ORIGINAL RESEARCH

Associations between ABCG2 gene polymorphisms and gefitinib toxicity in non-small cell lung cancer: a meta-analysis

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Background: Gefitinib is frequently used to treat patients with non-small cell lung cancer (NSCLC) and is excreted out from cells via the ATP-binding cassette transporter ABCG2. ABCG2 gene polymorphisms have been suggested to be associated with ABCG2 protein expression and function and may influence the risk of gefitinib toxicity in NSCLC patients. Previous studies on the associations between ABCG2 gene polymorphisms and the toxicity of gefitinib in NSCLC patients have produced conflicting results. The aim of this meta-analysis was to determine whether ABCG2 gene polymorphisms are associated with the risk of gefitinib-induced toxicity in NSCLC patients.

Methods: The PubMed and EMBASE databases were searched systematically for all eligible studies. A relative risk with corresponding 95% CI was calculated to evaluate the associations between ABCG2 gene polymorphisms and gefitinib-induced toxicity.

Results: Data were finally extracted from seven studies and 515 patients were found to meet the inclusion criteria of the meta-analysis. A dominant model showed that there was no significant association between the ABCG2 C421A polymorphism and the risk of gefitinib-induced toxicity, while the ABCG2 G34A polymorphism might be associated with an increased risk of skin toxicity in gefitinib therapy (relative risk =1.54, 95% CI 1.08–2.21, P=0.02). However, more reliable data are required to confirm the associations between the ABCG2 C421A and ABCG2 G34A polymorphisms and the toxicity of gefitinib in NSCLC patients.

Conclusion: While the ABCG2 C421A polymorphism might not be a reliable marker of gefitinib-related toxicity, the ABCG2 G34A genotype may be predictive of the skin toxicity of gefitinib in NSCLC patients. These conclusions need to be verified in further large-scale studies.

Keywords: gefitinib, ABCG2, meta-analysis, polymorphism, toxicity, NSCLC

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Introduction

Lung cancer is a very common carcinoma and also the leading cause of cancer-related deaths worldwide.[1] Non-small cell lung cancer (NSCLC) is the most common type, accounting for 80%–85% of cases of lung cancer.[2] Gefitinib (ZD1839; Iressa, Astrazeneca) is an oral inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase that has been used clinically for treating patients with NSCLC, and it exhibits strong antitumor activity.[3,4] However, gefitinib is also associated with side effects such as skin rash, diarrhea, hepatic impairment, and pulmonary toxicities.[5–7] Although most of these adverse events are usually mild and tolerable, the associated physical or psychosocial discomfort could decrease the quality of life, and the treatments might be discontinued when toxicities more severe than grade 2 occur.[8,9] Therefore, the development of an

accurate predictor of the toxicity response of gefitinib would contribute greatly to the treatment of NSCLC.

Gefitinib is a selective, reversible, EGFR tyrosine kinase inhibitor that blocks the EGFR signal transduction pathways related to the survival and proliferation of tumor cells.[4,10] Gefitinib is excreted from cells via the ATP-binding cassette transporter ABCG2. ABCG2 (formerly termed breast cancer resistance protein [BCRP]) localizes to the apical cell membrane and mediates the ATP-dependent unidirectional efflux of its substrates from cells, which leads to a reduction in intracellular cytotoxic effects and mediates concurrent resistance.[11–13] Gefitinib has a high-affinity interaction with ABCG2, and therefore, the expression of ABCG2 has an impact on gefitinib resistance.[14,15] In addition, ABCG2 gene polymorphisms affect the pharmacodynamics of gefitinib, which might also affect the toxicity of gefitinib.[16]

Previous studies have shown that several naturally occurring single-nucleotide polymorphisms (SNPs) in the ABCG2 gene may affect ABCG2 protein expression and function, of which ABCG2 C421A (rs2231142) and ABCG2 G34A (rs2231137) are two major nonsynonymous SNPs.[17–20] ABCG2 C421A, which is a C>A nucleotide transition at position 421 in exon 5 of the ABCG2 gene, has been related to low expression and activity of the ABCG2 protein, which results in higher accumulation of gefitinib.[21,22] Cusatis et al indicated that the ABCG2 C421A polymorphism increased the risk of gefitinib-induced diarrhea in NSCLC patients.[23] The ABCG2 G34A polymorphism, which results in a V12M substitution, disturbs apical plasma membrane localization and causes functional impairment of ABCG2, which may in turn influence the resistance to ABCG2 substrates.[22] Tamura et al found that the ABCG2 G34A polymorphism was significantly associated with the occurrence of skin rash in gefitinib-treated NSCLC patients and might be a reliable predictor of skin toxicity in gefitinib treatments.[8] These observations demonstrate that both ABCG2 C421A and ABCG2 G34A polymorphisms may affect gefitinib toxicity.

The effects of ABCG2 polymorphisms on the clinical outcome of gefitinib treatment in NSCLC (including gefitinib-induced toxicity) have been studied widely. Numerous genetic association studies have examined the possible linkages of the ABCG2 C421A and ABCG2 G34A polymorphisms with gefitinib toxicity in NSCLC patients. Although several of these studies found significant associations, most of them found no association. These inconsistent results may be due to differences in ethnicity, toxicity criteria, and sample sizes. Therefore, it remains uncertain if ABCG2 gene polymorphisms are related to gefitinib toxicity, and hence further studies are needed to clarify this association.

In this meta-analysis, we examined whether recent findings affect the strength of the association between the ABCG2 genotype and gefitinib toxicity in NSCLC patients. We first collected all published clinical studies of associations between ABCG2 polymorphisms and gefitinib toxicity in NSCLC patients, and then performed a meta-analysis of eligible studies that were selected by applying strict criteria to assess the effects of ABCG2 polymorphisms on gefitinib toxicity in NSCLC patients.

Material and methods
Literature and search strategy
Systematic literature searches were conducted using PubMed and EMBASE databases for relevant articles without language restrictions. The search terms were as follows: ("ATP-binding cassette subfamily G member 2" OR "ABCG2" OR "breast cancer resistance protein" OR "BCRP" OR "mitoxantrone resistance protein" OR "MRP" OR "ABCP") AND ("gefitinib" OR "Iressa" OR "ZD1839"). In addition, the reference lists of both original and review articles were investigated to ensure that all relevant studies were included. The literature search was last updated on March 2017.

Selection criteria
We included a full-length research study in the meta-analysis if it satisfied all of the following inclusion criteria: 1) assessing an independent association between the ABCG2 polymorphism and gefitinib toxicity in NSCLC patients, 2) providing either sufficient data on toxicity by genotype or sufficient information for such data to be calculated, and 3) when duplicate data were published, we selected the report involving the largest sample.

Data extraction and study quality assessment
The following information was extracted for each study: name of the first author, year of publication, sample size, disease type, country of origin, ethnicity, gefitinib therapeutic doses, toxicity category, and data on toxicity by genotype. The Newcastle–Ottawa Scale (NOS) was applied to assess the study quality from three broad perspectives, with the score ranging from 0 to 9.[24] The general methodological quality was considered to be moderate to good for studies that scored from 5 to 9 on the NOS.

Statistical analysis
Dominant genetic models for the ABCG2 C421A (CA+AA versus CC) and ABCG2 G34A (GA+AA versus GG) polymorphisms were applied to quantify the effect size in each study;

these models maximized the number of included studies. The associations between gefitinib toxicity and the ABCG2 C421A and ABCG2 G34A polymorphisms were analyzed using a dominant model by calculating the relative risk (RR) and its 95% CI. A Mantel–Haenszel estimate was used to pool the RR estimates of each study using a fixed-effects model. The statistical significance of the RR was determined by the Z-test, and $P<0.05$ was considered indicative of statistical significance. The presence of heterogeneity was assessed using Cochran's Q-statistic test and quantified using the I^2 statistic, where $P\leq0.10$ and/or $I^2>50\%$ indicates a high degree of heterogeneity.[25] A fixed-effects model was used to calculate pooled RRs when $P>0.10$; otherwise the random-effects model was applied. We performed subgroup analyses for skin toxicity, diarrhea, hepatotoxicity, and interstitial pneumonia. Sensitivity analysis was applied when possible by excluding individual studies. All of the statistical analyses were conducted using Review Manager Software (version 5.3).

Results
Study selection and characteristics
The detailed study selection process is depicted in Figure 1. The initial search identified 529 articles, which was reduced to 446 after removing duplicates. A further 149 articles

were excluded due to 142 articles being reviews or letters and 7 articles being abstracts. Of the remaining 297 articles, 128 did not involve NSCLC patients, 143 did not focus on gefitinib toxicity, and 16 were not relevant to ABCG2 gene polymorphisms, and thus all of these articles were also excluded. After performing full-text evaluations, 3 of the remaining 10 articles were excluded for reasons of no toxicity data (n=1), no ABCG2 genotype number (n=1), and being a duplicate study (n=1). This selection process, therefore, resulted in only seven studies finally qualifying for inclusion in the present meta-analysis.

The association between the ABCG2 C421A polymorphism and the risk of gefitinib toxicity was investigated in seven studies, while the ABCG2 G34A polymorphism was investigated in two studies.

The characteristics of these seven studies are listed in Table 1. Hirose et al[26] did not provide sufficient information regarding the gefitinib-induced adverse effects that were correlated with ABCG2 gene polymorphisms, which meant that the associations between ABCG2 polymorphisms and gefitinib toxicity were unclear, and hence that study was not included in the subsequent analysis. Four[8,27–29] of the remaining six clinical observation studies involved Asian populations and the other two[23,30] involved Caucasian
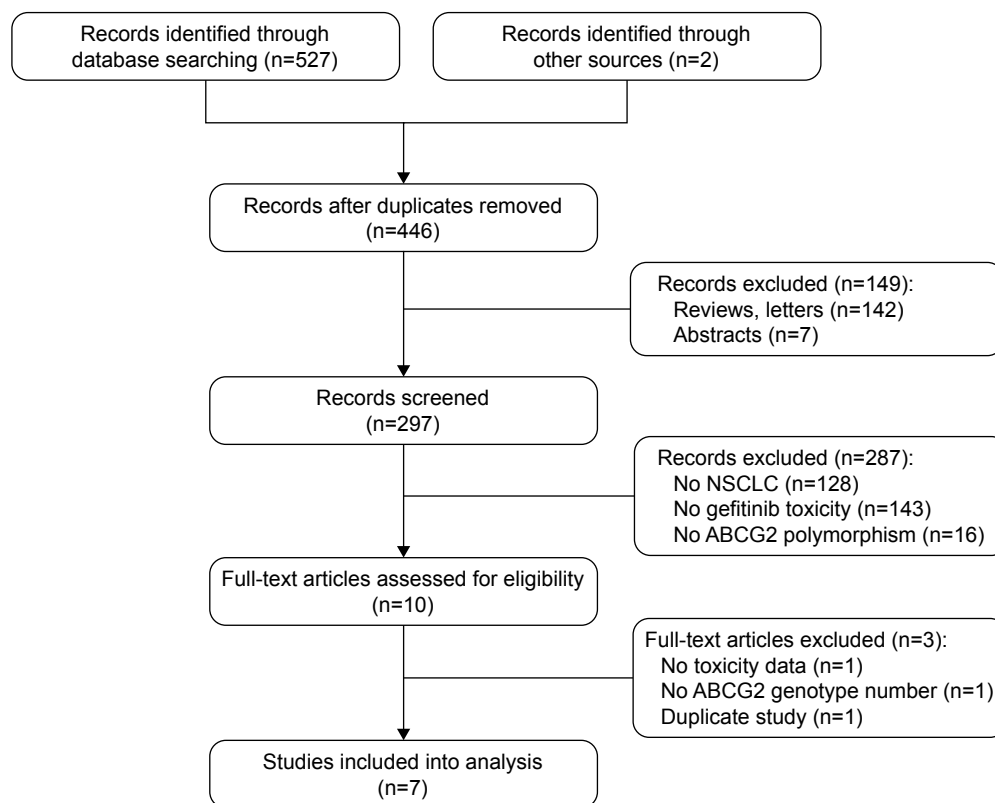
Figure 1 Flowchart of the study selection process in the meta-analysis.
Abbreviation: NSCLC, non-small cell lung cancer.

Table 1 Characteristics of the included studies of the associations between ABCG2 gene polymorphisms and gefitinib toxicity

Study	Year	Country	Ethnicity	Patients	Sample	Genotyping method	Gefitinib dose (mg)	NOS score
Akasaka et al[27]	2010	Japan	Asian	75 patients with advanced and/or recurrent NSCLC	Blood or tissue	PCR	250	7
Cusatis et al[23]	2006	Italy	Caucasian	173 patients with NSCLC	Blood	PCR	250	5
Kobayashi et al[28]	2015	Japan	Asian	31 patients with NSCLC	Blood	PCR-RFLP	250	7
Lemos et al[30]	2011	Italy	Caucasian	94 patients with NSCLC	Blood or tissue	Taqman probe	250	6
Ma et al[29]	2016	China	Asian	59 patients with NSCLC	Blood	Sequenom MassARRAY	250	7
Tamura et al[8]	2012	Japan	Asian	83 patients with NSCLC	Blood	Taqman probe	250	6
Hirose et al[26]	2016	Japan	Asian	35 patients with advanced NSCLC	Blood	PCR	250	6

Note: All cases came from hospital-based populations.
Abbreviations: NOS, Newcastle–Ottawa Scale; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

populations. The association between the ABCG2 C421A polymorphism and the risk of gefitinib toxicity was investigated in seven studies,[8,23,26–30] while the ABCG2 G34A polymorphism was investigated in two studies.[8,29] The results of the quality assessment are presented in Table 1 – the included studies scored from 5 to 7 on the NOS.

The studies investigated different types of toxicity. As different studies analyzed toxicity according to different evaluation criteria, we performed an in-depth analysis for each toxicity type (Tables 2 and 3). Because the patient data were insufficient for some types of toxicity, not all of them are listed

in Table 3. We performed meta-analyses for diarrhea, skin toxicity, hepatotoxicity and interstitial pneumonia. The results for each of these toxicities are presented separately below.

Diarrhea

Seven studies researched diarrhea,[8,23,26–30] of which six[8,23,27–30] provided sufficient data. Based on the data from 515 patients in these six studies, no association was observed between the ABCG2 C421A polymorphism and gefitinib-induced diarrhea (RR =0.97, 95% CI 0.53–1.79, P=0.52; Figure 2A). The forest plot showed considerable heterogeneity among

Table 2 Summary of the toxicity criteria and the number of toxicity events in the included studies

Study	Year	Toxicity criteria	Toxicity grade	Number of toxicity events			
				Skin toxicity	Diarrhea	Hepatotoxicity	Interstitial pneumonia
ABCG2 C421A							
Akasaka et al[27]	2010	NCI-CTCAE V3.0	Grade 0	19	53	62	69
			Grade 1+	56	22	13	6
Cusatis et al[23]	2006	NCI-CTCAE V2.0	Grade 0	44	104		
			Grade 1+	84	20		
Kobayashi et al[28]	2015	NCI-CTCAE V4.0	Grade 0	11	16	14	
			Grade 1+	20	15	17	
Lemos et al[30]	2011	NCI-CTCAE V3.0	Grade 0	41	52		
			Grade 1+	46	33		
Ma et al[29]	2016	NCI-CTCAE V4.0	Grade 0	13	26	42	
			Grade 1+	35	22	10	
Tamura et al[8]	2012	NCI-CTCAE V4.0	Grade 0–1	60	79	68	78[a]
			Grade 2+	23	4	15	5[b]
Hirose et al[26]	2016	NCI-CTCAE V4.0	Grade 0	44	30		
			Grade 1+	24	16		
ABCG2 G34A							
Ma et al[29]	2016	NCI-CTCAE V4.0	Grade 0	14	27	42	
			Grade 1+	35	22	11	
Tamura et al[8]	2012	NCI-CTCAE V4.0	Grade 0–1	60	79	68	78[a]
			Grade 2+	23	4	15	5[b]

Notes: [a]The number of Grade 0 interstitial pneumonia; [b]The number of Grade 1+ interstitial pneumonia.
Abbreviations: CTCAE, common terminology criteria for adverse events; NCI, National Cancer Institute.

Table 3 Toxicity types and findings in each study

Study	Country	Ethnicity	Toxicity type			
			Skin toxicity	Diarrhea	Hepatotoxicity	Interstitial pneumonia
ABCG2 C421A						
Akasaka et al[27]	Japan	Asian	NA	NA	NA	NA
Cusatis et al[23]	Italy	Caucasian	NA	+		
Kobayashi et al[28]	Japan	Asian	NA	NA	NA	
Lemos et al[30]	Italy	Caucasian	NA	NA		
Ma et al[29]	China	Asian	NA	NA	+	
Tamura et al[8]	Japan	Asian	NA	NA	NA	NA
ABCG2 G34A						
Ma et al[29]	China	Asian	NA	NA	+	
Tamura et al[8]	Japan	Asian	+	NA	NA	NA

Note: +, SNP associated with increased toxicity.
Abbreviations: NA, no association between SNP and toxicity; SNP, single-nucleotide polymorphism.
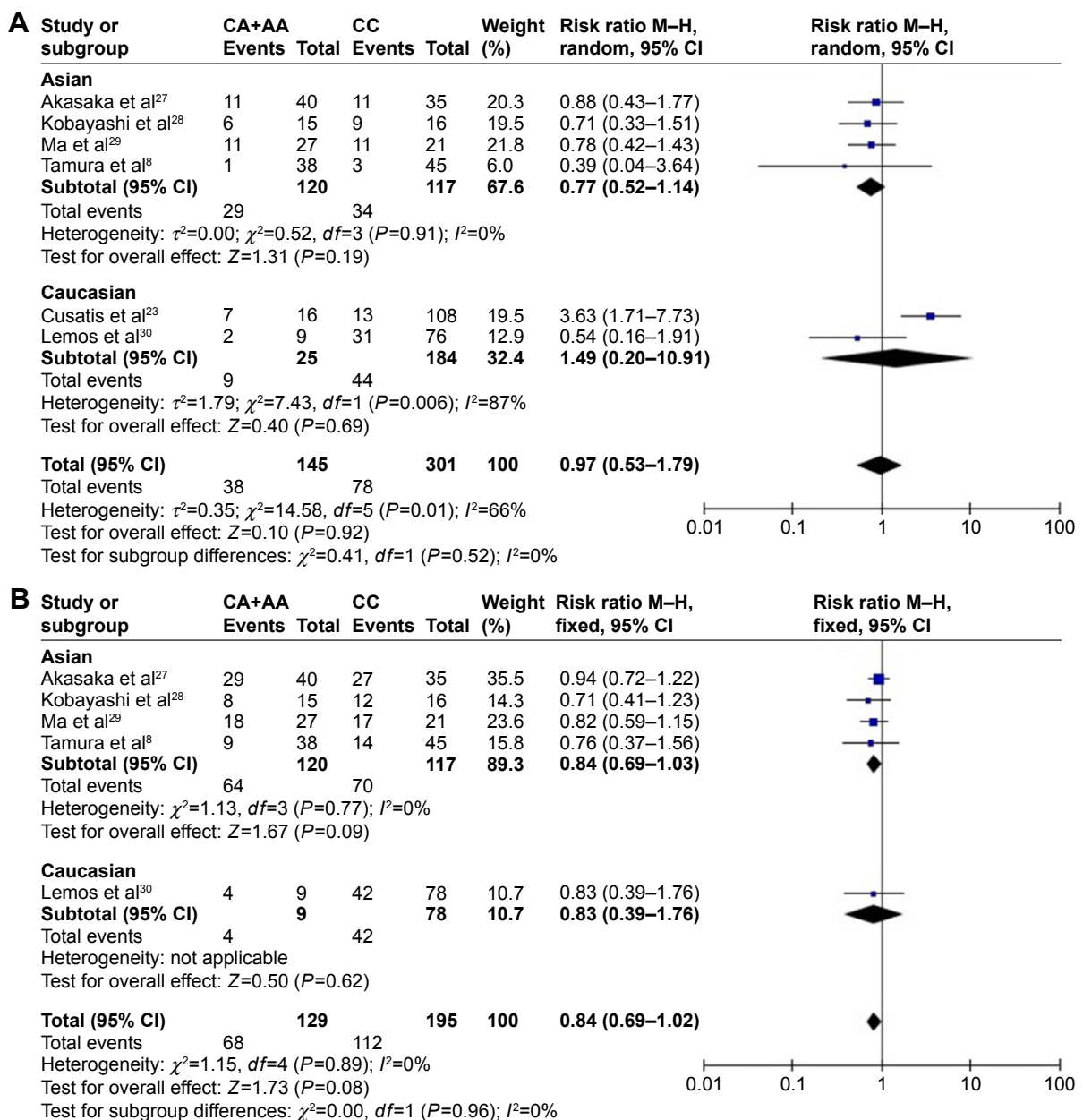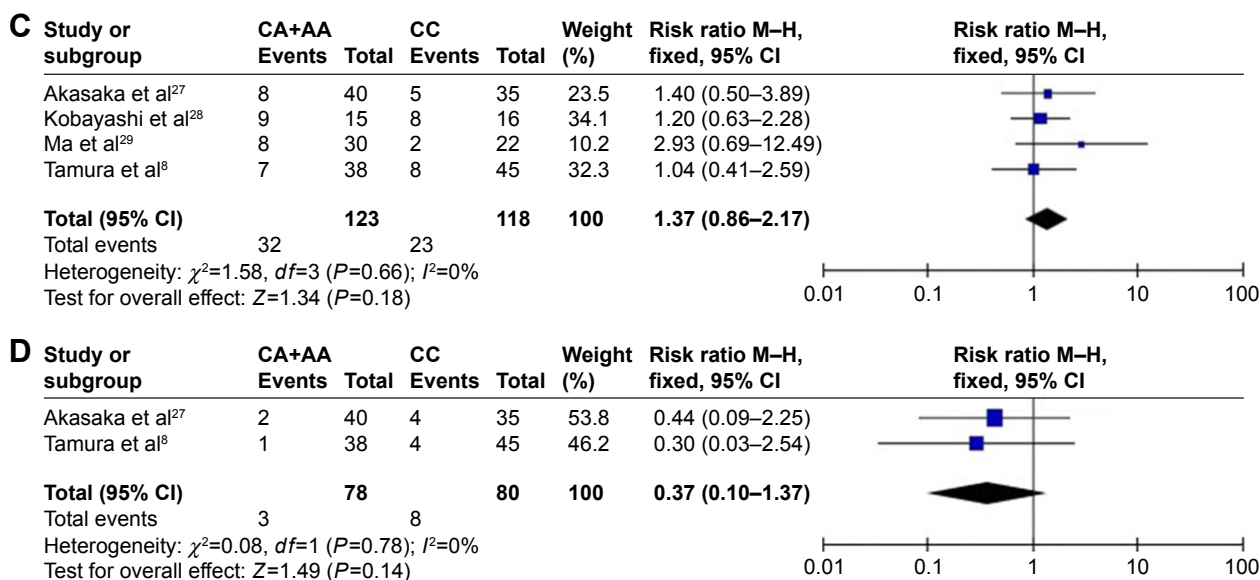

Figure 2 (Continued)

C

Study or subgroup	CA+AA Events	Total	CC Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Akasaka et al[27]	8	40	5	35	23.5	1.40 (0.50–3.89)
Kobayashi et al[28]	9	15	8	16	34.1	1.20 (0.63–2.28)
Ma et al[29]	8	30	2	22	10.2	2.93 (0.69–12.49)
Tamura et al[8]	7	38	8	45	32.3	1.04 (0.41–2.59)
Total (95% CI)		**123**		**118**	**100**	**1.37 (0.86–2.17)**
Total events	32		23			

Heterogeneity: $\chi^2=1.58$, $df=3$ ($P=0.66$); $I^2=0\%$
Test for overall effect: $Z=1.34$ ($P=0.18$)

Risk ratio M–H, fixed, 95% CI
0.01 0.1 1 10 100

D

Study or subgroup	CA+AA Events	Total	CC Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Akasaka et al[27]	2	40	4	35	53.8	0.44 (0.09–2.25)
Tamura et al[8]	1	38	4	45	46.2	0.30 (0.03–2.54)
Total (95% CI)		**78**		**80**	**100**	**0.37 (0.10–1.37)**
Total events	3		8			

Heterogeneity: $\chi^2=0.08$, $df=1$ ($P=0.78$); $I^2=0\%$
Test for overall effect: $Z=1.49$ ($P=0.14$)

Risk ratio M–H, fixed, 95% CI
0.01 0.1 1 10 100

Figure 2 Forest plots showing the associations between the ABCG2 C421A polymorphism and gefitinib-induced (**A**) diarrhea, (**B**) skin toxicity, (**C**) hepatotoxicity, and (**D**) interstitial pneumonia in NSCLC patients.
Abbreviations: M–H, Mantel–Haenszel method; NSCLC, non-small cell lung cancer.

the six studies ($I^2=66\%$, $P=0.01$; Figure 2A), and so the random-effects model was used. Stratification by ethnicity indicated no significant association between the ABCG2 C421A polymorphism and gefitinib-induced diarrhea in either the Asian (RR =0.77, 95% CI 0.52–1.14, $P=0.19$) or Caucasian populations (RR =1.49, 95% CI 0.20–10.91, $P=0.69$; Figure 2A).

The two studies[8,29] that evaluated the association between the ABCG2 G34A polymorphism and gefitinib-induced diarrhea involved 142 Asian participants. The pooled results indicated the absence of a significant association between the ABCG2 G34A polymorphism and gefitinib-induced diarrhea (RR =1.15, 95% CI 0.62–2.12, $P=0.65$; Figure 3A). The I^2-value indicated that there was no significant heterogeneity between the two included studies ($I^2=0\%$, $P=0.70$; Figure 3A).

Skin toxicity

Skin toxicity was addressed in seven studies,[8,23,26–30] of which five[8,27–30] provided sufficient data to explore the association between the ABCG2 C421A polymorphism and gefitinib-induced skin toxicity. A meta-analysis of these five studies, which involved data from 342 patients, revealed no significant association between the ABCG2 C421A polymorphism and gefitinib-induced skin toxicity (RR =0.84, 95% CI 0.69–1.02, $P=0.08$; Figure 2B). No significant heterogeneity was observed among the studies ($I^2=0\%$, $P=0.96$; Figure 2B), and so a fixed-effects model was applied. Stratification by

ethnicity indicated that there was no significant association between the ABCG2 CA+AA genotype and the skin toxicity of gefitinib in either Asians (RR =0.84, 95% CI 0.69–1.03, $P=0.09$; Figure 2B) or Caucasians (RR =0.83, 95% CI 0.39–1.76; Figure 2B).

The two studies that evaluated the association between the ABCG2 G34A polymorphism and gefitinib-induced skin toxicity were conducted on 142 Asian patients.[8,29] The meta-analysis indicated a significant association between ABCG2 G34A polymorphism and gefitinib-induced skin toxicity (RR =1.54, 95% CI 1.08–2.21, $P=0.02$; Figure 3B). No significant heterogeneity was observed between the two included studies ($I^2=40\%$, $P=0.20$; Figure 3B).

Hepatotoxicity

The association between the ABCG2 C421A polymorphism and gefitinib-induced hepatotoxicity was analyzed in four studies[8,27–29] involving Asian populations. These studies involved data from 248 participants, and no association between that polymorphism and hepatotoxicity was found (RR =1.37, 95% CI 0.86–2.17, $P=0.18$; Figure 2C). No significant heterogeneity existed among the four studies ($I^2=0\%$, $P=0.66$; Figure 2C), and so a fixed-effects model was used.

The association between the ABCG2 G34A variant and gefitinib-induced hepatotoxicity was evaluated by two studies[8,29] that involved 142 Asian patients. The pooled ORs revealed that there was no significant association between that polymorphism and gefitinib-induced hepatotoxicity
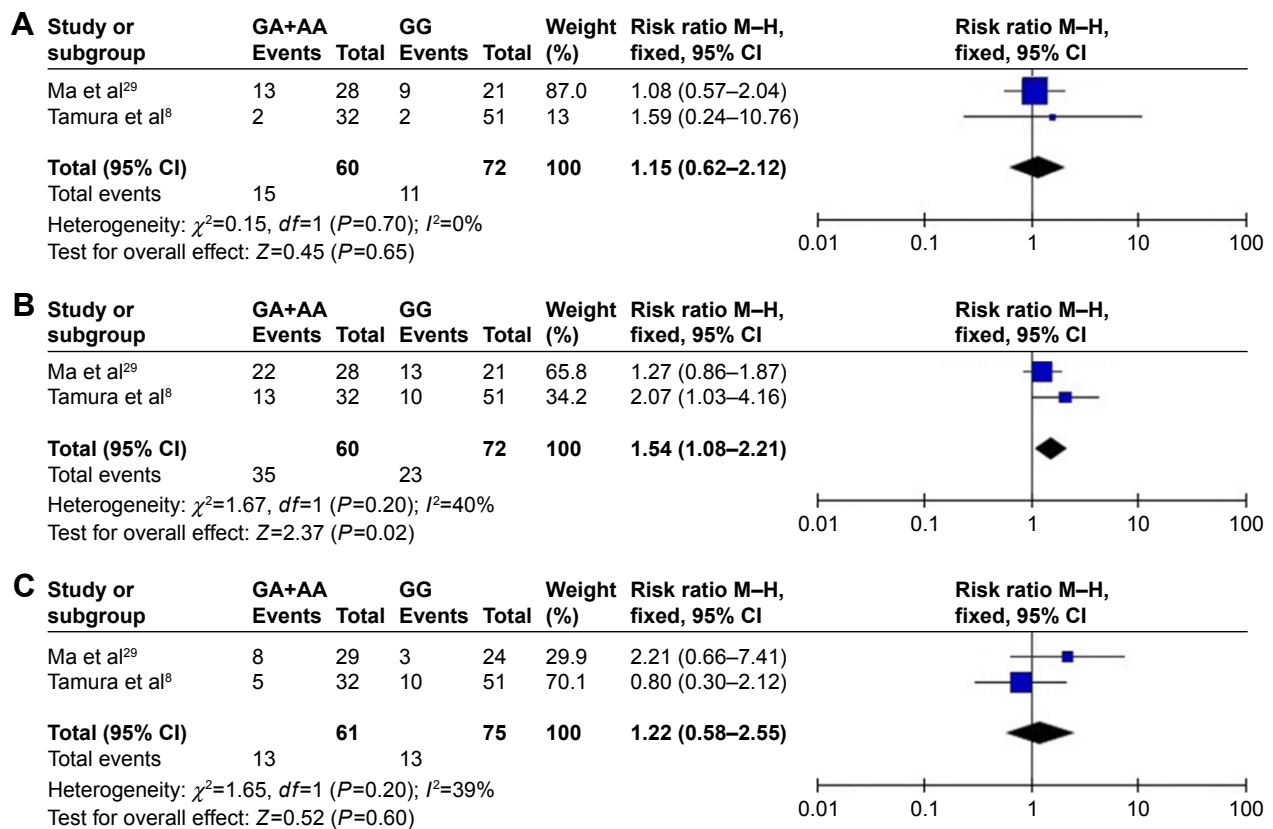
A

Study or subgroup	GA+AA Events	Total	GG Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Ma et al[29]	13	28	9	21	87.0	1.08 (0.57–2.04)
Tamura et al[8]	2	32	2	51	13	1.59 (0.24–10.76)
Total (95% CI)		60		72	100	1.15 (0.62–2.12)
Total events	15		11			

Heterogeneity: χ^2=0.15, df=1 (P=0.70); I^2=0%
Test for overall effect: Z=0.45 (P=0.65)

B

Study or subgroup	GA+AA Events	Total	GG Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Ma et al[29]	22	28	13	21	65.8	1.27 (0.86–1.87)
Tamura et al[8]	13	32	10	51	34.2	2.07 (1.03–4.16)
Total (95% CI)		60		72	100	1.54 (1.08–2.21)
Total events	35		23			

Heterogeneity: χ^2=1.67, df=1 (P=0.20); I^2=40%
Test for overall effect: Z=2.37 (P=0.02)

C

Study or subgroup	GA+AA Events	Total	GG Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Ma et al[29]	8	29	3	24	29.9	2.21 (0.66–7.41)
Tamura et al[8]	5	32	10	51	70.1	0.80 (0.30–2.12)
Total (95% CI)		61		75	100	1.22 (0.58–2.55)
Total events	13		13			

Heterogeneity: χ^2=1.65, df=1 (P=0.20); I^2=39%
Test for overall effect: Z=0.52 (P=0.60)

Figure 3 Forest plots showing the association between ABCG2 G34A polymorphism and gefitinib-induced (**A**) diarrhea, (**B**) skin toxicity, and (**C**) hepatotoxicity in NSCLC patients.
Abbreviations: M–H, Mantel–Haenszel method; NSCLC, non-small cell lung cancer.

(RR =1.22, 95% CI 0.58–2.55, P=0.60; Figure 3C). No obvious heterogeneity was indicated between the two included studies (I^2=39%, P=0.20; Figure 3C).

Interstitial pneumonia

Two studies[8,27] involving 158 patients evaluated the association between the ABCG2 C421A polymorphism and gefitinib-induced interstitial pneumonia, and both these studies involved Asian populations. The pooled results indicated that there was no significant association between that polymorphism and interstitial pneumonia (RR =0.37, 95% CI 0.10–1.37, P=0.14; Figure 2D). No significant heterogeneity was found (I^2=0%, P=0.78; Figure 2D), and so a fixed-effects model was used.

Given that the included studies may build analysis based on the different grade of toxicity events, we performed a stratified analysis by the grade of toxicity (Figure 4). The meta-analysis indicated no significant associations of the ABCG2 C421A polymorphism with diarrhea (grade ≥1; RR =1.03, 95% CI 0.54–1.96, P=0.94; Figure 4A), skin toxicity (grade ≥1; RR =0.85, 95% CI 0.70–1.04, P=0.11;

Figure 4B), hepatotoxicity (grade ≥1; RR =1.53, 95% CI 0.90–2.61, P=0.12; Figure 4C), and interstitial pneumonia (grade ≥1; RR =0.37, 95% CI 0.10–1.37, P=0.14; Figure 4D). Tamura et al[8] found ABCG2 421C>A polymorphism was not associated with grade 2 or worse diarrhea and skin toxicity. More studies are still needed to confirm the association between the ABCG2 C421A polymorphism and grade ≥2 toxicity in gefitinib-treated patients.

Discussion

Four of the seven studies included in this meta-analysis found no significant association between the ABCG2 C421A polymorphism and the toxicity of gefitinib in NSCLC patients.[8,27,28,30] Two of the studies did find such an association,[23,29] while the remaining study did not draw a definite conclusion.[26] Two of the seven studies[8,29] investigated the effect of the ABCG2 G34A polymorphism on gefitinib toxicity, and both found that this polymorphism is associated with the increased risk of gefitinib-related toxicity.

All of the studies included in the meta-analysis applied the same gefitinib dose of 250 mg/day. Since the included

studies evaluated toxicity using different criteria, we now consider their findings for each toxicity criterion.

Diarrhea

Five of the seven studies that researched diarrhea did not find any association between the ABCG2 C421A polymorphism and gefitinib-induced diarrhea.[8,27–30] Of the remaining two studies, Hirose et al[26] did not provide enough data on diarrhea or did not draw a definite conclusion, while Cusatis et al[23] demonstrated that patients with at least one variant ABCG2

C421A allele are at an increased risk of gefitinib-induced diarrhea. The reasons for these discrepancies remain to be investigated, but they could be due to ethnic factors, how the toxicity type was defined, or poor methodological quality associated with a small number of cases.

The allele frequencies of the ABCG2 C421A varies highly between different populations, reportedly being 35% in both Japanese and Chinese populations, in contrast to ~14% in Caucasians.[19] Subgroup analysis by ethnicity was performed for the ABCG2 C421A polymorphism. The result of our meta-analysis
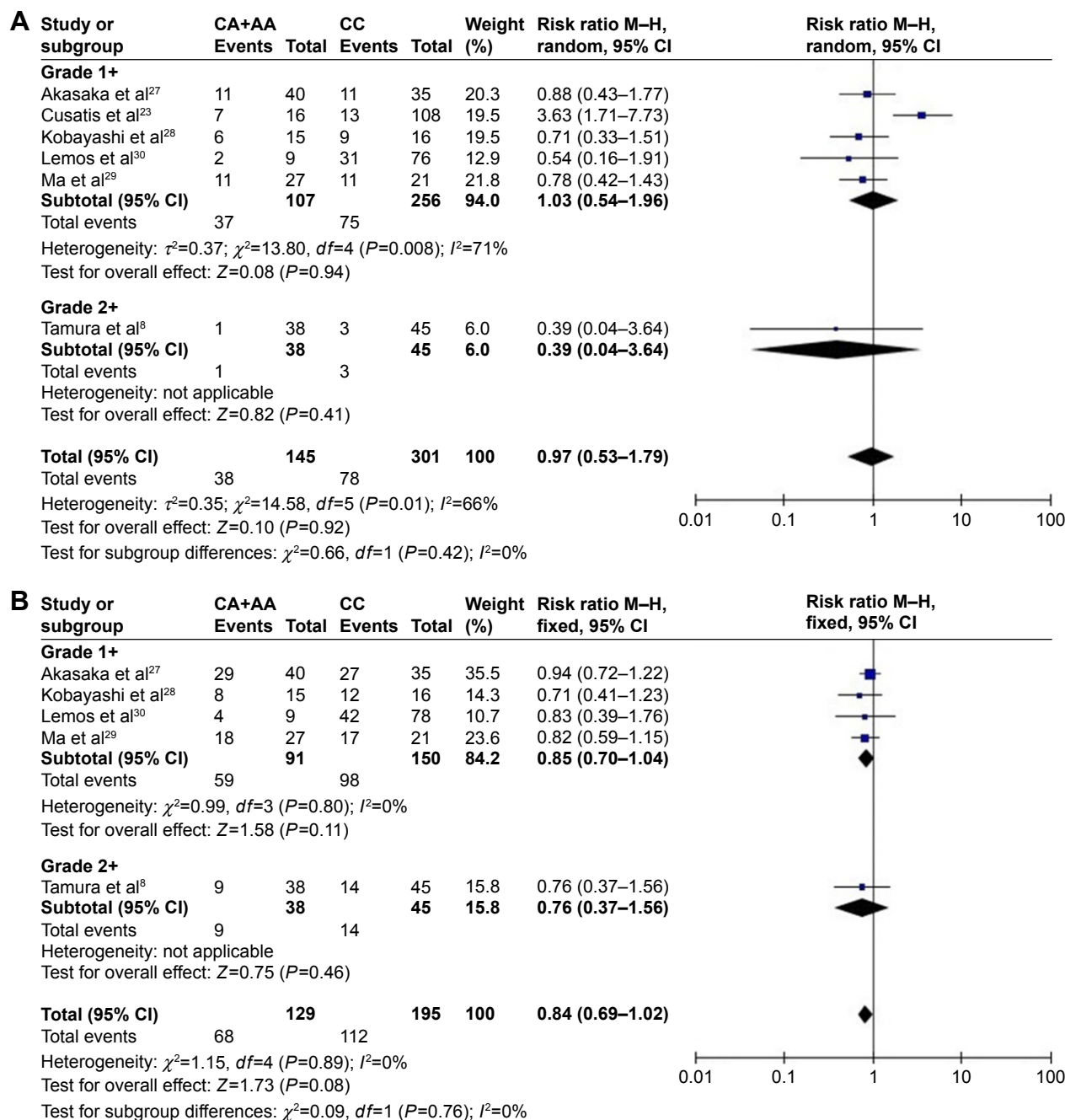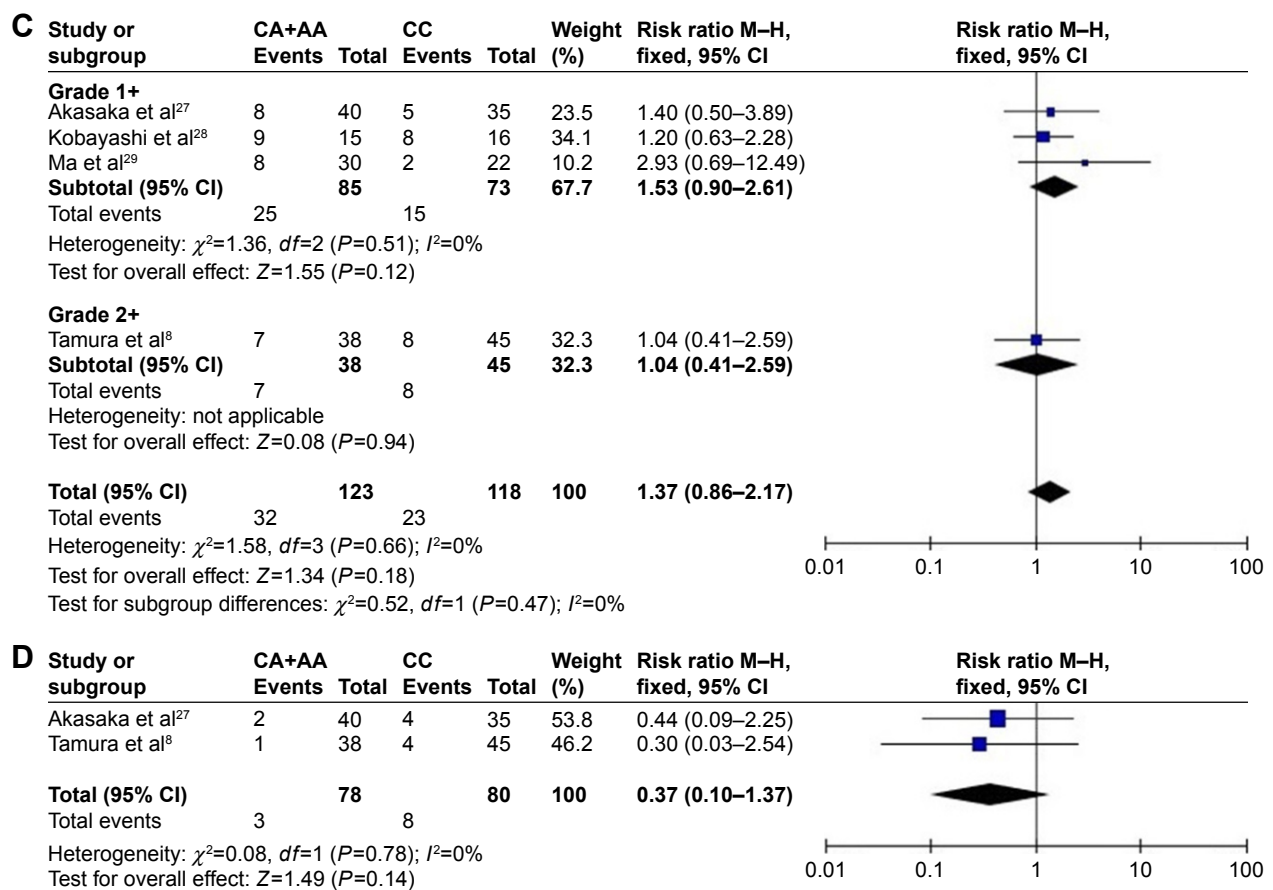
A

Study or subgroup	CA+AA Events	Total	CC Events	Total	Weight (%)	Risk ratio M–H, random, 95% CI
Grade 1+						
Akasaka et al[27]	11	40	11	35	20.3	0.88 (0.43–1.77)
Cusatis et al[23]	7	16	13	108	19.5	3.63 (1.71–7.73)
Kobayashi et al[28]	6	15	9	16	19.5	0.71 (0.33–1.51)
Lemos et al[30]	2	9	31	76	12.9	0.54 (0.16–1.91)
Ma et al[29]	11	27	11	21	21.8	0.78 (0.42–1.43)
Subtotal (95% CI)		**107**		**256**	**94.0**	**1.03 (0.54–1.96)**
Total events	37		75			

Heterogeneity: $\tau^2=0.37$; $\chi^2=13.80$, $df=4$ ($P=0.008$); $I^2=71\%$
Test for overall effect: $Z=0.08$ ($P=0.94$)

Grade 2+						
Tamura et al[8]	1	38	3	45	6.0	0.39 (0.04–3.64)
Subtotal (95% CI)		**38**		**45**	**6.0**	**0.39 (0.04–3.64)**
Total events	1		3			

Heterogeneity: not applicable
Test for overall effect: $Z=0.82$ ($P=0.41$)

Total (95% CI)		**145**		**301**	**100**	**0.97 (0.53–1.79)**
Total events	38		78			

Heterogeneity: $\tau^2=0.35$; $\chi^2=14.58$, $df=5$ ($P=0.01$); $I^2=66\%$
Test for overall effect: $Z=0.10$ ($P=0.92$)
Test for subgroup differences: $\chi^2=0.66$, $df=1$ ($P=0.42$); $I^2=0\%$

Risk ratio M–H, random, 95% CI
0.01 0.1 1 10 100

B

Study or subgroup	CA+AA Events	Total	CC Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Grade 1+						
Akasaka et al[27]	29	40	27	35	35.5	0.94 (0.72–1.22)
Kobayashi et al[28]	8	15	12	16	14.3	0.71 (0.41–1.23)
Lemos et al[30]	4	9	42	78	10.7	0.83 (0.39–1.76)
Ma et al[29]	18	27	17	21	23.6	0.82 (0.59–1.15)
Subtotal (95% CI)		**91**		**150**	**84.2**	**0.85 (0.70–1.04)**
Total events	59		98			

Heterogeneity: $\chi^2=0.99$, $df=3$ ($P=0.80$); $I^2=0\%$
Test for overall effect: $Z=1.58$ ($P=0.11$)

Grade 2+						
Tamura et al[8]	9	38	14	45	15.8	0.76 (0.37–1.56)
Subtotal (95% CI)		**38**		**45**	**15.8**	**0.76 (0.37–1.56)**
Total events	9		14			

Heterogeneity: not applicable
Test for overall effect: $Z=0.75$ ($P=0.46$)

Total (95% CI)		**129**		**195**	**100**	**0.84 (0.69–1.02)**
Total events	68		112			

Heterogeneity: $\chi^2=1.15$, $df=4$ ($P=0.89$); $I^2=0\%$
Test for overall effect: $Z=1.73$ ($P=0.08$)
Test for subgroup differences: $\chi^2=0.09$, $df=1$ ($P=0.76$); $I^2=0\%$

Risk ratio M–H, fixed, 95% CI
0.01 0.1 1 10 100

Figure 4 (*Continued*)

C

Study or subgroup	CA+AA Events	Total	CC Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI	Risk ratio M–H, fixed, 95% CI
Grade 1+							
Akasaka et al[27]	8	40	5	35	23.5	1.40 (0.50–3.89)	
Kobayashi et al[28]	9	15	8	16	34.1	1.20 (0.63–2.28)	
Ma et al[29]	8	30	2	22	10.2	2.93 (0.69–12.49)	
Subtotal (95% CI)		**85**		**73**	**67.7**	**1.53 (0.90–2.61)**	
Total events	25		15				
Heterogeneity: χ^2=1.36, df=2 (P=0.51); I^2=0%							
Test for overall effect: Z=1.55 (P=0.12)							
Grade 2+							
Tamura et al[8]	7	38	8	45	32.3	1.04 (0.41–2.59)	
Subtotal (95% CI)		**38**		**45**	**32.3**	**1.04 (0.41–2.59)**	
Total events	7		8				
Heterogeneity: not applicable							
Test for overall effect: Z=0.08 (P=0.94)							
Total (95% CI)		**123**		**118**	**100**	**1.37 (0.86–2.17)**	
Total events	32		23				
Heterogeneity: χ^2=1.58, df=3 (P=0.66); I^2=0%							
Test for overall effect: Z=1.34 (P=0.18)							
Test for subgroup differences: χ^2=0.52, df=1 (P=0.47); I^2=0%							

0.01   0.1   1   10   100

D

Study or subgroup	CA+AA Events	Total	CC Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI	Risk ratio M–H, fixed, 95% CI
Akasaka et al[27]	2	40	4	35	53.8	0.44 (0.09–2.25)	
Tamura et al[8]	1	38	4	45	46.2	0.30 (0.03–2.54)	
Total (95% CI)		**78**		**80**	**100**	**0.37 (0.10–1.37)**	
Total events	3		8				
Heterogeneity: χ^2=0.08, df=1 (P=0.78); I^2=0%							
Test for overall effect: Z=1.49 (P=0.14)							

0.01   0.1   1   10   100

Figure 4 Results of meta-analysis of the associations between the ABCG2 C421A polymorphism and gefitinib-induced (**A**) diarrhea, (**B**) skin toxicity, (**C**) hepatotoxicity, and (**D**) interstitial pneumonia in NSCLC patients.
Abbreviations: M–H, Mantel–Haenszel method; NSCLC, non-small cell lung cancer.

indicated that ABCG2 C421A polymorphism was not associated with the risk of diarrhea in Asian populations. There was significant heterogeneity between the two studies involving Caucasians. In contrast to Cusatis et al,[23] Lemos et al[30] observed no significant association between the ABCG2 C421A polymorphism and gefitinib-induced diarrhea in Caucasians. Because the allele frequency of the ABCG2 C421A is lower in Caucasians than in Asians, investigations involving larger numbers of Caucasian patients are necessary, which might lead to different conclusions. Therefore, more studies are still needed to confirm the association between the ABCG2 C421A polymorphism and gefitinib-related diarrhea.

Two studies involving Asian populations analyzed the association between the ABCG2 G34A polymorphism and gefitinib-induced diarrhea.[8,29] Both these studies found that this polymorphism was not associated with diarrhea, which is consistent with the results of our meta-analysis. Because there were no studies of the ABCG2 G34A polymorphism involving Caucasian populations, large-scale studies are still required to confirm the association between this polymorphism and gefitinib-induced diarrhea.

Skin toxicity

Seven studies investigated the association between the ABCG2 C421A polymorphism and the skin toxicity of gefitinib.[8,23,26–30] Cusatis et al[23] determined that this polymorphism was not associated with gefitinib-induced skin toxicity, but they did not provide sufficient data. The 2016 study of Hirose et al[26] also did not provide sufficient data on skin toxicity and no definite conclusion was drawn. The other five studies found no association between the ABCG2 C421A polymorphism and gefitinib-induced skin toxicity,[8,27–30] which is supported by the overall results of our meta-analysis. Stratification by ethnicity was applied to the ABCG2 C421A polymorphism, and this did not reveal any association between the ABCG2 421 CA+AA genotype and the risk of gefitinib-induced skin toxicity in Asians. Although the ABCG2 C421A polymorphism might not be associated with the skin toxicity in Caucasians, this result was based on only one study, and so more reliable data from large-scale studies are needed to confirm the effects of the ABCG2 C421A polymorphism on the skin toxicity of gefitinib therapy.

Two studies involving Asians analyzed the effect of the ABCG2 G34A polymorphism on gefitinib-induced skin toxicity.[8,29] Tamura et al found that this polymorphism was associated with skin toxicity and might be useful for predicting skin toxicity in gefitinib treatments.[8] Ma et al observed that the ABCG2 34 A allele tended to increase the risk of skin toxicity.[29] Pooled results showed that the GA+AA genotype of the ABCG2 G34A polymorphism might increase the risk of gefitinib-induced skin toxicity. A plausible mechanism is that the variant allele of ABCG2 G34A reduces the transporter activity, decreases drug efflux, and thus increases drug accumulation,[22] which may enhance the response to the drug. Consistent with the above result, it has been reported that the overall survival was longer in NSCLC patients carrying the ABCG2 GA or AA genotype than in those with the ABCG2 GG genotype.[31] In addition, the occurrence of skin rash was associated with improved survival in NSCLC patients treated with gefitinib.[32] However, because only two studies were included, further studies are needed to confirm the effects of the ABCG2 G34A polymorphism on the risk of gefitinib-induced skin toxicity.

Hepatotoxicity

Hepatotoxicity was investigated in four studies involving Asian populations.[8,27–29] Three of these studies found no obvious association between the ABCG2 C421A polymorphism and the risk of gefitinib-induced hepatotoxicity,[8,27,28] while Ma et al[29] demonstrated that the presence of the ABCG2 421 A allele increased the risk of gefitinib-induced hepatotoxicity. These different results might be related to differences in the period of toxicity assessments and the small size of the samples. The results from our meta-analysis indicated that there is no significant association between the ABCG2 C421A polymorphism and hepatotoxicity in Asians.

Two studies involving Asian populations investigated the effect of the ABCG2 G34A polymorphism on the risk of hepatotoxicity.[8,29] Ma et al[29] found that patients carrying the ABCG2 34 A allele tended to have an increased risk of gefitinib-related hepatotoxicity, while Tamura et al[8] found no such correlations. Our meta-analysis demonstrated no significant association between the ABCG2 G34A polymorphism and hepatotoxicity, but more studies are required to confirm this association.

Interstitial pneumonia

Two studies involving Asians investigated the effect of the ABCG2 C421A polymorphism on gefitinib-related interstitial pneumonia.[8,27] Neither of these studies found any significant association between ABCG2 C421A and interstitial pneumonia, which was consistent with the results of our meta-analysis.

Tamura et al[8] was the only study to have investigated the association of the ABCG2 G34A polymorphism with gefitinib-induced interstitial pneumonia, and they found that the ABCG2 G34A polymorphism had no effect on the risk of interstitial pneumonia. A meta-analysis could not be conducted due to lack of more studies and data.

The pharmacogenetics of erlotinib, an EGFR tyrosine kinase inhibitor similar to gefitinib, has been reported for toxicity. Several studies have researched the relationship between ABCG2 gene polymorphisms and the toxicity of erlotinib, which found no significant associations between the C421A and G34A loci of ABCG2 and the toxicity of erlotinib.[33,34]

Some limitations of the present meta-analysis should be noted. First, the studies included in our meta-analysis investigated only Asian or Caucasian patients, and so our conclusions apply only to these ethnic groups. Second, only small numbers of studies were included in our analyses, and so the conclusions need to be interpreted with caution. Clearly, it is necessary to conduct more studies to confirm our tentative conclusions. Third, due to such a small number of studies being included, publication bias in this meta-analysis could not be controlled. Finally, due to the lack of detailed original data at the individual level, a more precise analysis stratified according to age, sex, clinical manifestations, and environmental factors could not be conducted.

Conclusion

In summary, this meta-analysis has demonstrated that the ABCG2 C421A polymorphism might not be a reliable marker of gefitinib-related toxicity in NSCLC patients, while the ABCG2 G34A polymorphism might be associated with gefitinib-induced skin toxicity in NSCLC patients. Given that our analyses included only few studies and small samples, more large-scale studies are required to confirm these associations.

Disclosure

The authors report no conflicts of interest in this work.

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