Original Article

The analysis of pharmacokinetic and pharmacogenomic impact on gefitinib efficacy in advanced non-small cell lung cancer patients: results from a prospective cohort study

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Background: The current study is aimed to examine the impact of pharmacokinetics and gene polymorphisms of enzymes involving in absorption, distribution, metabolism and excretion (ADME) on the efficacy of gefitinib in non-small cell lung cancer (NSCLC) patients.

Methods: Eligible patients with indication of gefitinib treatment were prospectively enrolled in this study. Two peripheral blood samples at baseline and before cycle 2 day 1 were collected for the detection of single nucleotide polymorphisms (SNPs) of drug ADME enzymes and trough drug concentration (C_{trough}) at steady state. Thirteen SNPs were genotyped using the Sequenom Massarray system. C_{trough} was determined by validated high-performance liquid chromatographic method with tandem mass spectrometric (LC-MS/MS).

Results: Fifty-eight NSCLC patients were enrolled in this study. The median of C_{trough} was 175ng/mL (range from 47.8 to 470 ng/mL). The trough concentration was not associated with either objective response or progression free survival (PFS). C_{trough} was significantly lower in *CYP3A4* rs2242480 CC + CT genotype than in TT genotype (P=0.019) and in *ABCG2* rs2231142 AA genotype than in AC + CC genotype (P=0.031). *ABCB1* rs2032582 dominant model was significantly correlated with overall response rate (ORR) and patients with GG phenotype respond better than patients with GT + TT phenotypes (84.6% *vs.* 51.2%, P=0.032). ABCB1 rs10256836 recessive model was significantly correlated with PFS and patients with GG phenotype achieved longer PFS than patients with GC + CC phenotypes (17.40 *vs.* 10.33 months, P=0.040).

Conclusions: The C_{trough} of gefitinib was significantly different between *CYP3A4* and *ABCG2* genotypes, but not with the efficacy of gefitinib treatment. *ABCB1* rs2032582 and rs10256836 polymorphisms were correlated treatment outcome. Polymorphisms analysis of *ABCB1* could be a predictive biomarker for gefitinib treatment.

Keywords: Non-small cell lung cancer (NSCLC); gefitinib; pharmacokinetic; *ABCB1*; single nucleotide polymorphisms (SNPs)

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Introduction

Non-small cell lung cancer (NSCLC) patients containing sensitive somatic mutations of the epidermal growth factor receptor (*EGFR*) gene, such as the small deletions [747–750] and point mutations at codon 858 (L858R), are highly responsive to gefitinib (1,2). However, there are still 30% patients with *EGFR* mutations could not benefit from gefitinib treatment. Thus, it's important to identify biomarkers that allow clear separation of patients with or without a relevant chance of clinical benefit from this drug (2,3). Recently findings suggest that concomitant oncogenes strongly correlated with treatment outcome of gefitinib, and indicate poor clinical benefit, such as *TP53*, *KRAS*, *MYC*, *APC*, etc. (4,5). Some studies focus on the genes involved in drug targets or signaling pathways (6,7). The deletion polymorphism of *BIM*, a pro-apoptotic member of the *BCL2* family, could lead to intrinsic resistance to gefitinib (8,9).

However, very few studies focus on the pharmacogenomics impact of gefitinib on its treatment outcome. A different approach is to identify germline gene polymorphisms of enzymes involving in gefitinib absorption, distribution, metabolism and excretion (ADME) processes. The ADME of a drug is largely a host-mediated process. As a tyrosine kinase inhibitor, the efficacy of gefitinib *in vivo* could be significantly affected by genetic variability of these potential pharmacokinetics or pharmacogenomics linked enzymes (10,11). ATP-binding cassette (*ABC*) transporters function as substrate enzymes for multiple compounds and play a leading role in transporting drugs through the membrane between or out of body compartments (12-14). Cytochrome P450 proteins including *CYP3A5*, *CYP2D6*, *CYP3A4*, *CYP1A1*, *CYP1A2* and *POR* are the main metabolizing enzymes involved in gefitinib metabolism (15-17). A variety of single-nucleotide polymorphisms (SNPs) in these enzymes have been described to predict toxicities and efficacy of chemotherapeutic agents (18,19).

However, few studies have determined the impact of gene polymorphisms of enzymes in ADME processes on the efficacy of gefitinib in advanced NSCLC patients with *EGFR* mutations. Therefore, this study is aimed to investigate whether gene polymorphisms of enzymes in ADME processes could be used as predictive biomarkers for gefitinib.

Methods

Patients and study design

Patients were enrolled in this study prospectively. Key eligible criteria included histologically confirmed NSCLC, indication of gefitinib treatment (e.g., *EGFR* active mutation, unknown *EGFR* status failed standard treatment), evaluable disease according to the Response Evaluation Criteria in Solid Tumor (RECIST) v1.1, ≥18 years old and Eastern Cooperative Oncology Group (ECOG) performance score (PS) ≤2. Gefitinib was administrated 250 mg daily between 8 am to 9 am and consecutive treatment for one month was counted for one cycle. Anti-tumor activities were evaluated by investigators per RECIST v1.1 using image examination (computed tomography or magnetic resonance imaging scan) at baseline, first cycle (1 month), and every 2 cycles after. Overall response rate (ORR) included complete response (CR) and partial response (PR). Disease control rate (DCR) included CR, PR and stable disease (SD). Progression free survival (PFS) was defined as the time from the first dose to progression disease (PD), death and the start of second systemic treatment at the nearest evaluation before cut-off or the earliest event that shall prevail. This study was in accordance with the International Standards of Good Clinical Practice and approved by Sun Yat-sen University Cancer Center Independent Review Board (B2013-038-01) and registered on clinicaltrial.gov (NCT01994057). Written informed consents were provided by all patients.

Samples collection and detection

Two peripheral blood samples (2 mL) were collected for all patients using EDTA vacuum tubes. The first one was drawn before first gefitinib dose and the second one was drawn within 5 min before gefitinib administration at day 1 of cycle 2. Samples were stored at 2–8 ℃ and centrifuged within 4 h to separate plasma for somatic mutation detection. White blood cells were used for germline mutation detection. Both plasma and white blood cells were stored at –80 ℃ until analysis. The plasma of second blood was collected to measure the trough concentration of gefitinib at steady state using validated high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) (20). *EGFR* mutations in primary tumors,

metastatic lymph nodes or pleural effusion were detected using direct sequencing or real-time PCR (RT-PCR).

Genotype analysis

DNAs were extracted using the Genome TIANGEN Blood DNA Extraction Kit (AP348, China, Beijing). The quantity and quality of the extracted DNA were measured by calculating their optical density using ultraviolet spectrophotometer. SNPs were genotyped by matrix-assisted laser desorption/ionization time-of-flight platform using the Sequenom Massarray system (San Diego, CA, USA). Thirteen SNPs with allele frequency >5% in Chinese population were used for genotyping including *CYP1A1* (rs2606345, rs1048943), *CYP1A2* (rs762551), *CYP3A4* (rs2242480), cytochrome P450 reductase (*POR*) (rs1057868, rs17685), *UGT1A7* (rs6759892), *ABCB1* (rs10256836, rs1045642, rs1128503, rs2032582) and *ABCG2* (rs2231137, rs2231142). Since multiple genetic models have been adapted to explore the biological rationales behind the preference of these genetic models and there is no concrete evidence for each SNP, in this research, the allele with higher frequency was considered as the dominant allele and its homozygote and heterozygote genotypes were categorized as the dominant genotypes. Meanwhile, the homozygote genotype of the allele with lower frequency was considered as recessive genotype (21,22).

Statistical analysis

Clinical and experimental data were imported using EpiData 3.0. Statistical analyses were performed using SPSS v13 statistical software (USA). All descriptive data were expressed as mean ± standard deviation and categorical variables were presented as counts and percent. The distribution of genotypes was tested for Hardy-Weinberg equilibrium (HWE). Fisher's exact test and log-rank test were used to compare ORR and PFS in different genotypes and clinical characteristics. Univariate analysis was applied in SNPs and ORR correlation study as unadjusted results. With consideration of clinical confounding factors, multivariate logistic regression was used to analyze the correlation as adjusted results. Multivariate logistic regression was used to reanalyze the statistical significance of each marked SNP with ORR. Odds ratios (OR) and 95% confidence interval (95% CI) were estimated in all univariate analysis and multivariate analysis. The Wilcoxon Mann-Whitney U test and Kruskal-Wallis H test was adopted to test the difference in gefitinib trough concentration between different genotype groups. A two-tailed P value of 0.05 indicated statistical significance. The figures were drawn using the GraphPad Prism5.0.

Results

Patients

A total of 58 NSCLC patients from Sun Yat-sen University Cancer Center from July 2011 to January 2014 were consecutively enrolled into this study. Data cutoff was set on April 30, 2015. Patients' demographic and clinical characteristics are shown in *Table 1*. The population was made up of 48% male and 52% female, with a median age of 56 years at diagnosis. Most of them (90%) had adenocarcinoma. Twenty-two (38%) patients harbored EGFR exon 19 deletion and 27 (47%) harbored *EGFR* exon 21 L858R point mutation. Thirty-five (60%) patients achieved PR with ORR of 60% and DCR of 95%. The datasets with detailed disease description were listed in (http://cdn.amegroups.cn/static/application/05c49f1f89637a6bb3769bc81ba85e62/atm.2019.12.60-1.xlsx) with the private information blinded. The correlation of baseline clinical characteristics with ORR and PFS were analyzed and no significant clinical confounding characters had significant effect on gefitinib efficacy (*Figure 1*).

Genotypes and pharmacokinetics

The distributions of genotypes of all 13 SNPs were consistent with Hardy-Weinberg equilibrium (*Table S1*). Population distribution analysis indicated that the frequencies of SNPs in Chinese were highly consistent with those in Japanese NSCLC patients, but significantly different those in Caucasians and African Americans (*Table S2*).

Pharmacokinetic results are showed in *Figure S1*. The C_{trough} of gefitinib was in the range of 47.8–470 ng/mL with median value of 175 ng/mL and interquartile range of 130.5–236.6 ng/mL. Nonparametric tests were adopted to test the difference of C_{trough} of gefitinib among different genotype groups (*Table S3*). In *CYP3A4* rs2242480 dominant model, the C_{trough} of gefitinib was significantly lower in patients with CC + CT genotypes than patients with TT genotype (Wilcoxon Mann-Whitney U test, P=0.019, *Figure S2*). For *ABCG2* rs2231142, the C_{trough} of gefitinib was significantly different among patients with different genotypes (Kruskal-Wallis H test, P=0.045). The

Table 1 Patients' demographics and baseline clinical characteristics

Characters	No. of patients [%]
Gender	
Male	28 [48]
Female	30 [52]
Age, median (range), years	56 [31–77]
BSA, median (range), m^2	1.64 (1.26–2.06)
ECOG PS	
0–1	45 [78]
>2	10 [17]
Unknown	3 [5]
Smoking history	
Smoker	36 [62]
Non-smoker	18 [31]
Unknown	4 [7]
Disease staging	
Recurrent	21 [36]
IIIB or IV	37 [64]
Pathology	
Adenocarcinoma	52 [90]
Adenosquamous carcinoma	2 [4]
Squamous carcinoma	4 [7]
EGFR mutation status	
Exon19 deletions	22 [38]
Exon21 L858R	27 [47]
Other sensitive mutations*	6 [10]
Unknown	3 [5]
Treatment line of gefitinib	
1st	23 [40]
2nd or later	35 [60]
Platinum based chemotherapy	
With	18 [31]
Without	40 [69]
Best response to gefitinib	
Partial response	35 [60]
Stable disease	20 [34]
Progressive disease	3 [5]
Progression free survival	
≤10 months	24 [41]
>10 months	34 [59]

*, 3 patients with exon 21L861Q, 2 with exon 20S768I and 1 with exon 18G719X. Staging: according to IASLC 2007 staging method; BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group-performance status.

C_{trough} of gefitinib was significantly lower in patients with AA genotype than in patients with AC + CC genotype (Wilcoxon Mann-Whitney U test, P=0.031, *Figure S3*).

Association between gefitinib pharmacokinetics and efficacy

Nonparametric tests were adopted to test the difference in C_{trough} of gefitinib between patients with different gefitinib responses. The median C_{trough} of gefitinib was 173.9 ng/mL with interquartile range of 108–231 ng/mL in PR group and 191.5 ng/mL with interquartile range of 135.5–275.5 ng/mL in SD + PD group, respectively (P=0.236). X-Tile was used to determine the cutoff value of C_{trough} based on ORR (C_{trough} cutoff at 200 ng/mL) (23). Chi-square test showed that the ORR was not significantly different in patients with C_{trough} >200 ng/mL and patients with C_{trough} <200 ng/mL [64% (23/36) *vs.* 55% (12/22), P=0.480].

In addition, no significant correlation was found between patients' PFS and C_{trough} of gefitinib either. The median PFS of patients with C_{trough} >200 and <200 ng/mL was 11.80 and 14.00 months, respectively (P=0.78, *Table S4*, *Figure S4*).

Association between genotypes and efficacy

We analyzed the impact of gene polymorphisms on the efficacy of gefitinib treatment in all 58 patients. With consideration of clinical confounding factors, multivariate logistic regression was used to re-analyze each SNP and those factors with ORR as an adjusted result. The results indicated that 3 SNPs (*CYP1A2* rs762551 recessive model, *POR* rs1057868 dominant model and *ABCB1* rs2032582 dominant model) were significant high-risk determinants for ORR as shown in *Table 2*. Further multivariate logistic regression (stepwise method) was applied with considering 2 SNPs and clinical confounders as independent covariate factors. The results suggested only *ABCB1* rs2032582 dominant model was a significant high-risk determinant for ORR (GT + TT/GG: HR =0.111, 95% CI: 0.013 to 0.965). The response rate was significantly lower in patients with ABCB1 rs2032582 (GT + TT) genotypes than in patients with ABCB1 rs2032582 (GG) genotype, with ORR of 51.2% and 84.6%, respectively (P=0.032, *Tables 3*,*S5*).

All 58 patients were analyzed to identify the determinant of PFS in the 13 SNPs. Kaplan-Meier curves and log-rank test were applied to compare the PFS of patients with different gene polymorphisms in gefitinib treatment. The results indicated that *ABCB1* rs10256836 recessive model was significantly associated with PFS of patients subjected

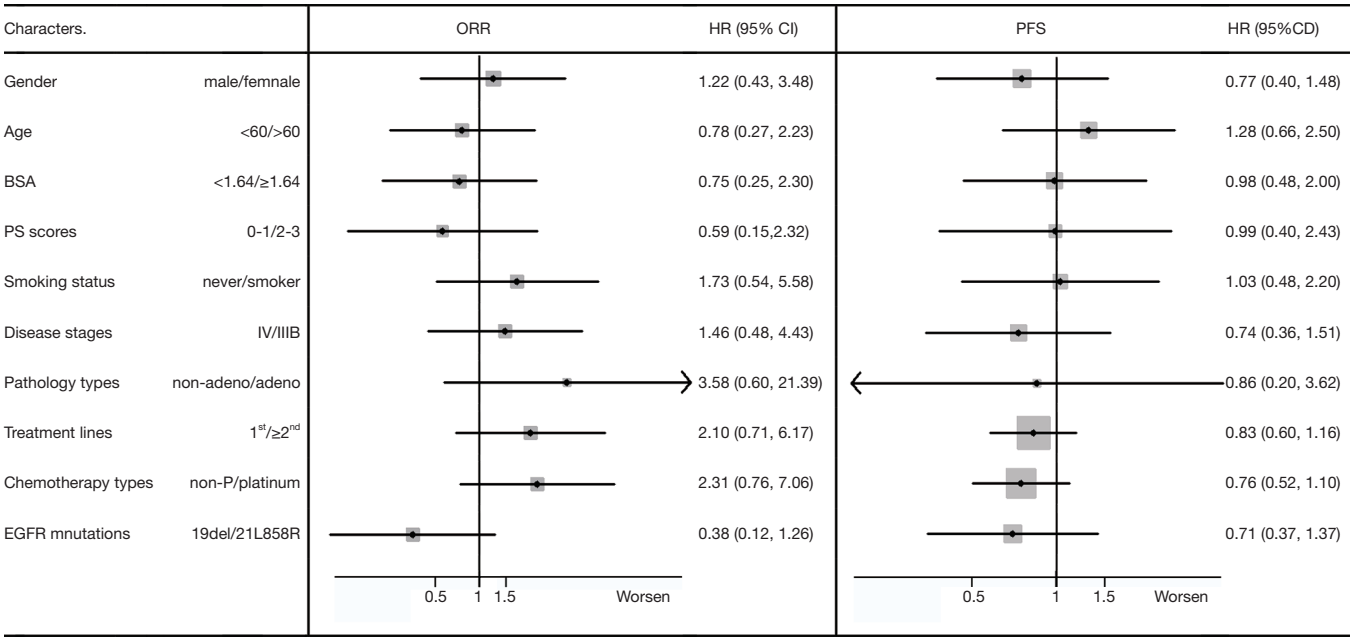

Figure 1 The impact of patients' demographics and clinical characteristics on Gefitinib efficacy (overall response rate and progression free survival). ORR, overall response rate; 95% CI, 95% confidence interval.

to gefitinib treatment, as shown in *Table 4* and *Figure 2*. *CYP3A4* rs2242480 dominant model was excluded due to unbalanced proportion of CC + CT (54/57) and TT genotype (3/57). For *ABCB1* rs10256836 recessive model, the median PFS was 10.33 months (95% CI: 7.58–13.09) for patients with GC + CC genotypes, significantly shorter than that of 17.40 months (95% CI: 12.19–22.61) for patients with GG genotype (P=0.04). The Cox regression with stepwise methods was adopted to select the significant determinant of PFS in gefitinib treatment (*Table S6*). The results showed that *ABCB1* rs10256836 recessive models were significantly associated with PFS of patients subjected to gefitinib treatment.

Discussion

Current study explored the impacts of blood C_{trough} of gefitinib and ADME enzymes on the outcomes of gefitinib treatment. The results from 58 patients indicated that the C_{trough} of gefitinib was associated with neither patient' response to gefitinib nor immediate survival, while ATP-binding cassette gene polymorphisms were significantly related with the outcome of gefitinib treatment.

In this research, the C_{trough} of gefitinib was significantly different between patients with *CYP3A4* rs2242480 and *ABCG2* rs2231142, implying that the blood C_{trough} of gefitinib might be influenced by the polymorphisms of metabolic enzymes in the liver and transporters in the kidney. Second, both preclinical and clinical researches revealed that the concentration of gefitinib in tumor and skin was much higher than its plasma concentration in xenograft mice and patients (24-26). Using positron emission tomography, ^{11}C and ^{14}C gefitinib was accumulated in tumor tissues and normal tissues such as skin and intestine rather than evenly distributed in blood, both in rat and human (25,27,28). Haura's study indicated that gefitinib level in tumor was approximately 40-fold higher than its plasma level (29). These findings indicated that the blood C_{trough} of gefitinib is not a good substitute indicator for its concentration in tumor, insistent with the previous research (20). Third, the ratio of gefitinib concentration in tumor to that in plasma is ranged from 1.12–250 to 1 (median 40 to 1), which demonstrated tremendous inter-individual variability (30). A previous research also indicated that the trough concentration at d8 and d15 was not associated with response rates or survival times with gefitinib (11). The polymorphisms of transporters (*ABCB1*) and metabolic enzymes (*CYP1A1*) in lung might contribute to the variability of tumor concentration (31). Therefore, it is reasonable to speculate that SNPs of these enzymes could

Table 2 Analysis of Association of SNPs gene models with ORR with/without consideration of potential confounders

Gene	Polymorphisms	Gene model	ORR		ORR	
			P-SV[#]	OR-SV[#] (95% CI)	P-MV*	OR-MV* (95% CI)
CYP1A1	rs1048943 (*CYP1A1**2C)	DOM	0.449	2.40 (0.248–23.181)	0.229	5.223 (0.354–77.086)
		REC	0.358	1.714 (0.543–5.410)	0.813	1.201 (0.264–5.465)
	rs2606345	DOM	0.168	2.963 (0.633–13.869)	0.292	3.300 (0.358–30.398)
CYP1A2	rs762551	REC	0.082	0.368 (0.120–1.134)	0.048	0.196 (0.039–0.983)
CYP3A4	rs2242480 (*CYP3A4**1G)	DOM	0.330	0.294 (0.025–3.454)	0.486	0.387 (0.027–5.589)
		REC	0.722	0.822 (0.279–2.419)	0.942	1.060 (0.220–5.101)
POR	rs1057868 (*POR**37)	DOM	0.273	2.588 (0.477–13.711)	0.039	14.729 (1.146–189.37)
		REC	0.336	0.547 (0.160–1.870)	0.682	0.726 (0.158–3.347
	rs17685 (*POR**11)	DOM	0.594	1.607 (0.281–9.188)	0.142	6.654 (0.529–83.747)
		REC	0.801	0.857 (0.258–2.844)	0.873	1.126 (0.263–4.821)
UGT1A7	rs6759892	DOM	0.468	0.667 (0.223–1.994)	0.907	1.089 (0.261–4.551)
ABCB1	rs10256836	REC	0.847	1.118 (0.359–3.484)	0.772	1.260 (0.263–6.029)
	rs1045642	DOM	0.298	0.425 (0.085–2.128)	0.679	0.674 (0.104–4.383)
		REC	0.419	0.615 (0.190–1.995)	0.187	0.277 (0.041–1.861)
	rs1128503	DOM	0.778	1.167 (0.399–3.408)	0.936	1.059 (0.263–4.254)
		REC	0.893	0.900 (0.193–4.194)	0.782	1.346 (0.164–11.043)
	rs2032582	DOM	0.068	0.219 (0.043–1.117)	0.020	0.033 (0.002–0.585)
		REC	0.050	0.259 (0.067–1.000)	0.136	0.286 (0.055–1.480)
ABCG2	rs2231137	DOM	0.431	1.556 (0.518–4.669)	0.399	1.764 (0.472–6.600)
		REC	0.829	0.762 (0.065–8.943)	0.742	1.757 (0.061–50.617)
	rs2231142	DOM	0.968	0.978 (0.329–2.907)	0.891	1.102 (0.275–4.411)
		REC	0.396	0.375 (0.039–3.605)	0.546	0.376 (0.016–8.967)

[#], single variant logistic analysis for the contribution of the SNPs gene models for the ORR; *, adjusted P value by multivariate logistic regression analysis (Enter method) for potential clinical confounders, including gender, BSA, age, PS, smoking history, staging, pathology, EGFR status, treatment lines and platinum chemotherapy. SV, single variant logistic analysis; MV, multivariate logistic regression analysis; ORR, overall response rate; OR, odds ratio; 95% CI, 95% confidence interval; DOM, dominant model; REC, recessive model; SNPs, single nucleotide polymorphisms.

Table 3 Response rate of gefitinib in *ABCB1* rs2032582 dominant model

Objective response	rs2032582 (GG)	rs2032582 (TT + GT)	P value
CR + PR	11 (84.6%)	22 (51.2%)	–
SD + PD	2 (15.4%)	21 (48.8%)	–
Total	13	43	0.032

CR, complete response; PR, partial response; SD, stable disease; PD, progression disease.

Table 4 Log-rank test for the association of SNPs gene models with PFS

Gene	Polymorphisms	Gene model	P	Median PFS (95% CI)
CYP1A1	rs1048943 (*CYP1A1**2C)	DOM	0.656	16.23 (9.44–23.02) *vs.* 14.00 (6.41–21.59)
		REC	0.443	17.03 (10.68–23.29) *vs.* 14.0 (4.60–23.40)
	rs2606345	DOM	0.868	16.23 (4.27–28.20) *vs.* 13.07 (6.90–19.24)
CYP1A2	rs762551	REC	0.926	13.53 (11.14–15.92) *vs.* 16.23 (8.50–23.97)
CYP3A4	rs2242480 (*CYP3A4**1G)	DOM	0.002	14.00 (8.58–19.42) *vs.* 5.37 (1.63–9.10)
		REC	0.327	17.90 (14.91–20.89) *vs.* 10.33 (8.42–12.24)
POR	rs1057868 (*POR**37)	DOM	0.874	12.40 (5.98–18.82) *vs.* 14.00 (6.67–21.33)
		REC	0.253	11.53 (9.56–13.50) *vs.* 17.03 (12.05–22.02)
	rs17685 (*POR**11)	DOM	0.423	12.40 (6.03–18.77) *vs.* 13.53 (0.36–26.71)
		REC	0.181	11.53 (9.56–13.50) *vs.* 16.23 (11.25–21.22)
UGT1A7	rs6759892	DOM	0.995	13.53 (8.52–18.55) *vs.* 14.00 (4.20–23.80)
ABCB1	rs1045642	REC	0.797	12.40 (9.46–15.34) *vs.* 14.00 (8.12–19.88)
	rs10256836	REC	0.040	17.40 (12.19–22.61) *vs.* 10.33 (7.58–13.09)
	rs1128503	DOM	0.721	16.23 (8.12–24.35) *vs.* 13.53 (7.51–19.56)
		REC	0.975	10.97 (0–24.27) *vs.* 14.00 (7.67–20.33)
	rs2032582	DOM	0.159	11.80 (6.86–16.74) *vs.* 17.03 (9.92–24.15)
		REC	0.322	11.80 (8.47–15.13) *vs.* 18.73 (16.52–20.54)
ABCG2	rs2231137	DOM	0.390	11.53 (8.22–4.85) *vs.* 17.50 (11.29–23.71)
		REC	0.652	8.23 (4.18–12.29) *vs.* 14.00 (7.94–20.06)
	rs2231142	DOM	0.554	12.40 (8.47–16.33) *vs.* 16.23 (9.32–23.15)
		REC	0.484	12.40 (7.96–16.84) *vs.* 13.53 (7.36–19.71)

SNPs, single nucleotide polymorphisms; PFS, progression free survival; 95% CI, 95% confident interval; DOM, dominant model; REC, recessive model.

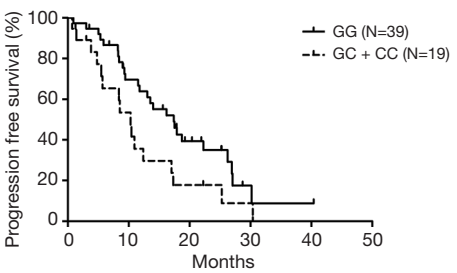

Figure 2 Kaplan-Meier curve and log-rank test for progression-free survival in advanced NSCLC patients treated with gefitinib: association with *ABCB1* rs10256836 recessive model (GG *vs.* GC + CC). NSCLC, non-small cell lung cancer.

cause variability of gefitinib concentration in tumor and affect tumor response.

ABC transporters distributed as membrane proteins in various organs, where they functioned as substrate enzymes for multiple compounds, and played a leading role in transporting drugs through the membrane between or out of body compartments (32,33). Thus, the genes polymorphisms of specific transporters that involved in these processes might have not negligible impact on the variation in drug response or toxicities. Vlaming's research indicated that human *ABCB1* and *ABCG2* transporters were over-expressed in NSCLC tumor cell lines (31).

Based on that, our hypothesis is that SNPs affect the activities of *ABCB1* or *ABCG2* in tumor tissues, causing abnormal variability of gefitinib concentration in tumor and eventually leading to different response to gefitinib. Our findings in this research indicated that *ABCB1* rs2032582 and rs10256836 gene polymorphisms were highly correlated with gefitinib efficacy. Gefitinib is a substrate of *ABCB1* (32,33). *ABCB1* rs2032582 G>T mutation G2677T/A is a nonsynonymous mutation in exon 21. Three nucleotides (G/T/A) were in rs2032582, encoding 3 amino acids (Ala893Ser/Thr). However, the function of this SNP is controversial. Some studies concluded that rs2032582 G>T mutation will weaken the drug efflux function of *ABCB1* (34,35). Based on that, GG phenotype in rs2032582 is probably the risk factor of gefitinib response. Clinical data indicated that rs10256836 G>C mutation increased the expression or activity of transporters proteins (36-38). Our results also indicated patients with rs10256836 GG genotype had longer PFS, consistent with the previous research. Previous research also suggested that *ABCB1* rs1128503 TT genotype was a significant high-risk determinant of both skin rash and diarrhea (39). In this research, neither *ABCB1* rs1128503 nor rs1045642 was significantly associated with ORR and PFS. However, only polymorphisms have been detected in this cohort, the gene expression data and concentration data from target tissue would be helpful to give a better illustration to the complete pharmacokinetics and pharmacogenomics inter-individual variability. *In vivo* and *in vitro* experiments were needed to confirm our findings.

Conclusions and limitation

Our study indicated that gefitinib trough concentration was significantly different between *CYP3A4* and *ABCG2* genotypes; blood gefitinib trough concentration was not associated with its efficacy. Moreover, pharmacogenomic analysis revealed that patients with GT + TT genotypes in *ABCB1* rs2032582 dominant model respond better to gefitinib and patients carried with GC + CC genotypes in *ABCB1* rs10256836 dominant recessive model had longer PFS after gefitinib treatment. However, our findings were based on a small sample prospective cohort study. Therefore, further randomized clinical trials must be conducted to confirm that correlation and to elucidate the biomarker function of ABC transporters family gene polymorphisms (*ABCB1* rs2032582 and rs10256836) in clinical practice.

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Footnote

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

Ethical Statement: The authors are accountable for all aspects of the work, and the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethical Committee of Sun Yat-sen University Cancer Center (B2013-08-01). Informed consent written informed consent was obtained from all participating subjects. This trial was registered at ClinicalTrials.gov. (NCT01994057, date of registration: 2013/11/23).

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Supplementary

Table S1 Genotype frequency and Hardy-Weinberg equilibrium test for SNPs

Gene	Rs	N	Genotype frequency			Minor allelic frequency	HWE P value
			wt/wt, n [%]	wt/m, n [%]	m/m, n [%]		
CYP1A1	rs1048943 (*CYP1A1**2C)	53	29 [54]	19 [37]	5 [9]	0.27	0.47
	rs2606345	58	50 [86]	7 [12]	1 [2]	0.08	0.23
CYP1A2	rs762551 (*CYP1A2**1F)	56	26 [47]	26 [46]	4 [7]	0.30	0.46
CYP3A4	rs2242480 (*CYP3A4**1G)	57	25 [45]	29 [50]	3 [5]	0.30	0.14
POR	rs1057868 (*POR**37)	55	18 [33]	28 [51]	9 [16]	0.43	0.73
	rs17685 (*POR**11)	53	17 [33]	29 [54]	7 [13]	0.40	0.32
UGT1A7	rs6759892	55	28 [52]	25 [45]	2 [3]	0.26	0.20
ABCB1	rs1045642	54	19 [35]	28 [53]	7 [12]	0.39	0.50
	rs10256836	58	39 [67]	18 [31]	1 [2]	0.17	0.49
	rs1128503	58	24 [42]	26 [44]	8 [14]	0.35	0.82
	rs2032582	56	12 [21]	32 [58]	12 [21]	0.50	0.28
ABCG2	rs2231137	56	25 [44]	27 [49]	4 [7]	0.32	0.36
	rs2231142	55	26 [46]	24 [45]	5 [9]	0.31	0.87

HWE, Hardy-Weinberg equilibrium.

Table S2 Comparison of genotypes distributions in different populations

Gene	Rs	Minor allelic frequency	Han Chinese	Japanese	Caucasian	African American
CYP1A1	rs1048943 (*CYP1A1**2C)	0.27	0.26	0.25	0.03**	0.01**
	rs2606345	0.08	0.03	0.03	0.45**	0.17
CYP1A2	rs762551 (*CYP1A2**1F)	0.3	0.31	0.36	0.32	0.44
CYP3A4	rs2242480 (*CYP3A4**1G)	0.3	0.28	0.30	0.20	0.12**
POR	rs1057868 (*POR**37)	0.43	0.42	0.40	0.29	0.08**
	rs17685 (*POR**11)	0.4	0.28	0.36	0.29	0.29
UGT1A7	rs6759892	0.26	0.23	0.21	0.45**	0.67**
ABCB1	rs1045642	0.39	0.42	0.46	0.45	0.07**
	rs10256836	0.17	0.11	0.14	0.30**	0.09
	rs1128503	0.35	0.29	0.41	0.42	0.14**
	rs2032582	0.50	0.38	0.48	0.44	0.13**
ABCG2	rs2231137	0.32	0.29	0.19	0.06**	0.06**
	rs2231142	0.31	0.29	0.31	0.09**	0.04**

**, the distribution of different alleles in different populations (Japanese/Caucasian/African American) compared with Han Chinese population by Chi-square test.

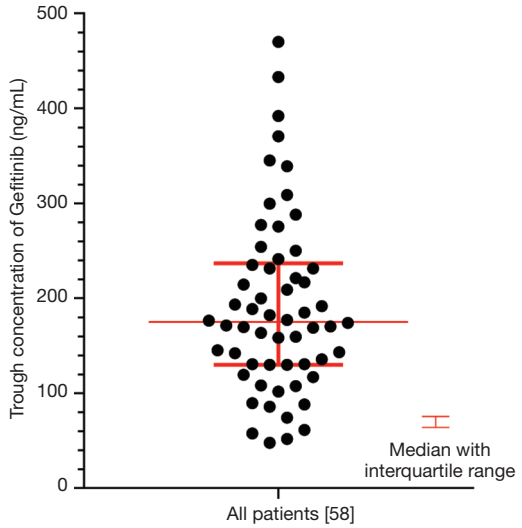

Figure S1 The distribution of gefitinib trough concentration in all 58 patients.

Table S3 The Wilcoxon Mann-Whitney U and Kruskal-Wallis H test for the gefitinib blood concentration among different genotype groups

Gene	Polymorphisms	Gene model	P#	P*
CYP1A1	rs1048943 (*CYP1A1**2C)	DOM	0.203	0.317
		REC	0.802	
	rs2606345	DOM	0.260	
CYP1A2	rs762551	REC	0.954	
CYP3A4	rs2242480 (*CYP3A4**1G)	DOM	0.019	0.078
		REC	0.531	
POR	rs1057868 (*POR**37)	DOM	0.302	0.546
		REC	0.963	
	rs17685 (*POR**11)	DOM	0.120	0.242
		REC	0.879	
UGT1A7	rs6759892	DOM	0.755	
ABCB1	rs1045642	DOM	0.479	0.743
		REC	0.986	
	rs10256836	REC	0.565	
	rs1128503	DOM	0.130	0.316
		REC	0.572	
	rs2032582	DOM	1.000	0.168
		REC	0.069	
ABCG2	rs2231137	DOM	0.585	0.239
		REC	0.154	
	rs2231142	DOM	0.064	0.045
		REC	0.031	

#, Wilcoxon Mann-Whitney U test for the gefitinib trough concentration among different genotype model groups; *, Krustal-Wallis H test for the gefitinib blood concentration among different genotype groups. DOM, dominant model; REC, recessive model.

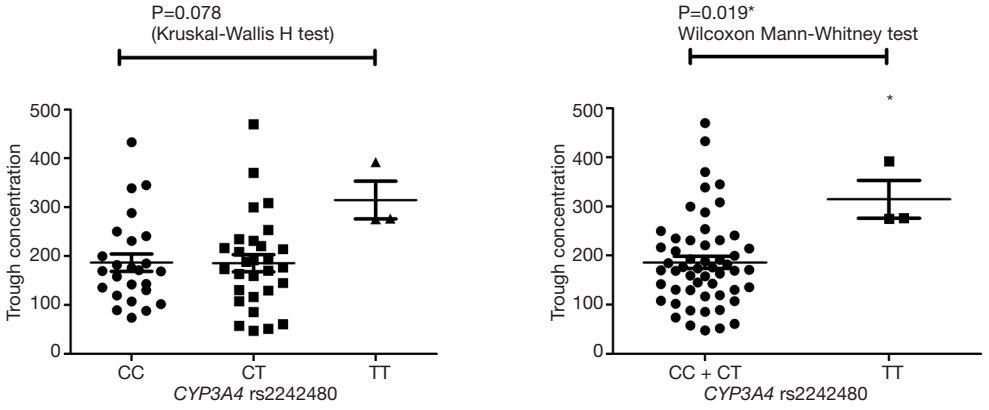

Figure S2 The distribution of gefitinib trough concentration in *CYP3A4* rs2242480 genotype groups. *, P value <0.05.

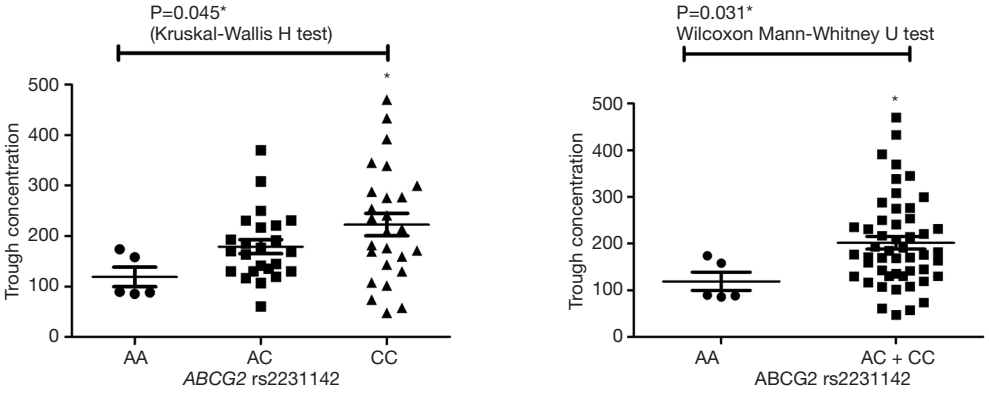

Figure S3 The distribution of gefitinib trough concentration in *ABCG2* rs2231142 genotype groups. *, P value <0.05.

Table S4 Log-rank test for PFS between patients with C_{trough} <200 ng/mL and C_{trough} >200 ng/mL

Group	Median	Standard deviation	95% confidence intervals	
			Lower	Upper
C_{trough} <200 ng/mL	14.000	2.880	8.355	19.645
C_{trough} >200 ng/mL	11.800	4.591	2.802	20.798
Total	13.533	3.073	7.510	19.556

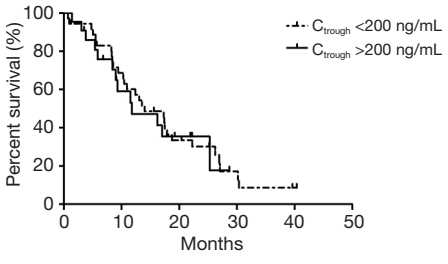

Figure S4 The Kaplan-Meier curve of the PFS in high trough concentration (>200 ng/mL) group compared with that in low trough concentration (<200 ng/mL) group. PFS, progression free survival.

Table S5 Multivariable logistic regression analysis of ORR

Parameter	P	OR	95% CI
Intercept	0.037	9.000	
rs2032582 (TT + GT/GG)	0.047	0.111	0.013 to 0.965

OR, odds ratio; 95% CI, confidence interval. ORR, overall response rate.

Table S6 Multivariable Cox regression of PFS

Parameter	Odds ratio	95% CI of OR	P
Rs2242480DOM	7.542	2.082 to 27.319	0.002
Rs10256836REC	2.170	1.136 to 4.146	0.019

OR, odds ratio; DOM, dominant model; REC, recessive model. PFS, progression free survival.