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Effects of pharmacokinetics-related genetic polymorphisms on the side effect profile of afatinib in patients with non-small cell lung cancer

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ABSTRACT

Objectives: Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) represent the first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer. Afatinib is a second-generation EGFR-TKI with excellent therapeutic effects. However, severe diarrhea and skin disorders are observed at high frequencies, often leading to treatment interruption because of low quality of life (QOL). The relationship between individual variations and the onset of these side effects remains to be elucidated. This study aimed to reveal the association among these side effects, pharmacokinetics, and related genetic polymorphisms.
Materials and methods: In total, 33 patients were recruited between July 2014 and June 2017. Afatinib plasma concentrations were measured at day 9 when the concentrations reached a steady state (early phase) and when the prescription dose was stable for more than 1 month (stable phase). We analyzed single nucleotide polymorphisms in the genes ATP-binding cassette sub-family B member 1 (*ABCB1*), *ABCG2*, and flavin-containing monooxygenase 3.
Results: The incidences of both diarrhea and acneiform eruption were greater than 80%. Afatinib plasma concentration and the severity of diarrhea in the early phase were correlated. Pharmacokinetics-related genetic polymorphisms influenced the severity of diarrhea. Particularly, the afatinib plasma concentration was higher and diarrhea was more severe in patients carrying the A allele of *ABCG2* C421A. Onset of side effects, genetic polymorphisms, and diarrhea in the maintenance phase or acneiform eruption in the early or maintenance phases were not correlated. The severity of diarrhea is influenced by drug plasma concentrations in the early phase and genetic polymorphisms related to afatinib pharmacokinetics.
Conclusion: Particular genetic polymorphisms can be screened before afatinib administration and the dose adapted to individual patients can be controlled, leading to reduced side effects, improved QOL, and better patient compliance to maintain the therapeutic effects.

1. Introduction

Lung cancer (LC) is the most common cancer worldwide with an estimated 2+ million newly diagnosed cases annually [1]. Despite the decreasing incidence of mortality, LC accounts for more than 1.2 million deaths annually, with an age-standardized 5-year net survival rate of only 10–20% [2]. There are two main types of LC: small cell lung cancer (SCLC) and non-SCLC (NSCLC). NSCLC accounts for approximately 85% of all LC cases [3]. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are administered as the first-line treatment for patients with advanced EGFR mutation-positive NSCLC. The frequency of EGFR mutations in NSCLC is greater in Asian vs. Caucasian populations (up to 50–60% vs. ˜10%, respectively) [4–6].

Clinical trials comparing first-generation EGFR-TKIs (gefitinib and

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erlotinib) with platinum-based first-line chemotherapy in common EGFR mutation-positive NSCLC unequivocally showed that the use of EGFR-TKIs improved the primary endpoint of progression-free survival (PFS) as compared to chemotherapy (9.2–13.1 vs. 4.6–6.3 months, respectively) and were better tolerated [7–9]. However, none of the first-generation TKI trials reported a benefit in overall survival (OS). The lack of an OS benefit for first-generation TKIs was confirmed in a meta-analysis of six trials that compared PFS and OS in patients with exon 21 and exon 19 EGFR mutation-positive NSCLC [10]. Additionally, some patients discontinued EGFR-TKI treatment because of the development of adverse events. Switching EGFR-TKI treatments because of adverse events is an effective option for patients with EGFR mutation-positive NSCLC [11].

Afatinib is a second-generation EGFR-TKI with excellent therapeutic effects. Particularly, of the many EGFR-TKIs, the use of afatinib leads to acquired resistance to EGFR-TKIs due to minor mutations to alleles of *EGFR* [12]. Afatinib showed an OS benefit in comparison with first-line chemotherapy in the LUX-Lung 3 and 6 trials [13]. In a pre-specified subgroup analysis of exon 19 EGFR mutation-positive NSCLC patients, a significant and clinically relevant median OS benefit of 12.2 and 13 months (LUX-Lung 3 and 6, respectively) was demonstrated with the use of afatinib [14,15]. This benefit was observed in both trials and in both Caucasian and Asian populations, as well as in a Japanese subgroup, despite a cross-over rate from chemotherapy to TKI of 100% [13]. Furthermore, Ezeife et al. reported the efficacy of afatinib re-challenge in heavily pretreated patients with advanced NSCLC [16]. However, many cases require a dose reduction from the standard prescription dose of 40 mg/day [17,18]. Especially, many Japanese patients require dose reductions due to severe side effects [19]. However, there are no reports about the cause of this inter-ethnic difference.

Despite the improvements in PFS and OS with afatinib, severe diarrhea and skin disorders, often leading to treatment interruption because of the low quality of life (QOL), are observed at high frequencies [14]. The relationship between individual variations and the onset of these side effects remains to be elucidated. Generally, the interindividual genetic variations in drug metabolizing enzymes and transporters influence the efficacy and toxicity of various drugs [20]. Genomic differences between individuals are present at approximately every 300–1000 nucleotides with over 14 million single nucleotide polymorphisms (SNPs) distributed throughout the entire human genome. The drug response of individual patients is primarily determined by the pharmacokinetic and pharmacodynamic properties of the prescribed drugs, which are either directly or indirectly affected by polymorphisms of drug metabolizing enzymes and transporters. Different populations have varied allele frequencies in genes of both drug metabolizing enzymes and transporters [21].

There are no reports about the correlation between genetic polymorphisms of drug metabolizing enzymes and transporters that influence the pharmacokinetics and side effects of afatinib. Therefore, the aim of the present study was to reveal the association between the side effects of afatinib, pharmacokinetics of afatinib, and pharmacokinetics-related genetic polymorphisms.

2. Patients and methods

2.1. Patients

The cohort of this prospective study included 33 Japanese patients, aged 54–74 years, with NSCLS who were administered afatinib at the Department of Respiratory Medicine, Gifu University Hospital during the study period of July 2014 to June 2017. The duration of evaluation for adverse effect was 3 months after the first administration of afatinib. All patients were fully informed of the purpose of the study and the risks involved, and gave written informed consent before being enrolled. Eligible patients were those with EGFR mutation-positive advanced NSCLC who were diagnosed based on the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. Patient demographics (age, body weight, height, and gender) and clinic courses (afatinib dose, degree of adverse event, and additional medical problems) were retrieved from medical records. The adverse events of afatinib were periodically assessed by each patient's physician according to Common Terminology Criteria for Adverse Events (v4.0) using a predefined format. A blood sample was collected for measurement of plasma afatinib concentrations and genotyping of ATP-binding cassette sub-family B member 1 (ABCB1), ABCG2, and flavin-containing monooxygenase 3 (FMO3).

The study protocol was approved by the Research Ethics Committees of Gifu Pharmaceutical University and the Gifu University School of Medicine, and was conducted in full concordance with the tenets of the Declaration of Helsinki, the Ethical Guidelines for Human Genome/Gene Analysis Research in Japan, and Japanese law.

2.2. Genotyping

Whole venous blood was collected into Venoject II vacuum tubes containing ethylenediaminetetraacetic acid-Na (final, 4.5 mM) (Terumo, Tokyo, Japan). A QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to extract leukocyte genomic DNA directly from the blood specimens. DNA samples were stored at −80 °C until analysis. Genotyping was performed using polymerase chain reaction–restriction (PCR), PCR–fragment length polymorphism, and allele–specific PCR assays. Supplementary Table 1 shows the primer sequences, PCR conditions, and restriction enzymes. The PCR products and restriction enzyme digestion products were electrophoresed on 2%–4% agarose gels, stained with ethidium bromide, and viewed under ultraviolet light.

2.3. Determination of afatinib plasma concentration

Measurement of afatinib trough concentrations in plasma was performed on day 9 (early phase) and the day when the prescription dose was stable for more than 1 month (stable phase). Blood samples were collected immediately prior to drug administration. Plasma concentrations of afatinib were analyzed by validated multiple reaction monitoring mode-based liquid chromatography-tandem mass spectrometry (LC–MS/MS) methods, as described in our previous report, but with minor modifications [22]. Briefly, an internal standard (deuterated gefitinib) was added to each plasma sample. After mixing, tert-butyl methyl ether was added and the tube was mixed vigorously and then centrifuged. The top organic layer was transferred to a polypropylene tube and dried under a stream of nitrogen gas. Samples were reconstituted with the mobile phase. Samples were injected onto the LC instrument for quantitative analysis. The LC–MS/MS system comprised a Nexera X2 Ultra High Performance Liquid Chromatograph (Shimadzu Corporation, Kyoto, Japan) and a LCMS-8040 Triple Quadrupole Mass Spectrometer equipped with an electrospray ion source (Shimadzu Corp., Kyoto, Japan). XBridge Shield RP18 Column (3.5 µm, 2.1 × 50 mm; Waters Corporation, Milford, MA, USA) was used for the chromatographic separation in the Nexera X2 UHPLC system.

2.4. Data analyses

The Spearman's correlation coefficient was used to test the association between the plasma concentration of afatinib and the severity of diarrhea. Genotyping data were tested for deviation from the Hardy–Weinberg equilibrium using the Fisher's exact test. The Mann–Whitney U test was used to compare the afatinib plasma concentration in different patient groups. A two-sided probability (p) value of < 0.05 was considered statistically significant. The statistical analyses were performed using the SPSS Statistics 19 software (SPSS Inc., Chicago, IL).

Table 1
Patients' characteristics.

	NSCLC Patients (n = 33)
Male/ Female	13/20
Age (years)	67.5 ± 9.6
Body weight (kg)	55.2 ± 12
Aspartate transaminase (IU/L)	24.8 ± 11
Alanine transaminase (IU/L)	20.4 ± 19
Total bilirubin (mg/dL)	3.9 ± 0.5
EGFR mutations	
exon18 G719X	3
exon19 deletions	14
exon21 L858R	15
exon21 L861Q	1
Initial dose	
40 mg	26
30 mg	7
Treatment line	
First-line	13
Second-line	8
After third-line	12
EGFR-TKIs before afatinib	
None	20
Gefitinib	7
Erlotinib	2
Gefitinib + Erlotinib	4

Values of age, body weight, transaminases, and bilirubin are expressed as mean ± S.D.

3. Results

3.1. Patient characteristics and genotypes

Table 1 shows the demographics of the 33 enrolled patients. Briefly, the average age was 67.5 (range, 54–74) years and the male:female ratio was 13:20. Genotyping was successful for all patients. Supplementary Table 2 shows genotype polymorphisms in afatinib pharmacokinetic-relative enzymes and transporters. No genotype distribution deviated from the Hardy–Weinberg equation.

3.2. Status of adverse effects and dose reduction

Fig. 1 shows the adverse effects of patients with NSCLC taking afatinib. The incidence of diarrhea was the highest for both ALL grade and grade ≥ 2 adverse events. Twenty-four patients (72.7%) required a dose reduction from the standard prescription dose of 40 mg. Only 9 (27.3%) patients were able to tolerate the standard prescription dose of 40 mg in the stable phase, while the dose was reduced to 30 mg in 13 (39.4%) and to 20 mg in 8 (24.2%). The prescription dose of 3 (9.1%) patients was reduced further to 20 mg every other day.

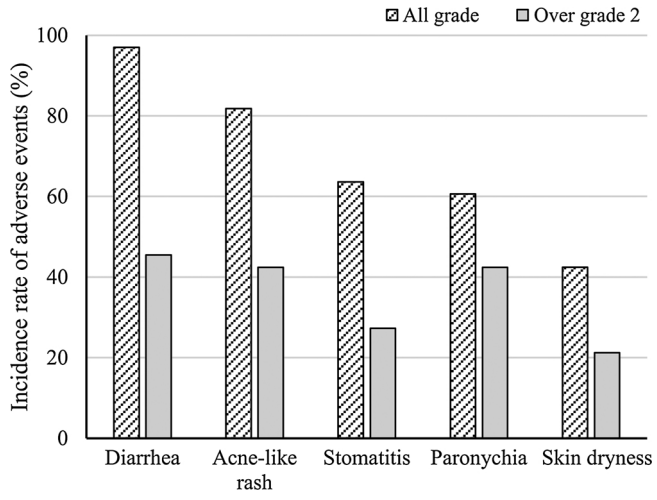

Fig. 1. Status of side effects among patients with NSCLC taking afatinib. The striped bars indicate the incidences of the all grade side effects and the gray bars indicate the incidences of grade ≥ 2 side effects.

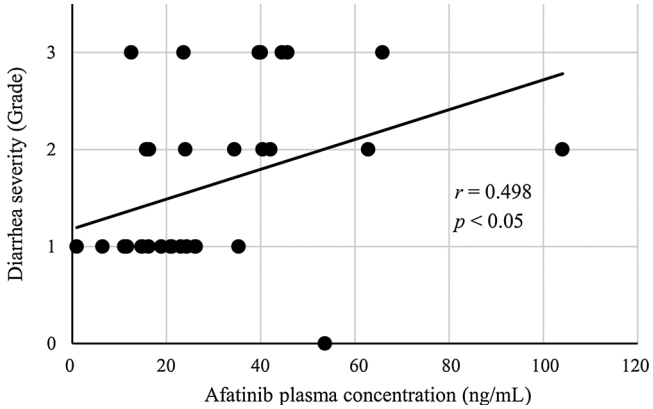

Fig. 2. Relationship between afatinib plasma concentration and the severity of diarrhea in the early phase after the start of administration (r = 0.498; p < 0.05; Spearman's correlation coefficient).

3.3. Relationship between afatinib plasma concentration and severity of diarrhea

The severity of diarrhea was evaluated based on the lowest grade during the administration period. There was a correlation between afatinib plasma concentration and the severity of diarrhea in the early stage after the start of administration. The grade of the diarrhea tended to become more severe in patients administered high concentrations of afatinib (Fig. 2).

3.4. Effects of pharmacokinetics-related genetic polymorphisms on the afatinib plasma concentration

There was a significant difference in afatinib plasma concentration adjusted by dose and body weight between patients with and without the mutant *ABCG2* C421A allele. In patients with the mutant *ABCG2* C421A allele, afatinib plasma concentrations were significantly higher than in those without the mutant allele. There were no significant differences in the incidences of other genetic polymorphisms (Fig. 3). Patients carrying the A allele (mutant allele) of *ABCG2* C421A experienced a two-fold greater incidence of severe diarrhea (grade ≥ 2), as compared with patients without the A allele. However, this difference was not statistically significant (data not shown).

We investigated the relationship among the total number of mutated alleles in five SNPs of three genes, the percentage of the patients administered with the standard dose, and the incidence of diarrhea. The proportion of patients with a mutated allele and who were able to tolerate the standard dose was low (Fig. 4a), while the incidence of severe diarrhea in this population was high (Fig. 4b). A reduction from the standard dose is required in cases of severe diarrhea in patients with a mutated allele.

3.5. Effect of dose reduction due to severe diarrhea on changes in afatinib plasma concentrations from the early to stable phase

In the early phase, the afatinib plasma concentration of patients with severe diarrhea (grade ≥ 2) was significantly greater than that of patients with mild diarrhea (grade ≤ 1). In the stable phase, there was no notable difference in afatinib plasma concentrations between the patient groups (Fig. 5). In the early phase, because of the dose reduction due to the occurrence of side effects, the afatinib plasma concentration

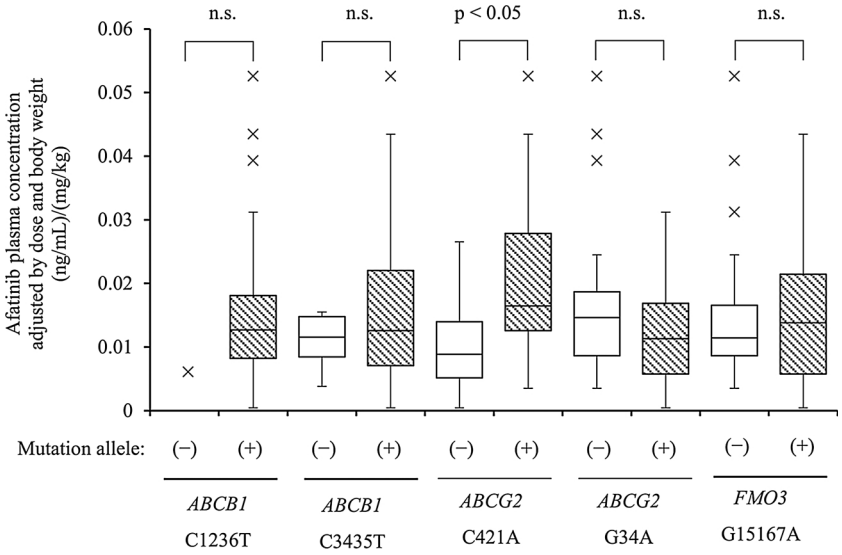

Fig. 3. Relationship between afatinib plasma concentrations and genetic polymorphisms in the five SNPs of the three genes affected afatinib pharmacokinetics in the early phase after the start of administration. Boxes indicate the 25% and 75% quantile ranges, and whiskers indicate the 5% and 95% quantiles. The Mann–Whitney U test was used to obtain the *p* values. n.s., not significant; (−), patients without mutation allele; (+), patients with mutation allele.

was decreased in the stable phase.

4. Discussion

Afatinib, an irreversible ErbB family blocker, inhibits signaling from all homo/heterodimers formed by ErbB receptors. In the randomized phase IIb LUX-Lung 7 trial, afatinib significantly improved PFS, time-to-treatment failure, and the objective response rate vs. gefitinib, a first-generation EGFR-TKI, in patients with EGFR mutation-positive NSCLC. In the patients given afatinib, the most common treatment-related grade 3 and 4 adverse events were diarrhea and rash [23]. In the subgroup analysis of the LUX-Lung 3 trial, adverse events leading to a dose reduction occurred in 76% of afatinib-treated Japanese patients. The most common adverse events leading to a dose reduction of afatinib were nail effects (32%), rash/acne (28%), and diarrhea (22%) [19]. Similar results were confirmed in the present study, in which 73% of patients required a dose reduction due to severe adverse events.

The recommendation dose of afatinib is 40 mg/day; however, it has been reported that many patients administered the prescribed dosage required a dose reduction due to adverse events. Furthermore, among these patients, plasma concentrations of afatinib tended to be high, suggesting that an appropriate plasma concentration of afatinib can be maintained by a dose reduction [18]. In the present study, there was a correlation between the afatinib plasma concentration and the severity of diarrhea in the early phase after the start of administration. With the use of an appropriate dose of afatinib from the early phase, it is possible to decrease the incidence of adverse events and improve the patient's QOL. Moreover, decreasing the incidence of adverse events caused by prolonged use of afatinib is expected to improve the PFS and OS rates.

Anti-cancer drug resistance is often associated with an adenosine triphosphate (ATP)-dependent decrease in cellular drug accumulation, attributed to the expression of certain ATP-binding cassette (ABC) transporter proteins. ABC transporters that confer drug resistance include P-glycoprotein (ABCB1), multidrug resistance protein 1 (MRP1, ABCC1), and the breast cancer resistance protein (BCRP, ABCG2). These ABC transporters promote the efflux of anti-cancer drugs from

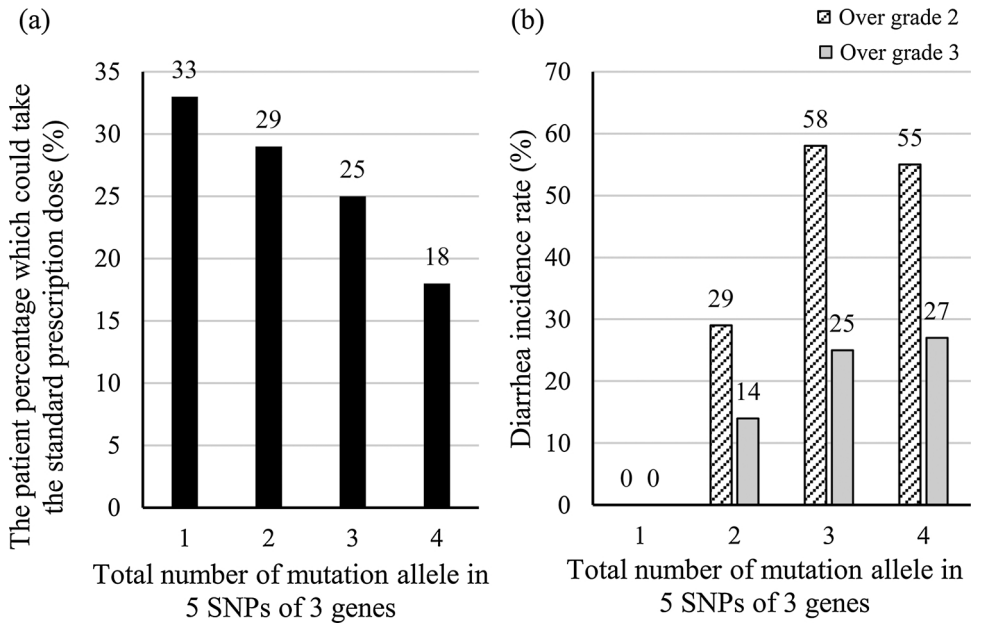

Fig. 4. Relationship among the total number of mutated allele in five SNPs, percentage of patients administered with the standard dose (a), and the incidence of grade ≥ 2 diarrhea (b) in the early phase after the start of administration.

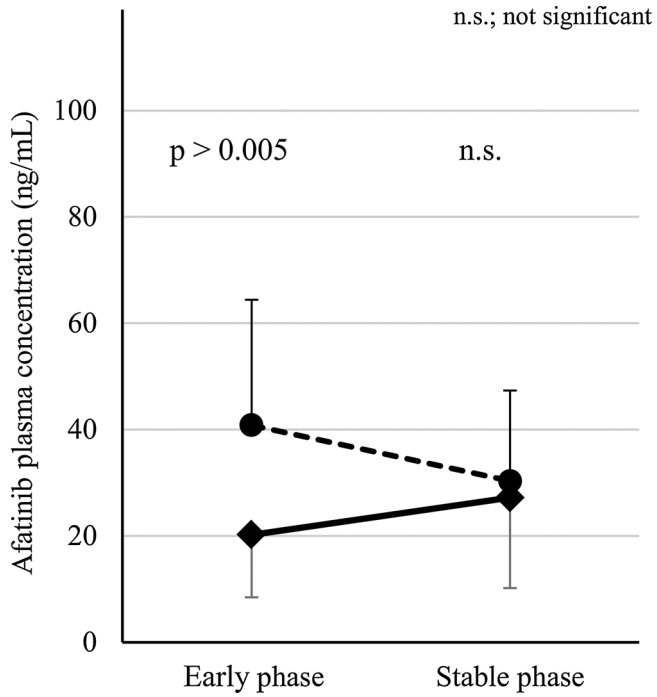

Fig. 5. Effect of dose reduction due to severe diarrhea on the afatinib plasma concentration from the early to stable phase. The dotted line indicates afatinib plasma concentrations of patients with grade ≥ 2 diarrhea (n = 18). The solid line indicates afatinib plasma concentrations of the patients with grade ≤ 1 diarrhea (n = 15). The values are presented as the mean ± S.D. The Mann–Whitney U test was used to obtain the *p* value. n.s., not significant.

cancer cells, resulting in a decrease in the cellular drug concentration. Furthermore, P-glycoprotein, MRP1, and BCRP are expressed in important tissues for drug absorption (e.g., intestine) and elimination (liver and kidney). These transporters cause a decrease in blood drug concentrations by inhibiting absorption from the intestine and promoting elimination of drugs into bile and urine [24]. It is reported that BCR-ABL TKIs, anti-cancer drugs for chronic myelogenous leukemia, such as imatinib, nilotinib, dasatinib, ponatinib and bosutinib, are substrates of P-glycoprotein and BCRP. Moreover, first-generation EGFR-TKIs for NSCLC, such as gefitinib and erlotinib, are substrates of these transporters [25–27]. Several studies have reported that afatinib can interact with P-glycoprotein and BCRP [28–30]. Additionally, Hoppe et al. reported that P-glycoprotein and BCRP had restricted the oral availability of afatinib in a mouse model. Furthermore, afatinib undergoes minimal metabolism by non-enzyme-catalyzed adduct formation with proteins or nucleophilic small molecules and is predominantly non-renally excreted via the entero-hepatic system. For instance, cytochrome P450 does not participate in afatinib metabolism [31]. A small percentage of afatinib is metabolized by FMO3 [32]. There were no patients with severe liver dysfunction in this study. The levels of transaminases in several patients were within the normal limits. Although few patients exceeded the baseline value, mild to moderate liver dysfunction has been reported to have no effect on the pharmacokinetics of afatinib [33].

Genetic polymorphisms of drug transporters and metabolic enzymes often cause individual variations in the pharmacokinetics of various drugs. Cusatis et al. reported the relationship between the pharmacogenetics of *ABCG2*, which codes for the BCRP protein, and adverse reactions to gefitinib [34]. In this study, the afatinib plasma concentration was higher and diarrhea was more severe in patients carrying the mutant allele of *ABCG2* C421A. Moreover, patients carrying the A allele of *ABCG2* C421A had a two-fold greater incidence of severe diarrhea (grade ≥ 2), as compared with patients not carrying the A allele. *ABCG2* C421A has been associated with lower BCRP protein expression. In patients carrying the mutant allele of *ABCG2* C421A, decreased expression of BCRP protein may increase gastrointestinal absorption of afatinib and decrease its excretion into bile, resulting in an increase in plasma concentration. The frequency of SNPs of *ABCG2* exhibits inter-ethnic differences. Notably, C421A is frequently observed in Asian compared with Caucasian population [35]. Therefore, plasma concentration of afatinib may be high in Asians, including the Japanese population. Furthermore, we investigated the relationship among the total number of mutated alleles in five SNPs of three genes (*ABCB1*, *ABCG2*, and *FMO3*), the percentage of patients administered with the standard dose, and the incidence of diarrhea. The percentage of patients with mutated alleles for whom the standard dose could be administrated was low, while the incidence of severe diarrhea was high. Hence, a reduction from the standard dose is required for patients with mutated alleles and severe diarrhea.

Severe diarrhea is a major adverse effect that appears in the early phase and is a dose-limiting factor of afatinib. Yang et al. reported that afatinib plasma concentrations with the 40 mg dose were higher at day 22 among patients who subsequently required a dose reduction to 30 mg vs. those who remained on 40 mg, with similar concentrations observed between the groups on day 43 [18]. However, because diarrhea often occurs within 7 days from the first administration of afatinib, it is necessary to investigate the pharmacokinetics of afatinib and the severity of diarrhea at an earlier phase. Therefore, we investigated the effect of dose reduction due to severe diarrhea on changes to the afatinib plasma concentration from the early phase (day 9) to the stable phase. In the early phase, because of the dose reduction due to adverse effects, the afatinib plasma concentration was decreased in the stable phase. Therefore, in the early phase, if diarrhea can be controlled by a dose reduction based on afatinib plasma concentrations, it may be possible to continue afatinib therapy for an extended period.

Limitations to this preliminary pilot study include the small sample size; thus, further large-scale studies are required to confirm these results.

In conclusion, the severity of diarrhea is influenced by afatinib plasma concentrations in the early phase, influenced by genetic polymorphisms related to afatinib pharmacokinetics. This particular genetic polymorphism can be tested before afatinib administration and the dose adapted to the individual patient can be controlled, leading to a reduced incidence of side effects, improved QOL, and better patient compliance to maintain the therapeutic effects.

Conflict of interest

The authors have no conflicts of interest associated with this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.05.013.

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