Original Article

Association of *CHEK2* polymorphisms with the efficacy of platinum-based chemotherapy for advanced non-small-cell lung cancer in Chinese never-smoking women

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Background: Cell cycle checkpoint kinase 2 (CHEK2) plays an essential role in the repair of DNA damage. Single nucleotide polymorphisms (SNPs) in DNA repair genes are thought to influence treatment effects and survival of cancer patients. This study aimed to investigate the relationship between polymorphisms in the *CHEK2* gene and efficacy of platinum-based doublet chemotherapy in never-smoking Chinese female patients with advanced non-small-cell lung cancer (NSCLC).

Methods: Using DNA from blood samples of 272 Chinese advanced NSCLC non-smoking female patients treated with first-line platinum-based chemotherapy, we have analyzed the relationships between four SNPs in the *CHEK2* gene and clinical outcomes.

Results: We found that overall survival (OS) was significantly associated with *CHEK2* rs4035540 (Log-Rank P=0.020), as well as the *CHEK2* rs4035540 dominant model (Log-Rank P=0.026), especially in the lung adenocarcinoma group. After multivariate analysis, patients with rs4035540 A/G genotype had a significantly better OS than those with the G/G genotype (HR =0.67, 95% CI, 0.48–0.93; P=0.016). In the toxicity analysis, it was observed that patients with the *CHEK2* rs4035540 A/A genotype had a higher risk of gastrointestinal toxicity than the G/G genotype group (P=0.009). However, there are no significant associations between chemotherapy treatments and genetic variations.

Conclusions: Our findings indicate that SNPs in *CHEK2* are related to Chinese advanced NSCLC never-smoking female patients receiving platinum-based doublet chemotherapy in China. Patients with rs4035540 A/G genotype have a better OS. And patients with rs4035540 A/A genotype have a higher risk of gastrointestinal toxicity. These results point to a direction for predicting the prognosis for Chinese never-smoking NSCLC female patients. However, there are no significant associations between chemotherapy treatments and SNPs in *CHEK2*, which need more samples to the further study.

Keywords: Cell cycle checkpoint kinase 2 (*CHEK2*); single nucleotide polymorphisms (SNPs); non-small-cell lung cancer (NSCLC); chemotherapy; never-smoking women

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Introduction

In China, lung cancer is the most common cause of the deaths by malignancies. Studies show that the incidence of lung cancer is closely related to the ability to repair DNA damage caused by environmental exposure and other factors. Non-small-cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases. As first-line therapy for advanced NSCLC, platinum-based combination chemotherapy has the effect of enhancing cancer patients' overall survival (OS) and quality of life (1). However, different groups of patients still have different prognoses. It is essential to find precise and effective biomarkers in order to establish personal treatment regimens for each patient. There is arising overall trend toward an increasing incidence and mortality of NSCLC among females, especially in those who never smoked (2). Previous studies have researched the prognosis factors in lung cancer for women, including performance status (PS) and clinical stages of the disease (3).

Single nucleotide polymorphisms (SNPs) are common in the human genome. There is growing evidence that demonstrates that the presence of some SNPs can predict NSCLC patients' response to chemotherapy (4-7). Evidence for genetic susceptibility to lung cancer will increase the chance to study prognosis factors for never-smoking women with NSCLC receiving chemotherapy.

There are some potential genes to be as the prediction biomarker for cancer till now, such as *BRCA1* (8), *XRCC1* (9,10), *ABCB1* (11), and *NDRG4* (12). High-lighting *BRCA1* promoter methylation may be a biomarker for effect and better prognosis of DNA damaging agents for breast cancer (8), *XRCC1* genetic variants may be the markers for predicting lung cancer susceptibility (9) and are associated to the OS of advanced NSCLC patients treated by gemcitabine/platinum (10). *ABCB1* C3435T gene polymorphism may as a potential biomarker of progress free survival in breast cancer patients (11). And as highly expressed methylated *NDRG4* gene in colorectal cancer (CRC) patients, the detection of methylated *NDRG4* could be used as a novel diagnostic technique for CRC (12).

Since our team has reported some articles about the relationship between treatments efficacy and SNPs in different genes, such as *RB1* (13), *WEE1* (7), etc. And *WEE1* as a G2/M checkpoint kinase can induce G2/M cell cycle arrest in the response to DNA damage. Cell cycle checkpoint kinase 2 (CHEK2) also is a G2/M checkpoint kinase, so we focused on it after *WEE1* SNPs research selectively. The checkpoint kinase 2 (*CHK2*, or *CHEK2*) gene on chromosome 22 is still playing an important role in tumor suppression (14). DNA damage can cause CHEK2 phosphorylation (15), activated CHEK2 can lead to phosphorylation of the CDC25 family, BRCA1, p53, and other similar functional effectors in order to start the cell cycle checkpoint regulation (15-17). Results from previous studies have shown a relationship between the *CHEK2* mutation and an increased risk for lung cancer (18,19), breast cancer (20,21), prostate cancer (22,23), colorectal cancer (24,25) and other cancers (26,27). In addition, there was a relationship between the decreased risk of endometrial cancer and the rs8135424 *CHEK2* SNP (28). And there was a significant risk association between *CHEK2* SNP rs17507066 and serous epithelial ovarian cancer (29). There are few previous studies which reported about the relationship about *CHEK2* SNPs with lung cancer, especially the rs4035540 in *CHEK2*. We have added a table to summary about the lung cancer related SNPs *CHEK2* gene (*Table S1*).

However, for NSCLC never-smoking female patients receiving platinum-based doublet chemotherapy, there is not enough convincing evidence to prove that a relationship between the *CHEK2* genetic polymorphisms and both the prognosis as well as the chemotherapeutic toxicity exists.

In this study, we have analyzed the association between four SNPs in the *CHEK2* gene and the efficacy of chemotherapy, as well as the toxicity. We analyzed the data collected from 272 advanced NSCLC never-smoking female patients to try to find the new research direction to predict survival, efficacy, and/-or toxicity for Chinese never-smoking females with NSCLC.

Methods

Patients

In this study, we enrolled 272 female patients who had been diagnosed with clinical stages III–IV NSCLC and were receiving platinum-based (carboplatin or cisplatin) chemotherapy. Enrolled patients were from Shanghai Pulmonary Hospital, Shanghai Zhongshan Hospital, Shanghai Chest Hospital, and Shanghai Changhai Hospital (all China) and would provide their written informed consent before being included in this study. Detailed inclusion criteria included several specific conditions: (I) stages III–IV NSCLC confirmed by at least one diagnostic criteria; (II) inoperable only; (III) platinum-based (cisplatin or carboplatin) chemotherapy as the first-line treatment; (IV) confirmed primary NSCLC by the histological test; (V) the Eastern Cooperative Oncology Group (ECOG) (30)

Table 1 SNPs of *CHEK2* gene

NCBI SNP ID	Location	Base change	Minor allele frequency	
			Current data	HCB
rs4035540	Intron	A/G	0.292	0.279
rs5762746	Intron	G/A	0.413	0.389
rs2236141	UTR-5	G/A	0.155	0.167
rs2236142	5' flank; upstream variant 2KB	C/G	0.399	0.389

SNP, single nucleotide polymorphism; CHEK2, cell cycle checkpoint kinase 2; UTR, untranslated region; HCB, Han Chinese Beijing population.

performance status (PS) from 0 to 2; (VI) no history of cancer in other organs; (VII) never received chemotherapy treatment previously; and (VIII) never smoking.

The ethics committee of Shanghai Pulmonary Hospital has approved this study. We have an approval number 2009FK31 from the Institution. Peripheral blood sample collection and the epidemiological information collection all had the informed consent of participants complying with the provisions of the ethics.

Treatment schedules and data collection

All enrolled patients received first-line platinum-based doublet chemotherapy with one of the following double chemotherapy regimens for 2 to 6 cycles: (I) carboplatin or cisplatin plus vinorelbine (NP/NC); (II) carboplatin or cisplatin plus gemcitabine (GP/GC); (III) carboplatin or cisplatin plus paclitaxel (TP/TC); or (IV) carboplatin or cisplatin plus docetaxel (DP/DC).

Following the Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.1 (31), patients' responses to platinum-based therapy were assessed after the first two chemotherapy cycles. All responses were re-assessed at least four weeks after initial assessment using the same criteria. For data analyses, subjects achieving a stable disease (SD), partial response (PR) or complete responses (CRs) were grouped as responders; subjects with progressive disease (PD) were considered non-responders. OS was defined as the first day the patients received chemotherapy treatments to the final follow-up or day of death.

Following the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 (32), chemotherapy toxicity was evaluated twice weekly. A toxicity grade of 3 or 4 recorded during the initial two cycles of therapy was collected for analysis.

Tag SNPs selection and genotyping procedure

Four tag SNPs (rs4035540, rs5762746, rs2236141, and rs2236142) from the gene region (including 2 kb upstream) of the human *CHEK2* (54163bp, 22q12.1) were selected from the Han Chinese population in Beijing (HCB) data downloaded from HapMap SNP databases (http://www.hapmap.org/) (*Table 1*). The tag SNPs were selected with a cutoff of 0.05 of a minor allele frequency (MAF) by Haploview (33) (http://www.broadinstitute.org/haploview/) or Hardy-Weinberg equilibrium P values <0.05 in the enrolled population. Haplotype blocks were concluded by the methods showed by Gabriel *et al.* (34).

We have collected the blood 3 to 5 milliliters by anticoagulant blood collection tube from each patient before the chemotherapy. And 400 microliters of blood were used for DNA extraction by the Human QIAamp DNA Blood Maxi Kit (Qiagen GmbH, Hilden, Germany). DNA has been checked by NanoDrop 2000 (Thermo Scientific, USA). When the value of OD 260/280 nm was in the range from 1.8 to 2, we indicated that the DNA was suitable for high-throughput sequencing.

In order not to affect the statistical efficiency, we used MAF >0.05, genotyping call rate >0.95 and the GenCall score >0.2 as the filter criteria for SNP genotyping by using the iSelect HD Bead-Chip (Illumina, CA, USA). The silica beads with 3.1 μm diameter and oligonucleotide probes were made at first. Each bead could be combined with thousands of the same probes at the 5' end of sequences. With the use of microporous etched optical fiber technology on a chip, we could easily handle and add the beads to the chip. Beads were combined with fiber microporous in a disorderly manner. Only one bead was actually placed in each of the microprobe. Results could be read with the Illumina BeadScan machine.

Statistical analysis

SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses. The OS of the enrolled patients was examined by the Kaplan-Meier and log-rank tests. Multivariate analyses were carried out using the Cox proportional hazard model by adjusting for clinical characteristics, such as age, TNM stage, pathologic types, adjuvant therapy and PS. Differences with P<0.05 were

Table 2 Clinical characteristics of the recruited non-smoking female patients with advanced NSCLC

Variables	N	mOS (95% CI) (m)[a]	P_{L-R}
Overall	272		
Age			0.012
≤57	146	26.47 (21.76–31.18)	
>57	125	18.87 (16.15–21.59)	
ECOG PS			0.180
0–1	238	22.80 (18.95–26.65)	
2	31	20.67 (14.83–26.50)	
Pathologic type			0.552
Adenocarcinoma	234	23.50 (19.53–27.47)	
Squamous carcinoma	20	23.83 (4.20–43.47)	
Adenosquamous carcinoma	3	15.30 (1.06–29.54)	
Others	15	19.10 (18.68–19.52)	
Adjuvant therapy			0.985
GP/GC	82	25.60 (18.65–32.55)	
TP/TC	72	21.37 (15.00–27.74)	
NP/NC	68	23.50 (14.44–32.56)	
DP/DC	20	20.87 (15.99–25.74)	
Other platinum combinations	30	21.43 (15.59–27.28)	
TNM stage			0.021
III	84	24.90 (18.56–31.24)	
IV	186	20.23 (16.426–24.04)	

[a], survival derived from Kaplan-Meier analysis. NSCLC, non-small-cell lung cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; TP/TC, carboplatin or cisplatin plus paclitaxel; NP/NC, carboplatin or cisplatin plus vinorelbine; GP/GC, carboplatin or cisplatin plus gemcitabine; DP/DC, carboplatin or cisplatin plus docetaxel; TNM, tumor-lymph-node metastasis; N, number; m, months; OS, overall survival; mOS, median time to OS; P_{L-R}, Log-Rank P.

considered statistically significant. Multivariate logistic regression analysis was used to estimate the response to therapy and the toxicity risk for each SNP with adjustment for clinical characteristics.

Results

Association between clinical characteristics and survival in enrolled patients treated with platinum-based chemotherapy

The cohort consisted of 272 NSCLC non-smoking female patients with a median age of 57 years, ranging from 26 to 80 years old. All patients had been diagnosed as IIIa/IIIb or IV clinical stage according to the ECOG PS 1 or 2.

The relationship of clinical characteristics with OS was analyzed using Kaplan-Meier curve test. No significant correlation was observed between the OS and pathologic types, adjuvant therapy, or ECOG PS (score 1 or 2) (*Table 2*). Since we focus on never-smoking female NSCLC patients, the clinical variable which may have effects on OS is the factor analyzed. The log-rank test suggested that younger patients (≤57 years old) had a significant better OS than elderly ones (*Table 2*, Log-Rank P=0.012; *Figure 1A*) and patients with TNM stage III had a significantly better OS than those with stage IV (*Table 2*, Log-Rank P=0.021; *Figure 1B*).

Furthermore, the multivariate Cox's proportional hazards regression analysis suggested that age classified according to a cutoff of 57 years old (*Table 3*, HR =1.62, 95% CI: 1.19–2.22; P=0.002) and TNM stage (*Table 3*, HR =1.63, 95% CI: 1.16–2.30; P=0.005) were independent predictive factors.

Association between CHEK2 tag SNPs and survival in enrolled patients treated with platinum-based chemotherapy

Analysis of the relationship by the log rank test between SNPs and OS showed that *CHEK2* rs4035540 (*Table 4*, Log-Rank P=0.020; *Figure 1C*) was significantly related NSCLC patients' OS. Furthermore, we also investigated the association between OS and both the *CHEK2* rs4035540 dominant model, as well as recessive model. As a result, the dominant model was also found to be a significant factor contributing to the OS of the enrolled patients treated with platinum-based chemotherapy (*Table 4*, Log-Rank P=0.026; *Figure 1D*).

Using multivariate Cox proportional hazard models, patients with rs4035540 A/G genotype had a significantly better OS than those with the G/G genotype (*Table 3*, HR =0.67, 95% CI, 0.48–0.93; P=0.016).

Association between CHEK2 tag SNPs and survival in NSCLC female patients classified by pathologic types

We further investigated the influence of SNPs on OS stratified by pathologic types (*Table 5*). The results suggested that the above rs4035540 (Log-Rank P=0.041) and rs2236142 (Log-Rank P=0.041), as well as the rs4035540 dominant model (Log-Rank P=0.025) were significantly associated with OS in non-smoking female patients with lung adenocarcinoma (*Table 5*, *Figure 2*).

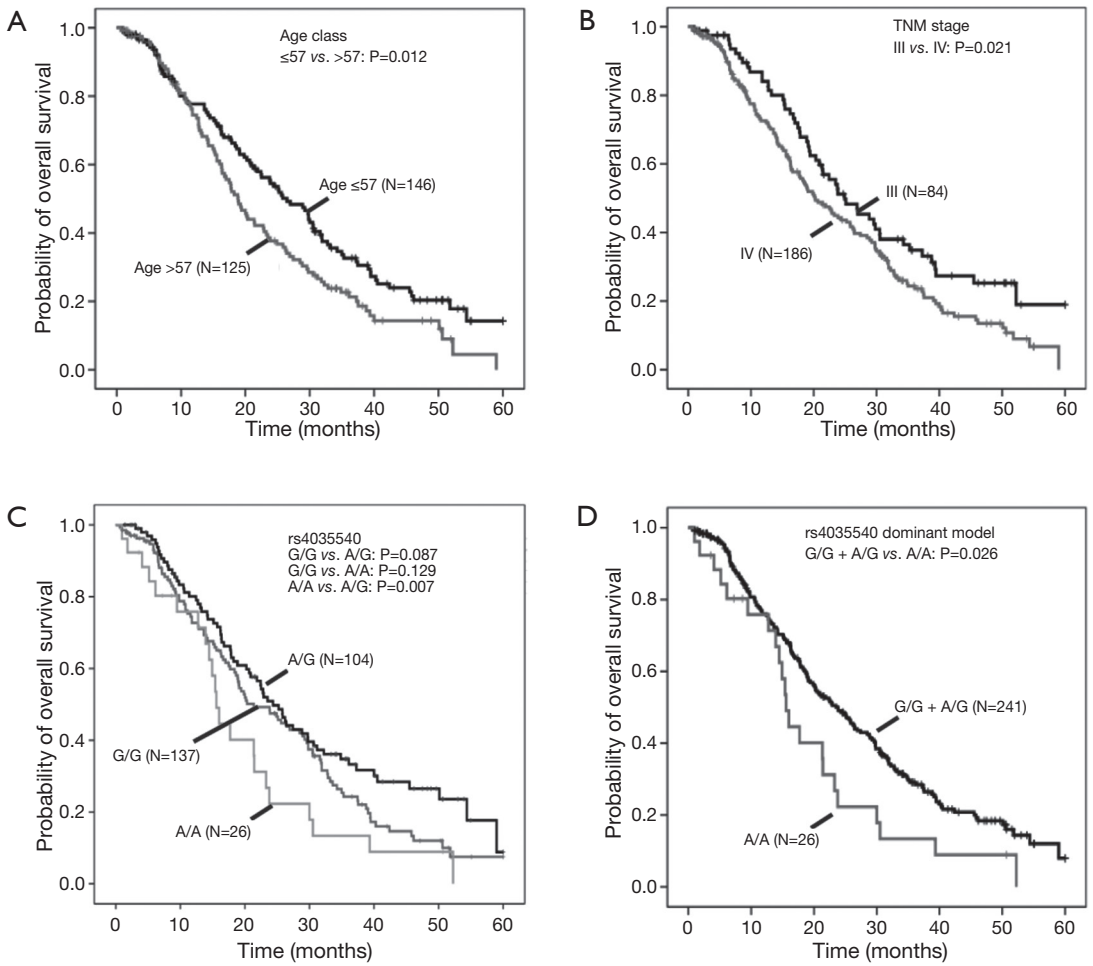

Figure 1 OS curves of significantly associated clinical characteristics and polymorphism in all patients. (A) OS and age classes; (B) OS and TNM stage; (C) OS and rs4035540; (D) OS and rs4035540 dominant model. P values: from the Kaplan-Meier and log-rank tests. OS, overall survival.

Toxicity

After analyzing the possible association of chemotherapy toxicity with different treatment regimens, it was shown that *CHEK2* rs4035540 was significantly associated with gastrointestinal toxicity. There was a significantly higher toxicity in the A/A genotype group than the G/G genotype group (adjusted OR =3.83, 95% CI, 1.36–10.56; P=0.009) (*Table 6*).

Chemotherapy response

There were no significant associations between chemotherapy treatments and genetic variations (*Table S2*). At the same time, we also found there was not any significant relationship between chemotherapy response and genetic variations grouped by the different regimens (*Table S3*).

Haploview analysis of CHEK2 tag SNPs

Among the four SNPs, the rs4035540 and rs5762746 were in strong LD (Linkage Disequilibrium) (|D'|=100%, r^2=0.563). And rs2236141 and rs2236142 were in LD (|D'|=100%, r^2=0.121). The results could be showed from the Haploview Software directly (*Figure S1*). Since only one tag SNP had the significance with the OS, there was no real meaning for analyzing the linkage disequilibrium between different SNPs.

Discussion

CHEK2 plays an essential role in the signaling pathway for DNA damage and cell checkpoint regulation. In order to maintain genomic stability when DNA damage occurs,

Table 3 Multivariate Cox's regression analysis of prognostic factors for OS in the 272 advanced NSCLC never-smoking female patients treated with platinum-based chemotherapy

Variables	HR (95% CI)[a]	P
Age		0.002
≤57	1	R
>57	1.62 (1.19–2.22)	
ECOG PS		0.838
0–1	1	R
2	1.05 (0.649–1.70)	
Pathologic types		0.334
Adenocarcinoma	1	R
Squamous carcinoma	1.25 (0.65–2.39)	0.509
Adenosquamous carcinoma	1.19 (0.29–4.89)	0.814
Others	1.74 (0.93–3.26)	0.082
Adjuvant therapy		0.827
GP/GC	1	R
TP/TC	1.17 (0.79–1.74)	0.428
NP/NC	1.10 (0.72–1.70)	0.657
DP/DC	1.03 (0.52–2.02)	0.943
Other platinum combinations	0.84 (0.48–1.48)	0.555
TNM stage		0.005
III	1	R
IV	1.63 (1.16–2.30)	
rs4035540		0.011
GG	1	R
AG	0.67 (0.48–0.93)	0.016
AA	1.34 (0.79–2.27)	0.272
rs5762746		0.322
AG	1	R
GG	0.82 (0.45–1.48)	0.506
AA	0.63 (0.32–1.26)	0.192
rs2236141		0.840
GG	1	R
AG	0.93 (0.65–1.35)	0.713
AA	1.36 (0.32–5.77)	0.678
rs2236142		0.376
CG	1	R
CC	1.04 (0.71–1.53)	0.826
GG	1.44 (0.86–2.38)	0.163

[a]HRs, 95% CIs and their corresponding P values were calculated using multivariate Cox proportional hazard models, adjusted for other variables. OS, overall survival; NSCLC, non-small-cell lung cancer; HR, hazard ratio, CI, confidence interval; TP/TC, carboplatin or cisplatin plus paclitaxel; NP/NC, carboplatin or cisplatin plus vinorelbine; GP/GC, carboplatin or cisplatin plus gemcitabine; DP/DC, carboplatin or cisplatin plus docetaxel.

Table 4 Association between *CHEK2* tag SNPs and OS in NSCLC patients

SNP and genotype	N	mOS (95% CI) (m)[a]	P_{L-R}
rs4035540			0.020
GG	137	21.37 (15.80–26.93)	
AG	104	24.37 (20.79–27.94)	
AA	26	15.57 (13.94–17.19)	
Dominant model			0.026
GG + AG	241	23.83 (19.89–27.78)	
AA	26	15.57 (13.94–17.19)	
Recessive model			0.315
GG	137	21.37 (15.80–26.93)	
AG + AA	130	22.50 (19.37–25.63)	
rs5762746			0.898
AG	130	22.80 (18.10–27.50)	
GG	90	23.83 (17.78–29.88)	
AA	44	21.37 (12.99–29.75)	
Dominant model			0.946
GG + AG	220	22.90 (19.08–26.72)	
AA	44	21.37 (12.99–29.75)	
Recessive model			0.680
GG	90	23.83 (17.78–29.88)	
AG + AA	174	22.43 (17.90–26.97)	
rs2236141			0.919
GG	188	21.87 (18.44–25.29)	
AG	70	25.83 (17.68–33.98)	
AA	6	27.00 (18.28–35.72)	
Dominant model			0.956
GG+AG	258	22.50 (18.91–26.09)	
AA	6	27.00 (18.28–35.72)	
Recessive model			0.702
AG + AA	76	26.90 (19.33–34.47)	
GG	188	21.87 (18.44–25.29)	
rs2236142			
CG	138	23.50 (19.26–27.75)	
CC	94	23.83 (17.60–30.07)	
GG	39	18.83 (13.63–24.03)	
Dominant model			0.182
CG + CC	232	23.83 (19.72–27.95)	
GG	39	18.83 (13.63–24.03)	
Recessive model			0.657
CG + GG	177	22.50 (18.84–26.16)	
CC	94	23.83 (17.60–30.07)	

[a], survival derived from Kaplan-Meier analysis. N, number; m, months; CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; OS, overall survival; NSCLC, non-small-cell lung cancer; mOS, median time to OS; P_{L-R}, Log-Rank P.

Table 5 Association between *CHEK2* tag SNPs and OS in enrolled patients with adenocarcinoma or non-adenocarcinoma NSCLC

Variables	Adenocarcinoma NSCLC patients			Non-adenocarcinoma NSCLC patients		
	N	mOS (95% CI) (m)[a]	P_{L-R}	N	mOS (95% CI) (m)[a]	P_{L-R}
rs4035540			0.041			0.271
GG	118	25.10 (17.05–33.15)		19	18.87 (11.56–26.18)	
AG	88	24.37 (20.44–28.29)		16	16.37 (0.00–39.21)	
AA	24	15.40 (13.81–16.99)		2	NA	
Dominant model			0.025			0.930
GG + AG	206	25.10 (20.84–29.36)		35	18.87 (14.98–22.76)	
AA	24	15.40 (13.81–16.99)		2	21.37	
Recessive model			0.627			0.126
GG	118	25.10 (17.05–33.15)		19	18.87 (11.56–26.18)	
AG + AA	112	22.80 (19.23–26.37)		18	21.37 (11.44–31.30)	
rs5762746			0.987			0.545
AG	115	22.80 (18.11–27.49)		15	16.23 (0.00–41.67)	
GG	75	26.47 (17.05–35.89)		15	19.07 (12.88–25.25)	
AA	37	19.27 (6.34–32.20)		7	21.37 (8.54–34.20)	
Dominant model			0.877			0.672
GG + AG	190	24.37 (20.05–28.69)		30	18.87 (12.80–24.93)	
AA	37	19.27 (6.34–32.20)		7	21.37 (8.54–34.20)	
Recessive model			0.996			0.271
GG	75	26.47 (17.05–35.89)		15	19.07 (12.88–25.25)	
AG + AA	152	22.80 (18.11–27.49)		22	16.37 (5.12–27.62)	
rs2236141			0.452			0.275
GG	164	NA		24	NA	
AG	59	NA		11	NA	
AA	5	NA		1	NA	
Dominant model			0.901			0.866
GG + AG	223	NA		35	NA	
AA	5	NA		1	NA	
Recessive model			0.235			0.113
AG + AA	64	28.47 (24.91–32.02)		12	16.37 (10.48–22.26)	
GG	164	22.50 (18.51–26.49)		24	21.37 (15.44–27.30)	
rs2236142			0.041			0.271
CG	118	23.87 (19.90–27.83)		20	19.10 (10.94–27.26)	
CC	81	26.90 (18.03–35.78)		13	18.87 (14.38–23.35)	
GG	35	17.70 (13.57–21.83)		4	21.43 (0.00–53.06)	
Dominant model			0.074			0.336
CG + CC	199	25.10 (20.57–29.63)		33	19.07 (15.396–22.74)	
GG	35	17.70 (13.57–21.83)		4	21.433 (0.00–53.06)	
Recessive model			0.966			0.175
CG + GG	153	22.80 (18.44–27.16)		24	21.37 (13.79–28.94)	
CC	81	26.90 (18.03–35.78)		13	18.87 (14.38–23.35)	

[a], survival derived from Kaplan-Meier analysis. N, number; m, months; CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; NSCLC, non-small-cell lung cancer; OS, overall survival; mOS, median time to OS; P_{L-R}, Log-Rank P.

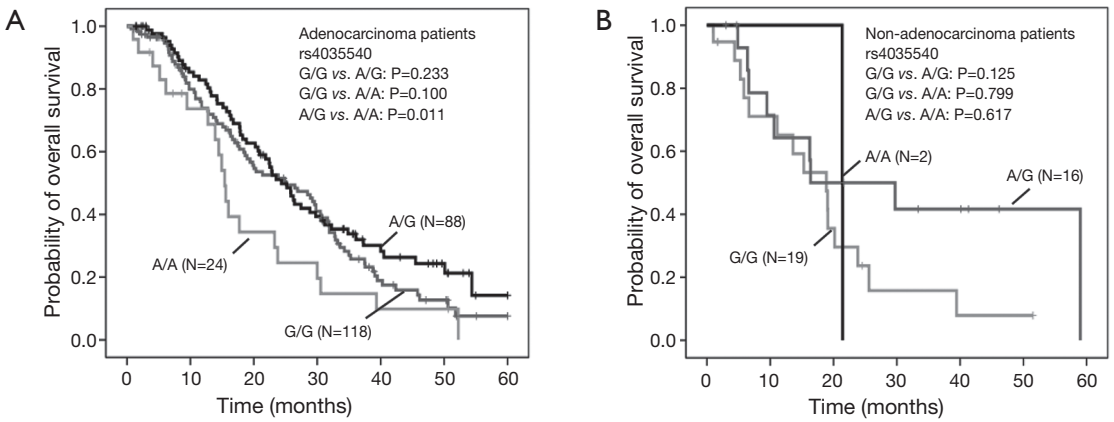

Figure 2 OS curves of significantly associated rs4035540 in all patients classified by pathologic types. (A) OS and rs4035540 in enrolled patients with lung adenocarcinoma; (B) OS and rs4035540 in enrolled patients with lung non-adenocarcinoma. P values: from the Kaplan-Meier and log-rank tests. OS, overall survival.

Table 6 Analysis of association between *CHEK2* SNPs and chemotherapy toxicity

Toxicity (SNP and genotype)	GasTox			HemTox			LeuTox		
	No/yes (N)	OR (95% CI)[a]	P[a]	No/yes (N)	OR (95% CI)[a]	P[a]	No/yes (N)	OR (95% CI)[a]	P[a]
rs4035540			0.023			0.277			0.298
GG	115/17	1	R	91/40	1	R	107/26	1	R
AG	89/14	1.02 (0.46–2.25)	0.960	78/23	0.60 (0.33–1.12)	0.109	88/15	0.57 (0.27–1.19)	0.135
AA	18/8	3.83 (1.39–10.56)	0.009	19/7	0.82 (0.31–2.16)	0.685	23/3	0.60 (0.16–2.27)	0.453
rs5762746			0.060			0.445			0.889
AG	109/20	1	R	91/33	1	R	105/23	1	R
GG	80/7	0.42 (0.17–1.09)	0.074	62/26	1.39 (0.72–2.66)	0.326	73/14	1.21 (0.55–2.69)	0.635
AA	34/10	1.54 (0.63–3.77)	0.343	34/10	0.83 (0.36–1.95)	0.671	37/7	1.14 (0.42–3.08)	0.800
rs2236141			1.000			0.349			0.303
GG	156/29	1	R	137/46	1	R	158/28	1	R
AG	58/10	0.99 (0.43–2.26)	0.984	44/22	1.56 (0.84–2.92)	0.163	53/14	1.80 (0.84–3.87)	0.132
AA	5/0	NA	NA	4/2	1.64 (0.28–9.54)	0.582	5/1	1.85 (0.19–18.35)	0.600
rs2236142			0.056			0.644			0.446
CG	117/20	1	R	99/34	1	R	116/20	1	R
CC	80/9	0.66 (0.28–1.58)	0.354	65/25	1.28 (0.68–2.42)	0.445	77/14	1.18 (0.54–2.59)	0.673
GG	29/10	2.29 (0.93–5.63)	0.071	26/13	1.38 (0.61–3.12)	0.441	29/10	1.80 (0.73–4.47)	0.204

[a]ORs, 95% CIs and their corresponding P values were calculated using multivariate logistic regression analysis, adjusted for age, pathologic type, ECOG PS, adjuvant therapy and TNM stage. CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; N, number; NA, not available; R, reference; NA, null data; OR, odds ratio; CI, confidence interval; GasTox, gastrointestinal toxicity; HemTox, hematological toxicity; LeuTox, leukotoxicity.

CHEK2 is activated via phosphorylation of downstream genes (14). The cell cycle checkpoint is an important mechanism in maintaining genomic stability. A DNA damage checkpoint can detect the DNA damage in the cell cycle and lead to cell cycle arrest to provide enough time to repair the damage (35). Many studies have reported that *CHEK2* gene mutations could increase the risk of some cancers (20,22,27,36-38). Many investigators have researched this area with special emphasis on *CHEK2* 1100delC.

There is a growing realization that genetic polymorphisms not only have effects on cancer development, but also on its prognosis. Numerous SNPs in *CHEK2* have been screened to reveal possible relationships with various cancers. In a study by Lawrenson *et al.* (29), the results indicated a significant risk association between *CHEK2* SNP rs17507066 and serous epithelial ovarian cancer (P=4.74E-7). Meanwhile, Gu *et al.* have reported that (39) about *CHEK2* gene with the results showing that the rs738722 C/T polymorphism and the rs2236142 G/C polymorphism might be protective factors against the risk of lymph node metastasis of esophageal cancer in the Chinese population. However, whether the polymorphism of the *CHEK2* gene is associated with the efficacy of platinum-based doublet chemotherapy in never-smoking NSCLC female patients has not yet been investigated.

Our study has provided evidence for a relationship between the *CHEK2* genetic polymorphisms and efficacy of platinum-based doublet chemotherapy in never-smoking Chinese NSCLC female patients. The A/G allele in rs4035540 in *CHEK2* has been verified to be an independent protective factor for the prognosis for never-smoking female NSCLC patients. At the same time, TNM stages and age classes could also be the independent factors for predicting prognosis for these Chinese patients. Different histological types of NSCLC may have different biological behavior. After the subgroup analysis classified by pathologic types, we found that *CHEK2* rs4035540 and rs2236142 may be significant in predicting OS in the never-smoking female patients lung adenocarcinoma group. Therefore, based on the findings of this study, we consider that *CHEK2* polymorphisms may affect the efficacy of platinum-based chemotherapy, and may play valuable roles in predicting the prognosis for NSCLC with never-smoking female patients.

In addition, although platinum-based doublet chemotherapy has been proved to be effective for NSCLC patients, adverse side effects still exist because of the platinum-related DNA damage (40). We analyzed the incidence of drug toxicities, including gastrointestinal, hematological and leukotoxicities. As a result, in our patients, we found that *CHEK2* rs4035540 A/A genotype group demonstrated higher gastrointestinal toxicity after treatment than the G/G genotype group. This finding suggests that it may help to identify patient subgroups with a strong risk for drug toxicity by testing for the presence of these polymorphisms for the selection of appropriate chemotherapy for the treatment of female NSCLC.

Our results suggest that genetic variations in the *CHEK2* gene may play a role in predicting the toxicity and prognosis of NSCLC. The clinical significance of *CHEK2* rs4035540 polymorphism in platinum-based chemotherapy of NSCLC has not been reported before. The screening of tagged SNPs of *CHEK2* in this study may bring a new evidence of the importance of *CHEK2* in NSCLC and the efficacy of treatments to clinical.

However, *CHEK2* rs4035540 is located in the intron area of the *CHEK2* gene. After the Haploview analysis, we made a very bold speculation that it may modify the expression levels of *CHEK2* gene based on linkage disequilibrium with multiple SNPs. And this speculation about the complex signaling pathway of which CHEK2 is involved is our following plan to research. Determining whether the *CHEK2* rs4035540 A/G genotype can be used a biomarker for the option of platinum-based regimen in NSCLC patients and the detailed mechanism are worth further studying in the future. The additional follow-up studies with a larger patient sample that includes different ethnic populations are warranted.

Conclusions

We demonstrated for the first time that polymorphisms in the *CHEK2* gene may predict the clinical outcomes for advanced Chinese never-smoked NSCLC female patients following platinum-based doublet chemotherapy. Our study provides evidence of a useful molecular research direction which may be easily applied in clinical situation. Consequently, in addition to the clinical stages and the pathologic types classified by lung adenocarcinoma, it may help identify patient subgroups with the risk of poor diseases outcomes by testing for the presence of these polymorphisms; the information can be used to tailor personal therapies in certain populations. The mechanisms for the effects of these SNPs on *CHEK2* biologic functions remain to be clarified in further studies to understand the role of the *CHEK2* gene in determining NSCLC outcomes after platinum-based doublet chemotherapy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the ethics committee of Shanghai Pulmonary Hospital (No. 2009FK31) and written informed consent was obtained from all patients.

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Supplementary

Table S1 *CHEK2* SNPs related to lung cancer

Study	SNPs	Effect size (95% CI)	P
Wang *et al.*, 2014 (18)	rs17879961	0.38[a]	1.27×10^{-13}
Brennan *et al.*, 2007 (19)	rs17879961	0.44 (0.31–0.63)	<0.00001
Zhang *et al.*, 2010 (41)	rs2236141	0.73 (0.57–0.95)	0.0018

[a], the full data cannot been extracted from the study. CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; CI, confidence interval.

Table S2 Analysis of association between *CHEK2* SNPs and therapy response

SNP and genotype	Response/no response (N)	OR (95% CI)[a]	P[a]
rs4035540			0.504
GG	106/26	1	R
AG	78/24	1.22 (0.63–2.37)	0.559
AA	18/7	1.80 (0.65–4.97)	0.254
rs5762746			0.417
AG	94/32	1	R
GG	72/16	0.62 (0.30–1.29)	0.199
AA	35/9	0.96 (0.4–2.33)	0.935
rs2236141			0.730
GG	136/44	1	R
AG	57/13	0.74 (0.36–1.55)	0.428
AA	6/0	NA	NA
rs2236142			0.895
CG	103/32	1	R
CC	71/19	0.85 (0.43–1.70)	0.649
GG	30/7	1.00 (0.38–2.62)	0.995

[a]ORs, 95% CIs and their corresponding P values were calculated using multivariate logistic regression analysis, adjusted for age, ECOG PS, pathologic type, adjuvant therapy and TNM stage. CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; N, number; NA, not available; R, reference; OR, odds ratio; CI, confidence interval.

Table S3 Stratification analysis of association between *CHEK2* SNPs and therapy response selected by adjuvant therapy

SNP and genotype	GP/GC group			TP/TC group			NP/NC group			DP/DC group		
	Response/no response (N)	OR (95% CI)[a]	P[a]	Response/no response (N)	OR (95% CI)[a]	P[a]	Response/no response (N)	OR (95% CI)[a]	P[a]	Response/no response (N)	OR (95% CI)[a]	P[a]
rs4035540			0.539			0.165			0.257			
GG	30/8	1		32/9	1		23/5	1		10/0	NA	NA
AG	24/6	0.52 (0.13–2.06)	0.349	17/7	1.56 (0.45–5.42)	0.483	27/3	0.49 (0.11–2.28)	0.362	3/4	NA	NA
AA	10/1	0.40 (0.04–3.85)	0.429	3/4	5.30 (0.94–29.81)	0.058	3/2	2.93 (0.38–22.46)	0.300	0	NA	NA
rs5762746			0.458			0.351			0.463			
AG	28/10	1		21/7			27/6	1		10/3	NA	NA
GG	19/4	0.72 (0.17–3.04)	0.658	22/8	1.26 (0.35–4.51)	0.723	18/1	0.27 (0.03–2.39)	0.237	4/0	NA	NA
AA	17/1	0.25 (0.03–2.27)	0.219	7/5	3.09 (0.64–14.83)	0.160	8/2	1.13 (0.19–6.70)	0.897	0/1	NA	NA
rs2236141			0.986			0.840						NA
GG	47/12	1		33/13	1		34/7	1		10/4	NA	NA
AG	16/3	1.14 (0.24–5.36)	0.868	14/7	1.44 (0.43–4.78)	0.555	18/2	0.52 (0.10–2.80)	0.446	3/0	NA	NA
AA	1/0	NA	NA	3/0	NA	NA	0	NA	NA	1/0	NA	NA
rs2236142			0.933			0.886			0.788			0.608
CG	36/9	1		25/11	1		26/5	1		9/2	1	
CC	21/5	0.98 (0.23–4.12)	0.974	19/7	0.77 (0.23–2.54)	0.665	17/2	0.59 (0.10–3.53)	0.562	4/1	1.421 (0.09–23.79)	0.807
GG	8/1	0.65 (0.07–6.42)	0.709	8/2	0.73 (0.12–4.43)	0.732	11/3	1.13 (0.20–6.35)	0.886	1/1	5.771 (0.18–181.6)	0.319

[a]ORs, 95% CIs and their corresponding P values were calculated using multivariate logistic regression analysis, adjusted for age, ECOG PS, pathologic type and TNM stage. CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; N, number; NA, not available; R, reference; NP/NC, carboplatin or cisplatin plus vinorelbine; GP/GC, carboplatin or cisplatin plus gemcitabine; TP/TC, carboplatin or cisplatin plus paclitaxel; DP/DC, carboplatin or cisplatin plus docetaxel; OR, odds ratio; CI confidence interval.

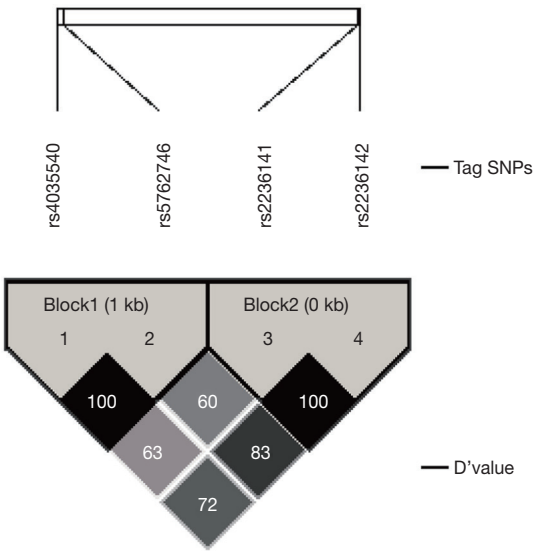

Figure S1 Linkage disequilibrium map of genotyped *CHEK2* SNPs. D' values (%) showed in this figure and the deeper the color represents the stronger LD. The figure could be showed from the Haploview Software directly. CHEK2, cell cycle checkpoint kinase 2; SNP, single nucleotide polymorphism; LD, linkage disequilibrium.