/m^2 on day 1 every 3 wks in combination with cisplatin (DP) or carboplatin AUC 5 (DC) 75 mg/m^2, also administered on day 1, every 3 wks. A few patients accepted other platinum-based combination therapies with other medicines. All treatment for the patients were maintained for at least two cycles and produced serious resistance or side effects.