ABSTRACT

The purpose of this study is to investigate associations between allelic variations of ABCG2 and ABCB1 with skin toxicity, diarrhea, liver injury and interstitial lung disease (ILD) in gefitinib-treated patients. A prospective clinical study of 83 Japanese patients with non–small–cell lung cancer was performed. Polymorphic loci in ABCG2 and ABCB1 were genotyped, and their effects on gefitinib toxicities were evaluated. ABCG2 34G>A was statistically associated with occurrence of skin rash; 13 (42%) of the 32 patients with at least one variant ABCG2 34G>A allele (G/A and A/A) developed grade 2 or worse skin rash, whereas only 10 (19%) of 51 patients homozygous for the reference allele (G/G) for the wild-type sequence for both alleles did so (P=0.046). There was no significant association between severe toxicities and polymorphisms of ABCG2 421C>A nor ABCB1 3435C>T. The results suggested that ABCG2 34G>A would be useful for predicting grade 2 or worse skin rash.

Key Words: ABCG2, ABCB1, Genetic polymorphisms, Gefitinib

INTRODUCTION

Gefitinib (Iressa), an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, has activity in patients with inoperable or recurrent non-small-cell lung cancer. EGFR-TKIs inhibit the intracellular tyrosine kinase domain of the EGFR and therefore block the signal transduction pathways implicated in the proliferation and survival of cancer cells. Recent clinical studies have demonstrated that gefitinib has consistent clinical benefit in patients with the activating mutations of the EGFR genes.

Skin rash and diarrhea are prominent adverse events of gefitinib treatment, which occurred in more than half patients treated with gefitinib. Liver injury (elevated levels of transaminases) is also a common toxicity, which is usually mild and tolerable. However, when grade 2 or especially more than grade 3 toxicities occur, the treatments might be discontinued. The etiology of these
toxicities is unknown, but it might be caused by inhibition of EGFR signaling. In this regard, Li et al. reported a strong association between gefitinib steady-state plasma concentrations and the severity of diarrhea, suggesting that the high concentration of gefitinib accumulation in cells resulted in disorders of skin, intestinal mucosa and liver cells. Interstitial lung disease (ILD) is relatively common in Japanese patients, and more so in older, smoking patients with preexisting ILD or poor performance status. ILD has high mortality in Japanese patients. Therefore, evaluation of the risk of developing ILD and other moderate or severe toxicities is important for treatment with gefitinib.

The ATP-binding cassette (ABC) transporters are a superfamily of transmembrane proteins that transport a wide variety of substrates including anticancer drugs across extracellular and intracellular membranes. In the human genome, 48 different ABC transporters have been identified and divided into seven subfamilies (A–G) based on sequence similarities. Many ABC transporter genes are associated with chemotherapeutic drug efflux. Among them, ABCG2 (BCRP: breast cancer resistance protein/MRP: mitoxantrone resistance protein) and ABCB1 (P-glycoprotein/MDR1: multidrug resistance protein 1) are demonstrated to be involved in transporting gefitinib.

Previous studies have shown that several naturally occurring variants in the ABCG2 gene may affect the expression and/or function of its encoded protein. Among these variants, two major functional variants, ABCG2 34G>A (rs2231137), resulting in a Val12Met substitution and ABCG2 421C>A (rs2231142), resulting in a Glu141Lys substitution were well studied and shown to be related to the adverse effect of many drugs that were transported by ABCG2. The ABCG2 C421A allele has been associated with low levels of ABCG2 expression and altered sensitivity to the anticancer drugs in vitro, compared with the wild type. In vitro study using HEK293 human embryonic kidney cells transfected with wild-type and mutant ABCG2 421C>A demonstrated that HEK293 cells transfected with this variant demonstrate reduced transport of gefitinib, and the presence of the variant has been associated with greater gefitinib plasma accumulation at steady state in patients receiving gefitinib therapy. And it has also been reported that the ABCG2 G34A allele, resulting in a Val12Met substitution, causes the apical plasma membrane dislocalization of ABCG2 and produces a protein with significantly reduced ability to transport several drugs.

The most extensively studied ABCB1 variant is a common synonymous C to T transition at nucleotide position 3435 in exon 26 (3435C>T) (rs1045642). Although this transition does not change its encoded amino acid, this variant (TT group) has been significantly associated with reducing mRNA expression and stability, and may have a reduced ability to transport drugs.

Cusatis et al. investigated associations between allelic variants of ABCG2, ABCB1 and EGFR with diarrhea and skin toxicity in gefitinib-treated patients. Although this transition does not change its encoded amino acid, this variant (TT group) has been significantly associated with reducing mRNA expression and stability, and may have a reduced ability to transport drugs.

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These previous reports led us to hypothesize that patients receiving gefitinib who have a variant of ABCG2 and/or ABCB1 may be especially vulnerable to toxicities of gefitinib. In the present study, we evaluated the association between three SNPs, ABCG2 421C>A, 34G>A and ABCB1 3435C>T polymorphisms and moderate or severe toxicities of gefitinib treatment in Japanese lung cancer patients.
PATIENTS AND METHODS

Study population characteristics

The study population was comprised of 83 Japanese patients with non-small cell lung cancer at Nagoya University Hospital, Aichi Cancer Center Aichi Hospital, Nagoya Ekisaikai Hospital and Aichi Cancer Center Hospital and Research Institute from August 2002 to May 2008. Patients received treatment with oral gefitinib at a dose of 250 mg once daily on a compassionate use basis until disease progression or toxicity. We defined “moderate/severe toxicity” in this research as liver injury of grade 2 or worse and/or diarrhea of grade 2 or worse and/or skin rash of grade 2 or worse and/or interstitial lung disease (ILD) of grade 1 or worse, classified in accordance with Common Terminology Criteria for Adverse Events (CTCAE v4.0) May 28, 2009 (JCOG/JSCO) (CTCAE v4.0 – JCOG). We paid close attention to adverse events (skin rash, diarrhea and liver injury) of grade 2 or worse and ILD of grade 1 or worse for which treatment may be difficult to continue. Diarrhea and skin toxicity are prominent gefitinib related adverse events that potentially limit its use. The frequency of liver injury in this study was higher than ever reported. We investigated the associations between allelic variants of ABCG2 and ABCB1 and moderate or severe toxicities of diarrhea, skin toxicity, liver injury and interstitial lung disease (ILD) in gefitinib-treated patients.

We reviewed the clinical records including patient characteristics (age, gender, primary disease, previous treatments, histological classification, American Joint Committee on Cancer (AJCC) system staging, Eastern Cooperative Oncology Group performance status and major complications) (Table 1). This study was reviewed and approved by each institutional review board (IRB) of each hospital, and signed informed consent was obtained from all patients.

Genotyping

Genomic DNA was prepared from whole blood (100–200 µl) using the QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). The polymorphisms, ABCG2 421C > A and ABCB1 3435C

| Variable                        | Value     |
|---------------------------------|-----------|
| Total No. patients enrolled     | 83        |
| Median age                      | 65 y (36-86) |
| Sex (male/female)               | 35 (42%)/48 (58%) |
| Histological classification     |           |
| Adenocarcinoma                  | 77 (92.8%) |
| Squamous cell carcinoma         | 2 (2.4%)  |
| Large-cell carcinoma            | 4 (4.8%)  |
| TNM classification              |           |
| IA                              | 2 (2.4%)  |
| IB                              | 1 (1.2%)  |
| IIIA                             | 2 (2.4%)  |
| IIIB                             | 15 (18%)  |
| IV                               | 43 (52%)  |
| post-ope                        | 20 (24%)  |
were genotyped by the TaqMan assay (Applied Biosystems). Genotyping was analyzed using allele discrimination plots using the SDS Software version 1.4.1 of Applied Biosystems. We sequenced ABCG2 34G>A after performing a single polymerase chain reaction (PCR) amplification reaction using multiplex primer sets.

The primer sequence (5’–3’) of ABCG2 34G>A (V12M)

S: TGCAGAAAGATAAAAAACTCTCCTCA
AS: CTCAACTGTTTTTCGACAAGG

The amplification reaction mixture (20 µl) contained 100 ng DNA in 0.2 mmol/L each of deoxynucleoside triphosphate, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl2, 1.6 µmol/L of each primer, and 1.3 units of Taq polymerase (Takara Shuzo, Otsu, Japan). PCR was performed using a GeneAmp PCR 9700 (Applied Biosystems, Foster City, CA, USA) with an initial denaturation step of 95°C for 4 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s. Cycle sequencing was performed with a dye-terminator sequence reaction (ABI Prism DNA Sequencing Kit, Perkin-Elmer, Foster City, CA) using an ABI PRISM 310 Genetic Analyzer.

Statistical analysis
Fisher’s exact tests were used to analyze the association between ABCG2 or ABCB1 genetic polymorphisms and toxicities. A difference was considered statistically significant when the two-tailed P value was under 0.05.

RESULTS

Gefitinib toxicities
We collected clinical information from all 83 patients (Table 1). After treatment of at least 2 months or more, we evaluated 83 patients for skin rash, diarrhea, liver injury and interstitial lung disease (ILD). Moderate or severe skin rash (grade 2 or worse) was experienced in 23 patients (27.7%). Diarrhea of grade 2 or worse was experienced in 4 patients (4.8%). Moderate or severe liver injury (grade 2 or worse) was experienced in 15 patients (18%). Interstitial lung disease (ILD) of grade 1 or worse was experienced in 5 patients (6%).

Genotyping of ABCG2 34G>A, ABCG2 421C>A and ABCB1 3435C>T
G/G allele (G/G) were found in 51 patients (61%), G/A alleles in 28 (34%), and A/A alleles in 4 (5%) for ABCG2 34G>A. C/C alleles were found in 45 (54%) patients, C/A alleles in 31 (38%), and A/A allele in 7 (8%) for ABCG2 421C>A. C/C alleles were found in 23 patients (28%), C/T alleles in 44 (53%), and T/T alleles in 16 (19%) for ABCB1 3435C>T. The frequencies of the ABCG2 or ABCB1 variants alleles were summarized in Table 2.

| Genotypes       | ABCG2 34G>A | ABCB1 3435C>T |
|-----------------|-------------|---------------|
|                 | GG          | GA            | AA            |
| G : A           | 0.78 : 0.22 | 51 (61%)      | 28 (34%)      | 4 (5%)        |
|                 |             |               |               |
| ABCG2 421C>A    | CC          | CA            | AA            |
| C : A           | 0.71 : 0.29 | 45 (54%)      | 31 (38%)      | 7 (8%)        |
|                 |             |               |               |
| ABCB1 3435C>T   | CC          | CT            | TT            |
| C : T           | 0.54 : 0.46 | 23 (28%)      | 44 (53%)      | 16 (19%)      |
Association SNP of ABC transporter and gefitinib toxicity

Fisher’s exact tests were performed to find any significant association between the occurrence of moderate or severe toxicities and SNPs at \(ABCG2\) 34G>A, \(ABCG2\) 421C>A and \(ABCB1\) 3435C>T (Table 3–5). Statistically significant associations were found between the occurrence of skin toxicity and \(ABCG2\) polymorphisms, 34G>A; 13 (42%) of the 32 patients with at least one variant \(ABCG2\) 34G>A allele (G/A and A/A) developed skin rash, against only 10 (19%) of 51 patients homozygous for the reference allele (G/G) for the wild-type sequence for both alleles did (\(P=0.046\)) (Table 3). Other polymorphisms were not associated with skin toxicity, liver injury, diarrhea and interstitial lung disease (ILD) (Table 3–5).

| Table 3 | Association between genetic polymorphisms \(ABCG2\) 34G>A and toxicity |
|---------|-------------------------------------------------------------|
|         | GG   | GA+AA | \(P\) value |
| Skin rash |      |       |             |
| Grade 2\(\leq\) (N = 23) | 10   | 13    | \(P=0.046\) |
| Grade 1\(\geq\) (N = 60)  | 41   | 19    |             |
| Diarrhea |      |       |             |
| Grade 2\(\leq\) (N = 4)   | 2    | 2     | \(P=0.638\) |
| Grade 1\(\geq\) (N = 79)  | 49   | 30    |             |
| Liver injury |      |       |             |
| Grade 2\(\leq\) (N = 15)  | 10   | 5     | \(P=0.773\) |
| Grade 1\(\geq\) (N = 68)  | 41   | 27    |             |
| Interstitial lung disease (ILD) |      |       |             |
| Grade 1\(\leq\) (N = 5)   | 4    | 1     | \(P=0.644\) |
| Grade 0 (N = 78)            | 47   | 31    |             |

| Table 4 | Association between genetic polymorphisms \(ABCG2\) 421C>A and toxicity |
|---------|-------------------------------------------------------------|
|         | CC   | CA+AA | \(P\) value |
| Skin rash |      |       |             |
| Grade 2\(\leq\) (N = 23) | 14   | 9     | \(P=0.473\) |
| Grade 1\(\geq\) (N = 60)  | 31   | 29    |             |
| Diarrhea |      |       |             |
| Grade 2\(\leq\) (N = 4)   | 3    | 1     | \(P=0.621\) |
| Grade 1\(\geq\) (N = 79)  | 42   | 37    |             |
| Liver injury |      |       |             |
| Grade 2\(\leq\) (N = 15)  | 8    | 7     | \(P=1\) |
| Grade 1\(\geq\) (N = 68)  | 37   | 31    |             |
| Interstitial lung disease (ILD) |      |       |             |
| Grade 1\(\leq\) (N = 5)   | 4    | 1     | \(P=0.369\) |
| Grade 0 (N = 78)            | 41   | 37    |             |
DISCUSSION

We evaluated the clinical impact on gefitinib-related adverse effects of genetic polymorphism of ABC transporters. We examined the association of three SNPs in gefitinib-related ABC transporters with moderate or severe toxicity, since the treatment with gefitinib could be tolerable with most G1 toxicities but ILD. To our knowledge, this is the first study of the clinical impact on gefitinib-induced toxicities of \( \text{ABCG2} \) \( ^{34} \text{G>A} \). Our results suggested that \( \text{ABCG2} \) \( ^{34} \text{G>A} \) would be useful for predicting grade 2 or worse skin rash. However, other genetic polymorphisms were not associated with other moderate or severe toxicities such as diarrhea, liver injury or interstitial lung disease (ILD).

Previous studies have showed that skin rash might be good predictive marker for the response to gefitinib treatment,\(^{18}\) although the exact mechanism was not identified. Interestingly, a weak association between the occurrence of skin rash (G1 or more than) and the response to gefitinib was found in the present study, although it is not statistically significant (\( P = 0.085 \)). This result is consistent with previous studies demonstrating that occurrence of skin rash was associated with improved survival with gefitinib for recurrent NSCLC patients.\(^{18}\) We also examined the association between \( \text{ABCG2} \) \( ^{34} \text{G>A} \) and disease control, which means responses to gefitinib were CR, PR and SD according to the RECIST criteria v1.1.\(^{19}\) The disease control rate (31 patients out of 32, 97%) among patients with at least one variant allele \( \text{ABCG2} \) \( ^{34} \text{G>A} \) was significantly higher than in patients with wild type (40 patients out of 51, 78%).

Variant allele of \( \text{ABCG2} \) \( ^{34} \text{G>A} \) caused apical plasma membrane dislocalization of ABCG2 and altered transporter function and sensitivity to anticancer drugs.\(^{13}\) A recent study suggested that gefitinib is also an inhibitor of ABCG2 function.\(^{20}\) In this regard, gefitinib may affect membrane localization of this variant type of ABCG2 more strongly than wild-type. Thus, further examination of the gefitinib effect on inhibition of ABCG2 is needed.

Cusatis \( et \ al. \) investigated the association of genetic factors with skin rash or diarrhea and indicated that \( \text{ABCG2} \) \( ^{421} \text{C>A} \) was statistically significantly associated with the occurrence of diarrhea.\(^{17}\) The frequencies of \( \text{ABCG2} \) \( ^{421} \text{C>A} \) are reported to be 20–40% in Asian population,\(^{21}\) which are higher than in Caucasians (14%) or African-Americans.\(^{22}\) Although the allele frequency of the variant \( \text{ABCG2} \) \( ^{421} \text{C>A} \) in our Japanese lung cancer population was higher than

| Table 5 | Association between genetic polymorphisms \( \text{ABCB1} \) \( ^{3435} \text{C>T} \) and toxicity |
|---------|--------------------------------------------------|
|         | CC | CT+TT | \( P \) value |
| Skin rash |     |       |               |
| Grade 2\( \leq \) (N=23) | 7  | 16  | \( P = 0.787 \) |
| Grade 1\( \geq \) (N=60)     | 16 | 44  |               |
| Diarrhea |     |       |               |
| Grade 2\( \leq \) (N=4)      | 1  | 3   | \( P = 1 \) |
| Grade 1\( \geq \) (N=79)     | 22 | 57  |               |
| Liver injury |     |       |               |
| Grade 2\( \leq \) (N=15)     | 3  | 12  | \( P = 0.542 \) |
| Grade 1\( \geq \) (N=68)     | 20 | 48  |               |
| Interstitial lung disease (ILD) | |      |               |
| Grade 1\( \leq \) (N=5)      | 3  | 2   | \( P = 0.127 \) |
| Grade 0 (N=78)                | 20 | 58  |               |
in Cusatis’s study,\textsuperscript{17} we were unable to confirm the association between \textit{ABCG2} 421C>A and diarrhea. Akasaka \textit{et al.} also reported that the allele frequency of the variant \textit{ABCG2} 421C>A was higher and not associated with diarrhea and skin toxicity in the Japanese patients.\textsuperscript{23} The reason for this inconsistency remains to be investigated. One possible explanation is that other genetic factors such as polymorphisms of proteins related with EGFR signal pathway, might be more strongly involved in the adverse effects of gefitinib than this ABC transporters genotype. For example, the length of a tandem repeat (CA)n in intron 1 of \textit{EGFR} has been inversely related to \textit{EGFR} mRNA expression and protein levels. Several studies reported that the EGFR intron 1 polymorphism is associated with the occurrence of skin rash with gefitinib treatment.\textsuperscript{24}

Another possible explanation might be lower allele frequency of the variant \textit{ABCG2} 421C>A in Caucasian populations compared with two Japanese studies. In a previous positive study, 14 patients had variant alleles of \textit{ABCG2} 421C>A and seven patients developed diarrhea, while 108 patients had only wild-type allele. There is a possibility that the significant association may be by accident due to the low frequency of variant alleles and that investigation of the larger Caucasians patients with more variant alleles might have resulted in a different conclusion. In another EGFR-TKI erlotinib study in a Caucasian population, there was no association between genetic polymorphisms of \textit{ABCG2} 421C>A and toxicities, although \textit{ABCG2} 16702G>A was associated with grade 2 or more skin rash.\textsuperscript{25}

\textit{ABCB1} 3435C>T has been significantly associated with reducing mRNA expression\textsuperscript{15} and stability,\textsuperscript{16} and may have a reduced ability to transport drugs. However, the determination of polymorphisms of \textit{ABCB1} 3435C>T would not be useful for predicting moderate or severe toxicities induced by gefitinib in this study.

In summary, our study suggested that \textit{ABCG2} 34G>A might be the predictor of skin toxicity in gefitinib treatments, although the effects of other variants are not consistent with previous study. Comprehensive analysis of the genes related with gefitinib metabolism is necessary for more exact prediction of its adverse effect.

REFERENCES

1) Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, Schiller JH, Kelly K, Sandler A, Albain KS, Cellis D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. \textit{JAMA}, 2003; 290: 2249–2258.

2) Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek K, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Neyereislova A, Dong RP, Baselga J. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial. \textit{J Clin Oncol}, 2003; 21: 2237–2246.

3) Sato M, Shames DS, Gazdar AF, Minna JD. A translational view of the molecular pathogenesis of lung cancer. \textit{J Thorac Oncol}, 2007; 2: 327–343.

4) Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers—a different disease. \textit{Nat Rev Cancer}, 2007; 7: 778–790.

5) Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakanami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K, Fukuoka M; West Japan Oncology Group. Gefitinib versus cisplatin plus docetaxel in patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial. \textit{J Clin Oncol}, 2003; 21: 2237–2246.

6) Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okiangta S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saiyo Y, Hagiwara K, Morita S, Nukiwa T; North-East Japan Study Group. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. \textit{N Engl J Med}, 2010; 362: 2380–2388.

7) Li J, Karlsson MO, Brahmer J, Spitz A, Zhao M, Hidalgo M, Baker SD. CYP3A phenotyping approach
predict systemic exposure to EGFR tyrosine kinase inhibitors. *J Natl Cancer Inst*, 2006; 98: 1714–1723.

8) Ando M, Okamoto I, Yamamoto N, Takeda K, Tamura K, Seto T, Ariyoshi Y, Fukuoka M. Predictive factors for interstitial lung disease, antitumor response, and survival in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol*, 2006; 24: 2549–2556.

9) Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res*, 2001; 11: 1156–1166.

10) Li J, Sparreboom A, Zhao M, Robey RW, Bates SE, Baker SD. Gefitinib and erlotinib, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, are substrates for the breast cancer resistance protein (BCRP)/ABCG2 transporter. *Clin Cancer Res*, 2005; 11S: 120 (Abstract A251).

11) Kim HS, Sunwoo YE, Ryu JY, Kang HJ, Jung HE, Song IS, Kim EY, Shim JC, Shon JH, Shin JG. The effect of ABCG2 V12M, Q141K and Q126X, known functional variants in vitro, on the disposition of lamivudine. *Br J Clin Pharmacol*, 2007; 64: 645–654.

12) Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, Miki Y, Sugimoto Y. C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. *Mol Cancer Ther*, 2002; 1: 611–616.

13) Mizuara S, Aozasa N, Kotani H. Single nucleotide polymorphisms result in impaired membrane localization and reduced aptase activity in multidrug transporter ABCG2. *Int J Cancer*, 2004; 109: 238–246.

14) Lepper ER, Nooter K, Verweij J, Acharya MR, Figg WD, Sparreboom A. Mechanisms of resistance to anticancer drugs: the role of the polymorphic ABC transporters ABCB1 and ABCG2. *Pharmacogenomics*, 2005; 6: 115–138.

15) Song P, Lamba JK, Zhang L, Schuetz E, Shukla N, Meibohm B, Yates CR. G2677T and C3435T genotype and haplotype are associated with hepatic ABCB1 (MDR1) expression. *J Clin Pharmacol*, 2006; 46: 373–379.

16) Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet Genomics*, 2005; 15: 693–704.

17) Cusatis G, Gregorc V, Li J, Sprefacio A, Ingersoll RG, Verweij J, Ludovini V, Villa E, Hidalgo M, Sparreboom A, Baker SD. Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. *J Natl Cancer Inst*, 2006; 98: 1739–1742.

18) Mohamed MK, Ramalingam S, Lin Y, Gooding W, Belani CP. Skin rash and good performance status predict improved survival with gefitinib in patients with advanced non-small cell lung cancer. *Ann Oncol*, 2005; 16: 780–785.

19) Therase P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Glabbeke MV, Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst*, 2000; 92: 205–216.

20) Ozvegy-Laczka C, Hegedus T, Várády G, Ujhelly O, Schuetz JD, Váradi A, Kéri G, Orfi L, Német K, Sarkadi B. High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. *Mol Pharmacol*, 2004; 65: 1485–1495.

21) Lee SS, Jeong HE, Yi JM, Jung HJ, Jang JE, Kim EY, Lee SJ, Shin JG. Identification and functional assessment of BCRP polymorphisms in a Korean population. *Drug Metab Dispos*, 2007; 35: 623–632.

22) Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD, and Schuetz EG. Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics*, 2003; 13: 19–28.

23) Akasaka K, Kaburagi T, Yasuda S, Ohmori K, Abe K, Sagara H, Ueda Y, Nagao K, Imura J, Imai Y. Impact of functional ABCG2 polymorphisms on the adverse effects of gefitinib in Japanese patients with non-small-cell lung cancer. *Cancer Chemother Pharmacol*, 2010; 66: 691–698.

24) Huang CL, Yang CH, Yeh KH, Hu FC, Chen KY, Shih JY, Lin ZZ, Yu CJ, Cheng AL, Yang PC. EGFR intron 1 dinucleotide repeat polymorphism is associated with the occurrence of skin rash with gefitinib treatment. *Lung Cancer*, 2009; 64: 346–351.

25) Rudin CM, Liu W, Desai A, Karrison T, Jiang X, Janisch L, Das S, Ramirez J, Pounkuzhal B, Schuetz E, Fackenthal DL, Chen P, Armstrong DK, Brahmer JR, Fleming GF, Vokes EE, Carducci MA, Ratain MJ. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. *J Clin Oncol*, 2008; 26: 1119–1127.