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Establishment and application of a predictive model for gefitinib-induced severe rash based on pharmacometabolomic profiling and polymorphisms of transporters in non-small cell lung cancer

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ABSTRACT

Background: Rash is a well-known predictor of survival for patients with gefitinib therapy with non-small cell lung cancer (NSCLC). However, whether patients with more severe rash obtain the more survival benefits from gefitinib is still unknown, and predicted model for severe rash is needed.
Methods: The relationship between gefitinib-induced rash and progression free survival (PFS) was primarily explored in the retrospective cohort. The association between rash and gefitinib/metabolites concentration and genetic polymorphisms were determined by pharmacometabolomic and pharmacogenomics methods in the exploratory cohort and validated in an external cohort.
Results: The survival for patients with rash was significantly higher than that of patients without rash ($p = 0.0002$, $p = 0.0089$), but no difference was found between grade 1/2 or grade 3/4. Only the concentration of gefitinib, but not its metabolites, was found to be associated with severe rash, and the cutoff value of gefitinib was 204.6 ng/mL conducted by ROC curve analysis (AUC=0.685). A predictive model for severe rash was established: gefitinib concentration (OR = 11.523, 95% CI = 2.898-64.016, $p = 0.0016$), *SLC22A8* rs4149179(CT vs CC, OR = 3.156, 95% CI = 0.958–11.164, $p = 0.0629$), *SLC22A1* rs4709400(CG vs CC, OR = 10.267, 95% CI = 2.067–72.465, $p = 0.0087$; GG vs CC, OR = 5.103, 95% CI = 1.032–33.938, $p = 0.061$). This model was confirmed in the validation cohort with an excellent predictive ability (AUC = 0.749, 95% CI = 0.710–0.951).
Conclusions: Our finding demonstrated that the incidence, not the severity, of gefitinib-induced rash predicted improved survival, the gefitinib concentration and polymorphisms of *SLC22A8* and *SLC22A1* were recommended to manage severe rash.

Introduction

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers and is a leading cause of death from cancer around the world [1]. NSCLC patients with activating epidermal growth factor receptor (EGFR) mutants, such as in-frame deletions within exon 19 or the L858R mutant within exon 21, are highly responsive for gefitinib and erlotinib [1,2]. Gefitinib (Iressa®, AstraZeneca UK Limited) is the first following accelerated approval by the US Food and Drug Administration (FDA) in 2003 [3] and further approved for the first-line treatment of patients with *EGFR* exon 19 deletions or exon 21 (L858R) in metastatic NSCLC [4]. However, gefitinib-induced rash (30%–87%), mainly featured by papulopustular rash (or skin rash), is the most common adverse reaction [2,5,6], moreover, the severe rash, with the incidence of 7%–22%, can result in treatment interruptions or discontinuation [1,7,8]. Therefore, this often-stigmatizing toxicity represents a serious threat to the patients' quality of life and may lead to dose-reduction or even suspension of the antineoplastic therapy [1]. Meanwhile, man-

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agement of targeted therapies-induced dermatologic toxicities was estimated at a median of $1920 per patient [9]. However, the incidence of rash was a potential predictive biomarker for identifying which patients were likely to gain benefits from gefitinib [10,11]. Unfortunately, whether patients with more severe rash obtain the more survival benefits from gefitinib has never elucidated.

The mechanism of gefitinib-induced rash is complex. The observed skin toxicities may be related with off-target effects of gefitinib, such as inhibit the proliferation, differentiation and survival of normal keratinocyte [12]. On the other hand, the single nucleotide polymorphisms (SNPs) of metabolizing enzymes (CYPs, UGTs) and transporters (ABCBs, SLCs) of gefitinib are associated with the incidence of drug-induced rash in previous researches [13–19], which implied the exposure of gefitinib or its metabolites may contribute to gefitinib-induced rash. Meanwhile, as far as we know, these associations with gefitinib induced severe rash (grade 3/4) is still lacking. In fact, Studies on gefitinib and its metabolites are insufficient due to its accelerated approval [3]. Gefitinib is mainly metabolizing by CYP3A4, CYP3A5 and CYP2D6 [20]. Morpholine ring oxidation (M537194) and oxidative de-fluorination (M387783) are produced by CYP3A4-dependent metabolism [20]. M523595, M387783, M537194 are first determined in human plasma by D. McKillop [21]. M605211 is first determined in human plasma in our institution by liquid chromatography with tandem mass spectrometry (LC-MS/MS) [22]. However, little is known whether gefitinib-related severe rash is associated with exposures of gefitinib or its metabolites.

In this study, to primarily explore whether patients with more severe rash obtain the more survival benefits from gefitinib, we did a retrospective analysis in 162 patients with EGFR activating mutation. Our results indicated that the grade 3/4 of rash were disassociated with survival of patients with gefitinib therapy compared to grade 1/2 of rash. In order to filter patients with the gefitinib-induced grade 3/4 rash and improve quality of life in patients, we establish a predictive model for gefitinib-induced severe rash (grade 3/4) in an exploratory cohort. The predictive model was validated in an external cohort with an excellent accuracy. Taken together, our study demonstrated that the incidence, not the severity, of skin rash predicted improved survival, the gefitinib concentration and polymorphisms of *SLC22A8* and *SLC22A1* were recommended to manage severe rash.

Materials and methods

Study population

All eligible patients were 18 years old or older with at least grade 1 rash. All patients were histologically confirmed to be stage IV or IIIB NSCLC with a minimum one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and *EGFR*-activating mutation (exon 19 deletion, exon 21 L858R or other rare mutations), and Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1. The main exclusion criteria were: presence of other histologically confirmed tumors; presence of any organ dysfunctions; uncontrolled pleural or pericardial effusion and systemic disease; known gefitinib and any excipient alleging; and lactation or pregnancy. The study was approved by Human Ethics of Sun Yat-sen University Cancer Center and conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonization. The informed consents have been obtained from the participants. This study was registered at ClinicalTrials.gov (NCT01994057).

All patients eligible were administered gefitinib 250 mg/day (AstraZeneca, Macclesfield, UK) as first-line therapy until disease progression or treatment discontinue for toxicities or other reasons. We obtained patients' peripheral blood 30±10 days after gefitinib administration for analyzing gefitinib and its main metabolites. Rash was assessed according to National Cancer Institute Common Terminology Criteria version 4.0 (NCI CTCAE 4.0) on the visit of each treatment cycle. In brief, papules and/or pustules covering of body surface area (BSA), symptoms of pruritus or tenderness, limiting self-care age-appropriate instrumental (ADL), local superinfection with oral antibiotics indicated, life-threatening consequences should be considered.

Determination of gefitinib and its metabolites in plasma

We established a method for determining exposures of gefitinib and its main metabolites, included M523595 (M1), M605211 (M2), M537194 (G235) and M387783 (G236) in our previous study [22]. The parent/ product irons of metabolites were 447.60/128.28, 433.59/128.27, 461.62/142.22, 421.57/320.36 and 445.67/128.27, respectively. Gefitinib and metabolites were extracted with 500ul tert-butyl methyl ether by vortex-mixing for 5 min followed centrifuging at 15,000 rpm for 10 min. The resultant residue, dried in a vacuum, was resuspended by 100 ul mobile phase (water: acetonitrile (35:65, v/v) with 0.1% formic acid). 10 ul supernatant was inject into a TSQ Ultra triple-quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Boston, USA) after centrifuging at 15,000 rpm in 4 °C for 10 min. Gefitinib and its four metabolites were separated on a X-Terra RP18 column (50 × 2.1 mm, 3.5 μm, Waters) at 40 °C within 3 min. The calibration ranges were 0.05–100 ng/ml for M605211 (M2), M537194 (G235) and M387783 (G236), and 0.5–1000 ng/ml for gefitinib and M523595 (M1).

DNA isolation and genotyping

Genomic DNA was extracted and purified from peripheral blood leukocytes with Tiangen Blood DNA Purification Kit (AP348, Beijing, China). Briefly, 200 μL peripheral blood leukocytes were digested with proteinase K, disrupted with GD and purified with PW. All DNA were kept in −20 °C until analysis.

Nine 9 SNPs, including SNPs in SLC22A8 rs4149179(PMID: 27274832 and 25788532), ABCC1 rs129081 (PMID:32621177 and 22261339), ABCB1 rs1128503(PMID: 27089937), CYP3A4 rs2242480(PMID: 32042822), SLC22A1 rs4709400(PMID: 26464716), ABCC4 rs2274405(PMID:21266046), SLCO1B3 rs4149117(PMID: 29054076 and 23340295), UGT1A1 rs10929303(PMID: 28433553 and 21997136), ABCB1 rs2032582(PMID: 32042822), selected according to previous studies, were analyzed by using a previously published Agena MassARRAY System technique (Agena Bioscience Applications and Technology, USA) [15], and the location of these SNPs were shown in Table S1. The Assay Design Suite 2.0 was used to design the primers. *EGFR* mutations, which are the most common somatic mutations in exons 18, 19, 20 and 21, were detected by using ADx ARMS *EGFR* mutation detection kit (AmoyDx, Xiamen, China). The proportions of different *EGFR* mutation types were shown in Table 1.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (IBM®), GraphPad 7.0 (San Diego, CA, USA) and R 3.6.0. Codominant, dominant, recessive, over-dominant and log-additive genetic models were analyzed in R 3.6.0 with package SNPassoc1.9-2 [23]. The association ~~of~~ between concentrations of gefitinib and its metabolites and gefitinib-induced rash were analyzed in ggpur package and visualized by ggplot2 [24]. Odds ratios (ORs) and 95% confidence intervals (CIs), generalized linear regression was conducted in R 3.6.0 with packages Glmnet 3.0.2 [25] for predicting gefitinib-induced severe rash. All the codes used in this study was shown in the supplementary file.

Table 1
Patients' characteristics.

Variables	No. of patients(%)		p
	Exploratory dataset	Validation dataset	
Weight, mean(range), kg	61.0(41.4–94.0)	60.4(38.0–94)	0.744
Height, mean(range), cm	162.1(150.0–181.0)	162.6(181.3)	0.757
Gefitinib, mean(range), ng/mL	282.7(59.2–816.5)	259.3(57.3–710.0)	0.319
M1, mean(range), ng/mL	143.5(45.1–607.1)	141.8(58.3–485.8)	0.924
M2, mean(range), ng/mL	14.0(3.0–73.5)	13.3(0.6–32.2)	0.536
G235, mean(range), ng/mL	7.2(1.3–35.7)	7.0(1.7–21.3)	0.880
G236, mean(range), ng/mL	1.4(0.4–4.8)	1.3(0.3–4.0)	0.686
Sex			0.691
Female	73(60.8)	37(57.8)	
Male	47(39.2)	27(42.2)	
Age, year			0.561
≥60	56(46.7)	27(42.2)	
<60	64(56.3)	37(57.8)	
Smoking			0.206
Never smoking	101(84.2)	49(76.6)	
Smoking	19(15.8)	15(23.4)	
EGFR			0.624
19 exon del	64(53.3)	38(59.4)	
21 exon L858R	52(43.3)	24(37.5)	
Other	4(3.4)	2(3.1)	
TNM			0.772
IIIB	8(6.7)	5(7.8)	
IV	112(93.3)	59(92.2)	
Rash			0.564
Grade 0/1/2	98(81.7)	50(78.1)	
Grade 3/4	22(18.3)	14(21.9)	

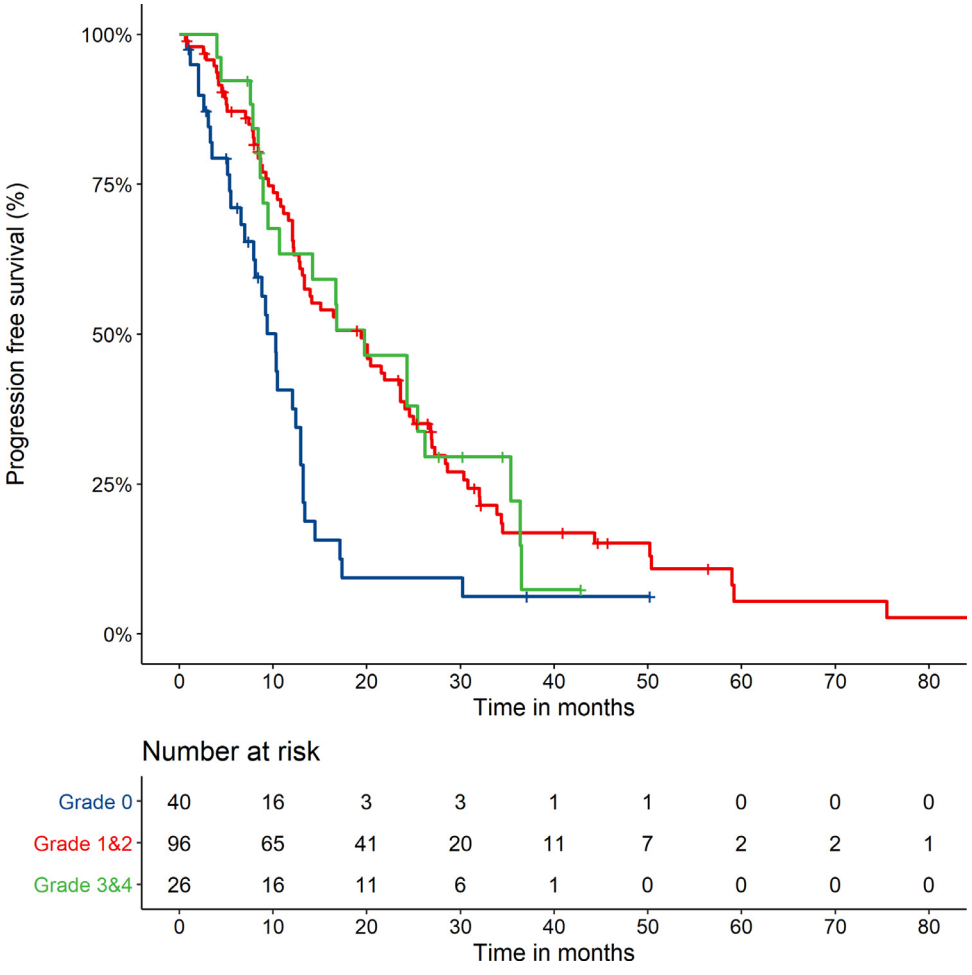

Fig. 1. Skin rash status predicted survival of gefitinib. The median PFS were 10.30(95% CI, 7.93-12.97), 19.40(13.33-24.07) and 19.77(10.67-36.40) months for grade 0, grade 1&2 and grade 3&4, respectively. The log-rank *p* values were 0.0002, 0.0089 and 0.995 for grade 0 vs grade 1&2, grade 0 vs grade 3&4 and grade 1&2 vs grade 3&4, respectively.

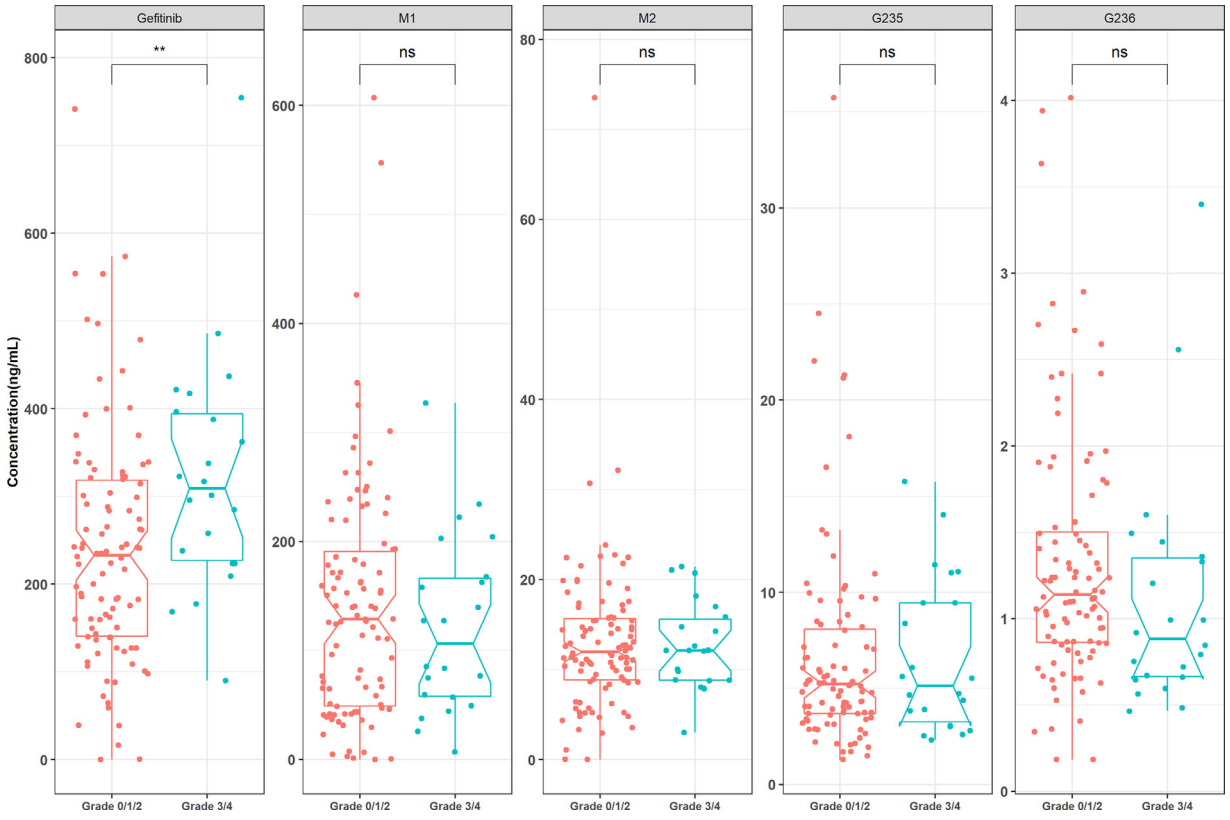

Fig. 2. The concentration of gefitinib was associated with gefitinib-induced grade 3&4 rash.
**: $p < 0.01$; ns: no significance.

Results

Patients

A total of 346 patients were enrolled in this study, including 162 patients in the retrospective cohort, 120 patients in the exploratory cohort and 64 patients in the validation cohort. In the exploratory dataset, 73 females (60.8%) and 47 males (39.2%) patients were enrolled during study, 22(18.3%) patients experiencing grade 3/4 rash (Table 1). 64 patients were enrolled in validation data set, 14(21.9%) were developed grade 3/4 rash (Table 1). The mean concentration of gefitinib, M1, M2, G235 and G236 were 282.7(59.2–816.5) ng/mL, 143.5(45.1–607.1) ng/mL, 14.0(3.0–73.5) ng/mL, 7.2(1.3–35.7) ng/mL and 1.4(0.4–4.8) ng/mL in exploratory dataset, respectively. The mean concentration of gefitinib M1, M2, G235 and G236 were 259.3(57.3–710.0) ng/mL, 141.8(58.3–485.8) ng/mL, 13.3(0.6–32.2) ng/mL, 7.0(1.7–21.3) ng/mL, 1.3(0.3–4.0) ng/mL in validation dataset, respectively. None statistic difference was found for all variables in Table 1 between exploratory and validation dataset.

Severe rash did not improve PFS compared to grade 1/2

As shown in Fig. 1, skin rash status predicted improved survival in NSCLC patients with gefitinib, however, compared to grade 1/2, severe rash (grade 3/4) was not associated with better PFS. The median PFS were 10.30(95% CI, 7.93–12.97), 19.40(13.33–24.07) and 19.77(10.67–36.40) months for grade 0, grade 1/2 and grade 3/4, respectively. The log-rank p values were 0.0002, 0.0089 and 0.995 for grade 0 vs grade 0/1/2, grade 0 vs grade 3/4 and grade 1/2 vs grade 3/4.

The concentration of gefitinib was associated with gefitinib-induced severe rash

As shown ~~as~~ in Fig. 2, the mean concentration (±SD) of G235, G236, Gefitinib, M2 and M1 were 6.620 ± 4.036 ng/mL, 1.113 ± 0.705 ng/mL, 323.100 ± 138.400 ng/mL, 12.600 ± 4.878 ng/mL and 121.500 ± 81.500 ng/mL in patients with grade 3/4 rash, respectively. 6.704 ± 5.468 ng/mL, 1.327 ± 0. 764 ng/mL, 239.300 ± 134.100 ng/mL, 13.070 ± 8.616 ng/mL and 140.800 ± 110.00 ng/mL among patients with grade 0/1/2 rash, respectively. Only the concentration of gefitinib was associated with gefitinib induced severe rash ($p = 0.007$) by Wilcox test.

SNPs in transporters were associated with severe gefitinib-induced rash

As shown in Table S1, only four transporters SNPs were associated with gefitinib-induced severe rash without consideration of clinical confounding factors in the exploratory dataset ($p < 0.1$), including *SLC22A8* rs4149179 ($p = 0.078$), *ABCC1* rs129081 ($p = 0.092$), *ABCB1* rs1128503 ($p = 0.012$) and *SLC22A1* rs4709400 ($p = 0.035$). We further analyzed the association between SNPs and concentrations of gefitinib. *UGT1A1* rs10929303 were correlated with gefitinib plasma exposure and concentration of gefitinib in patients with TT genotypes were significantly lower compare to any other genotypes ($p < .01$); gefitinib exposures in *SLC22A1* rs4709400 CC genotype carriers were significantly higher compare to CG carriers ($p < 0.01$) (Fig. 3). The association between SNPs and metabolites were shown in Figure S1.

Developing predictive model and validating in the validation dataset

We evaluated the predictive performance of clinical confounding factors, including height, weight, sex (male, female), *EGFR* sensitive mutation type (21 exon L858R,19 exon del and others), age (≥60, <60

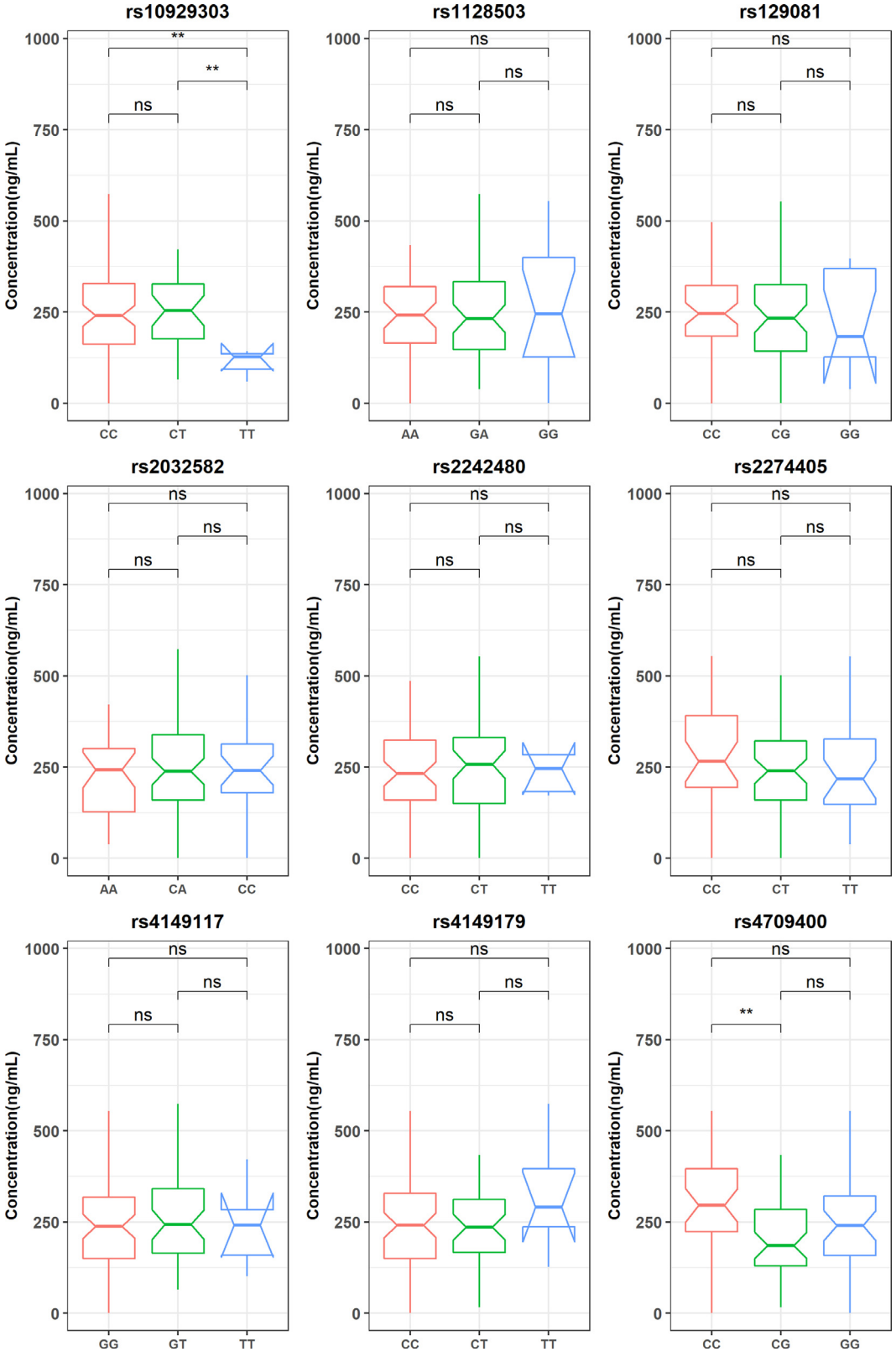

Fig. 3. The relationships gefitinib concentration and were analyzed by conditional plots.
$^{*}p < 0.05$; $^{**}p < 0.01$; ns, no significance.

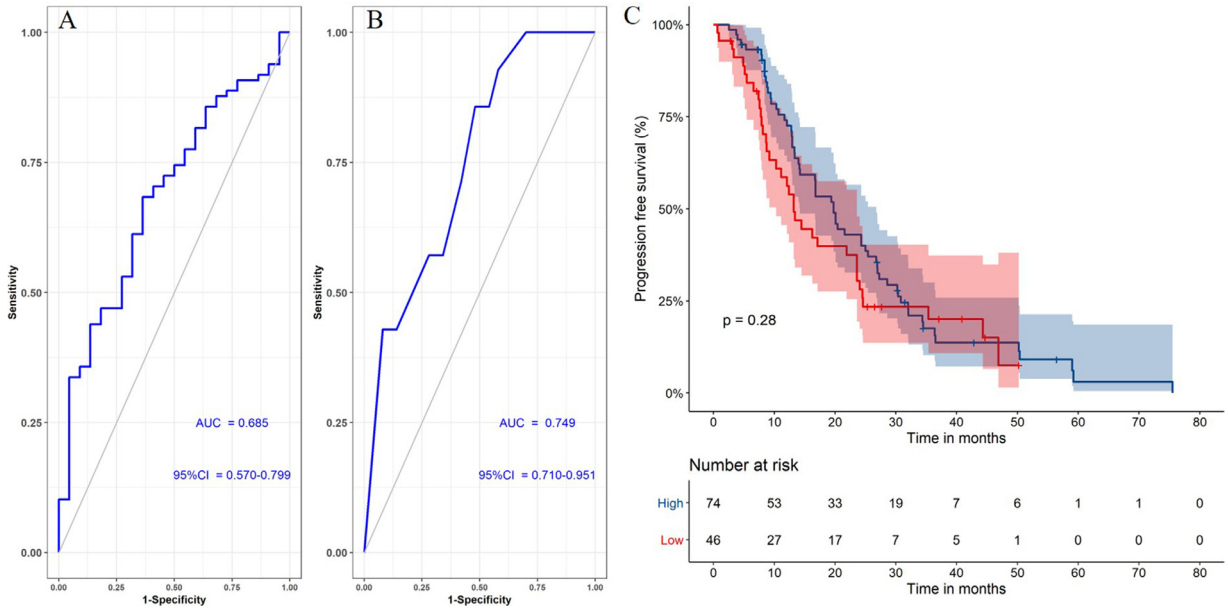

Fig. 4. Receiver operating characteristic curve for the concentration of gefitinib for severe rash and for validation cohort with established model. **A**, the sensitivity and specificity were 43.9% and 86.4%, and the cut off value for gefitinib was 204.6 ng/ml; **B**, the sensitivity and specificity were 42.6% and 92.0% in validation cohort with predictive models; **C**, patients were grouped by gefitinib concentration into gefitinib high and low group, the median PFS were 19.77(95%CI:14.23-27.77) and 13.20(95%CI:10.30-23.60), $p = 0.28$.

Table 2
Establish a predictive model for gefitinib induced severe rash by generalized linear model with clinical confounding factors.

Variables	*P*	OR
Gefitinib(ng/ml)	–	–
≥204.6 vs <204.6	0.0016	11.523(2.898–64.016)
SLC22A8 rs4149179		
CT vs CC	0.0629	3.156(0.958–11.164)
TT vs CC	0.632	0.504(0.0170–6.204)
SLC22A1 rs4709400		
CG vs GG	0.0087	10.267(2.067–72.465)
CC vs GG	0.061	5.103(1.032–33.938)

years old), tumor stages(IV, IIIB), smoking status(never smoked, smokers). Generalized linear model was used to build the predictive model in the exploratory dataset. The gefitinib cutoff value of 204.6 ng/mL (AUC=0.685, 95%CI: 0.570–0.799, sensitivity= 43.9%, specificity= 86.4%) was determined by conducting ROC curve analysis for severe rash in NSCLC patients (Fig. 4A) and was introduced in the following model. From Table 2, variables included in the model were gefitinib concentration (OR = 11.523, 95%CI = 2.898–64.016, $p = 0.0016$), *SLC22A8* rs4149179(CT vs CC, OR = 3.156, 95%CI = 0.958–11.164, $p = 0.0629$), *SLC22A1* rs4709400(CG vs CC, OR = 10.267, 95%CI = 2.067–72.465, $p = 0.0087$; GG vs CC, OR = 5.103, 95%CI = 1.032–33.938, $p = 0.061$). As shown in Fig. 4B, this model showed excellent predictive ability for discriminating between gefitinib-induced grade 0/1/2 and grade 3/4 gefitinib-induced rash in the validation dataset (AUC = 0.749, 95%CI = 0.710–0.951).

We further analyzed the association of gefitinib concentration with PFS in NSCLC patients. Regarding 204.6 ng/ml as the cutoff value, patients were grouped into gefitinib high and low group, the median PFS were 19.77(95% CI:14.23–27.77) and 13.20(95%CI:10.30–23.60), respectively. ($p = 0.28$) (Fig. 4C).

Discussion

Rash is a well-known predictor of PFS for patients with EGFR-TKIs therapy. However, whether severe rash (grade 3/4) is a benefit factor for PFS compared to grade 0 and grade 1/2 rash remains unclear. In this study, the grade 3/4 of rash were disassociated with PFS of patients with gefitinib therapy compared to grade 1/2 of rash. To filter patients with the gefitinib-induced grade 3/4 rash, a predictive model was established by incorporating with gefitinib concentration, *SLC22A8* rs4149179 and *SLC22A1* rs4709400 in this study.

The present study showed the occurrence of rash was significantly associated with greater PFS which is good agreement with other studies for patients with gefitinib therapy [26]. Grade 3/4 rash did not improve PFS compared to patients suffering from grade 1/2 of skin rash (Fig. 1). The median PFS of grade 3/4 19.77(95%CI:10.67–36.40) was a little longer than that in patients with grade 1/2 rash (median PFS=19.40 month (95%:13.33–24.07)), but no statistical significance was found ($p = 0.995$). A significant increase in the probability of PFS was found to be associated with increased severity of erlotinib-induced rash (grade 2+ vs grade 1, $p = 0.019$) [27]. This discrepancy could be attributed to different drugs, different races (oriental and non-oriental) and different grouping ways (grade3/4 vs grade 0/1/2 for gefitinib, grade 2+ vs grade 1 for erlotinib).

It's known that patients with grade 3/4 rash often lead to drug discontinuation/ interruption. To improve the quality of life, predictive model is warranted for filtering patients who will develop severe rash on gefitinib. The present study established predictive model with gefitinib steady state trough concentrations, *SLC22A8* rs4149179 and *SLC22A1* rs4709400. In this study, severity of rash is associated with levels of gefitinib steady state trough concentrations (grade3/4 vs grade0/1/2, $p = 0.015$). The association between gefitinib exposure and incident of gefitinib-induced adverse reaction is still controversy due to small sample size [19,28]. Meanwhile, trough concentration of gefitinib was disassociated with gefitinib-induced skin rash(grade 1+ vs grade 0, grade 2+ vs grade 0&1) [15]. To date, the mechanism of gefitinib-induced rash is considerably complicated and needs to be answered. In terms of dermatologic toxicities, EGFR-TKIs-induced rash was correlated with abnormal chemokine expression, and further expression of microbial defense genes and epidermal differentiation were altered in keratinocytes [29,30]. The expression of microbial defense genes was associated with EGFR-TKIs exposure in vitro [30]. It is possible that increased gefitinib concentration was attributed to the inhibition of microbial defense genes and induced rash.

One important question is whether decreasing the dose of gefitinib in patients with grade 3/4 rash is needed. Regarding to gefitinib dosage, Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL-1 and –2) and NEJ002, the 500 mg dose was correlated with higher commonly rash, but the PFS and overall survival (OS) were similar [31-33]. What's more, steady state trough concentration of gefitinib was disassociated with PFS of patients in NSCLC patients with EGFR sensitive mutation [34,35], which was similar in this study (Fig. 4C). Furthermore, dose reduction may not lead to inferior PFS due to severe toxicities compared to patients with full dosage in a retrospective study [36]. Taken together, it is possible to reduce dosage of gefitinib in patients with grade 3/4 rash in future after prospective validation study to improve the quality of life and reduce management costs of patients because of skin toxicity.

On the other hand, patients with steady state trough concentration ≥ 200 ng/mL suffered more rash compared with <200 ng/mL in *EGFR* wild-type volunteers [37]. In the present study, the cutoff value of steady state trough concentration of gefitinib was 204.6 ng/mL with a moderate accuracy in patients with activating *EGFR* mutation suffering grade 3/4 rash (AUC = 0.685, 95%CI: 0.570–0.799, sensitivity = 43.9%, specificity = 86.4%). It's hard for us to come to a conclusion with such a similar result between incidence rate of rash in *EGFR* wild type (grade 1+ vs grade 0) and EGFR sensitive mutation (grade 3/4 vs grade 0/1/2) due to differences of drug sensitivity in our study. Therapeutic drug monitoring steady state trough concentration of gefitinib might be necessary in patients who received gefitinib.

The accuracy of gefitinib cutoff value was not enough for application with a moderate accuracy in the present study. The two SNPs on *SLC22A8* [38] and *SLC22A1* [39], involved in gefitinib and its metabolites transporting, were included in the predictive model. The model was established based on exploratory dataset by generalized linear regression with a moderate accuracy (AUC = 0.749) in the validation cohort. Previous studies showed that cationic charge and high lipophilicity in the structure of gefitinib might have the interaction of SLC transporters [40], which implied that gefitinib might be a substrate of *SLC22A1* and *SLC22A8*. SLC transporters, unlike ABC transporters, are involved in the absorbs of endogenous and exogenous molecules into cells, including steroids, prostaglandins, gut microbiome and drugs [41]. Thus, functional variations in *SLC22A1* and *SLC22A8* may have impacts on exposure of gefitinib in skin, furthermore, have influences on inflammation through regulating internal environment variations [42]. *T>C* rs4149179 and *C>G* rs4709400 are located in 5′ UTR on *SLC22A8* and *SLC22A1,* which is regulatory region on genes [43], might have an impact on expression of *SLC22A8* and *SLC22A1* and further studies are warranted in future.

Conclusion

The analyses and models presented here suggest physicians and patients should not always view the rash as a much desirable outcome. The patients who developed non-severe rash are likely benefit from gefitinib, have good quality of lives and less health care costs. In conclusion, we developed a predictive model involved variations on *SLC22A1/SLC22A8* and gefitinib concentration with an excellent accuracy in the validation dataset for predicting gefitinib-induced grade 3/4 rash. Prospective research is warranted in future.

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Contributions

Yan Huang, Fei Wang and Wei Feng: Methodology, Resources and Project administration; Min Huang, Xueding Wang and Li Zhang: Funding acquisition, Writing - Review & Editing; Shaoxing Guan and Xi Chen: Investigation, Formal analysis, Data Curation and Writing - Original Draft; Shu Liu and Wenfeng Fang: Visualization; Wei Zhuang, Hongyun Zhao and Xiaoxu Zhang: Validation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

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