Side effects of tyrosine kinase inhibitors therapy in patients with non-small cell lung cancer and associations with *EGFR* polymorphisms: A systematic review and meta-analysis

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Abstract. Rash and diarrhea are common side effects of tyrosine kinase inhibitor (TKI) therapy administered to patients with non-small cell lung cancer (NSCLC). The polymorphisms of the epidermal growth factor receptor (*EGFR*) gene may be a potential predictor of these side effects. The aim of the present meta-analysis was to examine the association of *EGFR* polymorphisms and TKI-associated toxicities. Electronic databases (PubMed, Scopus and ISI Web of Science) were searched for relevant studies. According to the inclusion and exclusion criteria, a search of the databases identified 4,918 results, among which 6 clinical trials were obtained with 1,318 patients with NSCLC. A total of 9 *EGFR* single nucleotide polymorphisms (SNPs) associated with TKI toxicity were identified including, rs11568315, rs712829, rs712830, rs2227983, rs2075102, rs2293347, rs11977388, rs4947492 and rs884225. The data associated with skin toxicity from rs11568315, rs712829 and rs712830 were analyzed in the present meta-analysis. Data from rs11568315 were also analyzed in relation to diarrhea. Among all the examined SNPs, statistically significant results were obtained under the dominant genetic model for CA repeats in rs11568315 (SS vs. SL+LL) with skin toxicity. The long CA repeat (SL+LL) carriers were more likely to experience skin toxicity associated with TKIs (P=0.005). By contrast, there was no significant result for diarrhea (P=0.661) under dominant genetic model for CA repeats.

Introduction

As the most prevalent form of lung cancer, non-small cell lung cancer (NSCLC) is reported to be one of the deadliest types of cancer in the world, with 2,206,771 newly diagnosed cases and 1,796,144 new deaths recorded in 2020 (1,2). In patients with advanced NSCLC, platinum-based chemotherapy is the first line treatment, but it is usually cytotoxic and has a short progression-free survival (PFS) time of 3-5 months and an overall survival (OS) time of ≤10 months (3). Targeted therapy has been developed to prevent epidermal growth factor receptor (EGFR) activation (3-6). EGFR is a transmembrane protein and a potent transducer of altered signals in tumor cells. There are two ways of blocking the EGFR: Either by blocking the ligand from binding to the receptor extracellular domain with anti-EGFR monoclonal antibodies (cetuximab) or by reversibly binding the small molecule tyrosine kinase inhibitors (TKIs) to the receptor intracellular tyrosine kinase domain (4,5). Thus, the introduction of the first TKI generation drugs, gefitinib and erlotinib, resulted in markedly higher treatment response rates (73.7% for TKI compared with 30.7% for chemotherapy), and the median PFS time increased to 10-13 months for patients with NSCLC (6-8).

For accurate therapeutic decisions to be made in the management of patients with NSCLC, it is essential to find molecular markers that can identify patients who will respond most effectively to treatment. Promising molecular identifiers include mutations in the *EGFR* gene. In patients carrying exon 19 deletions and point mutations in exon 21, a significant clinical benefit following treatment with TKIs was observed (9,10). However, acquired resistance in connection with *EGFR* T790 mutations limited the efficacy of the EGFR-TKI (11,12). The role of polymorphisms [including single nucleotide polymorphisms (SNPs) and short tandem CA repeats] of the *EGFR* gene as another potential molecular target that improved clinical outcomes is well-established (13-16). Recently, a meta-analysis elucidated that among rs712829 (-216G>T), rs11568315 (CA repeat), rs2293347 (D994D) and rs4947492, -216G>T and variable CA repeat polymorphisms significantly affected OS and PFS time in gefitinib- or erlotinib-treated patients with NSCLC (17).

EGFR-TKI therapy is associated with side effects, primarily in the form of skin or gastrointestinal toxicities (e.g., skin rash or diarrhea). Although skin toxicities are not lethal or dose-limited, they frequently occur with EGFR-TKIs and affect patient quality of life (18). Among usual skin toxicities, such as xerosis, pruritus, paronychia, mucositis and increased

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growth of eyelashes or facial hair, skin rash is the most prevalent (19-22). Notably, patients with NSCLC that develop skin rashes are better responders to EGFR-TKI therapy and have a longer median overall survival time (18-23). *EGFR* SNPs have been examined in association with survival in NSCLC (17,18); they may provide insight into therapy outcomes, particularly the potential side effects associated with TKIs (23-28). Literature analysis discovered notable inconsistency in previously published reports. While some studies found associations with *EGFR* genotypes and TKI toxicity (23-26), others did not (27). Additionally, previous meta-analyses investigated *EGFR* mutations, but not *EGFR* polymorphisms and therapy side effects in patients with NSCLC (29,30), or toxicity in relation to radiotherapy (31). With regards to these discrepancies and the role of *EGFR* SNPs as potential determinants of treatment outcome, the aim of the present meta-analysis was to determine whether the molecular mechanisms involving *EGFR* SNPs were associated with EGFR-TKI therapy side effects.

Materials and methods

Search strategy and study selection. The present study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) (32). The systematic search for the relevant studies was performed using electronic databases, PubMed, Scopus and ISI Web of Science. Searches were performed considering *EGFR* polymorphisms and side effects of TKI therapy in patients with NSCLC. The search had the following retrieval strategy for the PubMed database: [('receptor, epidermal growth factor' (MeSH Terms) OR *EGFR* (All Fields)) AND (gene(tiab) OR 'polymorphism, genetic'(MeSH Terms)) AND ('carcinoma, non-small-cell lung' (MeSH Terms) OR NSCLC (All Fields)) AND ('drug therapy' (Subheading) OR treatment (All Fields) OR 'erlotinib hydrochloride' (MeSH Terms) OR 'gefitinib' (MeSH Terms) OR TKI OR 'TK inhibitors' OR 'tyrosine kinase inhibitors' OR 'Tyrosine-kinase inhibitor') AND response (All Fields)) OR Prognosis (MeSH)) OR toxic (MeSH)) OR toxicity (MeSH)) OR side effect (MeSH)) AND (humans (MeSH))]. The Scopus and ISI Web of Science databases were also searched with necessary modifications to the PubMed search query. The full search string is available from the corresponding author upon request. Finally, additional studies were searched for in the bibliographies of the selected eligible studies or reviews.

Selection criteria. All studies fulfilling the following inclusion criteria were eligible: i) Studies published from January 1, 2009, to February 13, 2019; ii) studies published in English; iii) studies involving human subjects; iv) patients >18 years old with histopathologically confirmed NSCLC who received EGFR-TKI therapy and v) clinical trials or observational studies that investigated associations between *EGFR* polymorphisms and any side effects of TKI therapy. In the systematic review, studies were excluded based on the following criteria: i) Meta-analyses, editorials, letters, commentaries, systematic or narrative reviews; ii) not in the English language; iii) duplicate publications or studies involving animal or cell experimental models; iv) studies investigating *EGFR* polymorphisms and TKI adverse effects but not reporting their associations; v) single study reports of *EGFR* polymorphisms associated with TKI toxicities (skin toxicity or diarrhea), or other side effects (such as hepatotoxicity) due to being unable to make comparisons due to the lack of data from other studies and vi) randomized control trial (RCT) studies that did not report genotype numbers data, even though the odds ratio (OR) was reported.

Data extraction. Extracted studies from the electronic databases were first merged and duplicates were removed. A total of 2 authors (JO and JT) independently performed a manual search of titles and abstracts of potentially eligible studies according to the inclusion and exclusion criteria. Any discrepancies were resolved by discussion or by consulting the third author (VJ). Finally, the following data were extracted from the full texts based on the prior determined datasheet: The first author, year of publication, country, study type, study period, number of patients, median age, sex and ethnicity of patients, percentage of smokers, clinical stage, histology, median follow-up (in months), TKI treatment dosage, additional therapy, toxicity assessment, adverse effects of treatment, available *EGFR* genotype, variant location, SNP database identifier and number of patients/genotype.

Quality assessment. The Newcastle-Ottawa Quality Assessment Scale (NOS) (33) for cohort studies and the Jadad Scale for RCTs (34) were used to assess the methodological quality of the studies included. For the NOS scale, the overall maximum quality score was 9 points; for the Jadad Scale, the score was 5 points. The reviewers (JO and JT) independently evaluated the quality of the studies with discrepancies resolved by consensus.

Statistical analysis. When ≥2 studies had available *EGFR* polymorphic genotypes associated with TKI therapy side effects, meta-analysis was conducted. To examine heterogeneity between the eligible studies, Cochran's Q statistics and I^2 statistics were applied. I^2 was interpreted as follows: 0, no heterogeneity; 25, low heterogeneity; 50, moderate heterogeneity and 75%, high heterogeneity (35). The random effect model was used when there was significant heterogeneity between studies ($P<0.05$; $I^2>50\%$), otherwise, the fixed effect model was applied (36). Galbraith's plot was used to identify potential sources of heterogeneity (37). If heterogeneity was present, subgroup analyses of OR were conducted according to the available *EGFR* SNPs. The dominant genetic model (wild-type homozygote vs. heterozygote + mutant homozygote) of all three *EGFR* SNPs (rs11568315, rs712829 and rs712830) was used to calculate OR. The available adverse effects for the analysis were skin toxicity (skin rash) and gastrointestinal toxicity (diarrhea). For comparison, the adverse effects were combined and used as any grade vs. the absence of adverse effects. Sensitivity analysis was also performed to determine whether the results would be affected by excluding the study with the smallest sample size. The publication bias of the enrolled studies was tested with Begg's and Egger's tests, as well as funnel plots (38,39). $P<0.05$ was considered to indicate a statistically significant difference. STATA software package v.15 (StataCorp LP) was used for all statistical analysis.

Results

Study selection. The initial search of databases identified 4,918 results (PubMed, 881; ISI Web of Science, 395; Scopus,

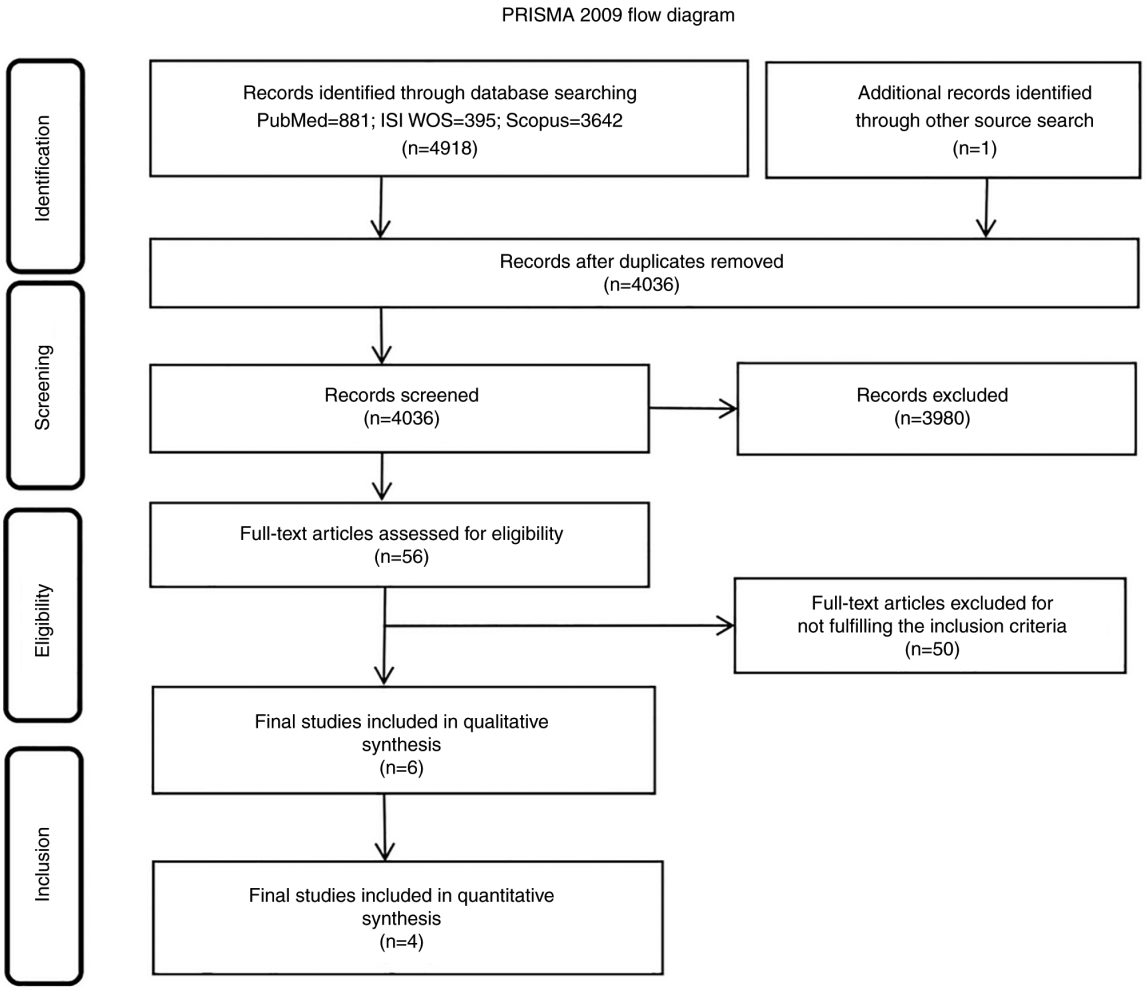

Figure 1. Flowchart of the study selection process. WOS, Web of Science; PRISMA, Preferred reporting items for systematic reviews and meta-analyses.

3,642; Fig. 1). An additional study was included after reading the bibliographies of the full-text articles. After merging into the single datasheet and removing duplicates, 4,036 studies remained. Of these, 3,980 were excluded and 56 full-text articles were used to assess eligibility. Of these 56 articles, 50 were excluded due to not fulfilling the inclusion criteria. Finally, 6 clinical trials were included in the systematic review which contained 1,318 patients with NSCLC. A total of 4 studies were included in the meta-analysis.

Characteristics of the studies. The six studies from the search included four cohort studies (23,25,26,28) containing 316 patients and 2 RCTs (24,27) containing 1,002 patients. The studies were published from 2009-2017, with sample sizes ranging from 52-760 patients. A total of two studies were from Asia (Taiwan and China), two from Europe (Germany and Italy), one from Canada and one RCT was from a consortium of counties (Canada, Italy, South Korea and Brazil). The number of male patients in the studies was 33-67%. The percentage of smokers was 12-76%, while the median age was 56-68 years. Most of the patients had adenocarcinoma histology and were in clinical stages IV, IIIB and IIIA (23-28). Only one study reported a median follow-up of 12 months (23). The EGFR-TKI therapy type for patients with NSCLC in all examined reports was gefitinib (250 mg/day) and erlotinib (150 mg/day), except in one study where cetuximab (250 or 500 mg/m^2) or panitumumab (6 mg/kg) was prescribed (25). Additionally, four studies reported patients that had been previously treated with cisplatin (24-27). Adverse effects were skin (rash) and gastrointestinal toxicity (diarrhea and hepatotoxicity). Toxicity assessment was conducted using the National Cancer Institute's Common Terminology Criteria for Adverse Events (40). The quality of the studies was rated acceptable using the NOS and the Jadad scale (33,34). Adequacy of follow-up was the lowest rated aspect. The characteristics and quality assessment of the included studies are presented in Table I.

A total of nine *EGFR* SNPs (rs11568315, rs712829, rs712830, rs2227983, rs2075102, rs2293347, rs11977388, rs4947492 and rs884225) relative to TKI toxicity was identified in the literature search, which were provided by seven studies (23-28,41). Of these, four studies reported exact numbers of patients/genotype of *EGFR* SNPs (rs11568315, rs712829 and rs712830) associated with TKI-caused toxicity and were included in quantitative synthesis (23,25,26,28). Genotypes for all *EGFR* SNPs for the meta-analysis were merged according to the dominant genetic model. The data for the *EGFR* SNPs genotype and skin toxicity, diarrhea or hepatotoxicity caused by *EGFR* TKI therapy are presented in Table II. Certain studies or sets of data were excluded from further analyses. The reasons are outlined below.

Table I. Characteristics of the included studies.

First author, year	Country	Study type	Study period	Patients, n	Median age, years (range)	Males, %	Ethnicity, %	Smokers, %	Clinical stage (%)[a]	Histology (%)	Median follow-up, months	TKI treatment (dose)	Additional therapy	Toxicity assessment	Adverse effect	Overall quality score	(Refs.)
Huang *et al*, 2009	Taiwan	Cohort	May 2005-September 1, 2006	52	66 (39-86)	33	NR	NR	IIIB (14), IV (87)	Adenocarcinoma (96), Other (4)	12	Gefitinib (250 mg/day)	NR	NCI CTCAE v3.0[c]	Skin	NOS[h]: 7	(23)
Giovannetti *et al*, 2010	Italy	Cohort	NR	96	64 (NR)	57	NR	69	IIIB (9), V (91)	Adenocarcinoma (56), Other (44)	NR	Gefitinib (250 mg/day)	85% of patients were previously treated with chemotherapy[b]	NCI CTC manual version[d]	Skin, diarrhea	NOS: 7	(26)
Liu *et al*, 2012	Canada	RCT Phase III	NR	242	60 (NR)	64	East Asian (6), Other (94)	76	IIIB (NR), IV (NR)	Adenocarcinoma (54), Other (46)	NR	Erlotinib (NR)	93% patients were previously treated with chemotherapy	NCI v2.0.[e]	Skin	Jadad[i]: 4	(24)
Parmar *et al*, 2013	Germany	Cohort	September 2008-November 2010	109	68 (43-86)	67	NR	12; 49.5 former	IIIA (2); IIIB (11), IV (83), Unknown (5)	NR	NR	Gefitinib (250 mg/day), erlotinib (150 mg/day), cetuximab (250 mg/ m^2, 500 mg/m^2 weekly or biweekly or panitumumab (6 mg/kg) biweekly	31% patients were previously treated with chemotherapy	NCI CTC v3.0[f]	Skin	NOS: 6	(25)

Table I. Continued.

First author, year	Country	Study type	Study period	Patients, n	Median age, years (range)	Males, %	Ethnicity, %	Smokers, %	Clinical stage (%)[a]	Histology (%)	Median follow-up, months	TKI treatment (dose)	Additional therapy	Toxicity assessment	Adverse effect	Overall quality score	(Refs.)
Kim *et al*, 2017	Canada, Italy, South Korea and Brazil	RCT Phase III	NR	760	62 (56-66)	66	Caucasian (96), East Asian (3), Other (1)	26; 53 former	IIIB (11), IV (89)	Adenocarcinoma (56), Other (44)	NR	Erlotinib (NR)	50% of patients were treated with erlotinib, followed by cisplatin and gemcitabine at progression; 50% were treated with cisplatin and gemcitabine, followed by erlotinib at progression	NR	Skin, diarrhea	Jadad: 3	(27)
Ma *et al*, 2017	China	Cohort	2011-2014	59	56 (31-77)	49	NR	61	IIIB or IV (64)	Adenocarcinoma (90), Other (10)	NR	Gefitinib (250 mg/day)	NR	CTCAE v4.0[g]	Skin, diarrhea, hepatotoxicity	NOS: 7	(28)

Data is provided to 2 significant places. [a](23-28), [b]additional 127 chemotherapy-treated/gefitinib-non-treated patients with NSCLC were used as a comparison, [c](23), [d](26), [e](24), [f](25), [g](28), [h](33), [i](34). CTC, Common Toxicity Criteria; CTCAE, Common Terminology Criteria for Adverse Events; NCI, National Cancer Institute; NOS, Newcastle-Ottawa Quality Assessment Scale; NR, not reported; RCT, randomized clinical trial; TKI, tyrosine kinase inhibitor.

Table II. *EGFR* genotypes and adverse effects of tyrosine kinase inhibitor therapy.

A, Patients with skin toxicity (n=880, 78.36%)

dbSNP-ID	Variant type, location, and/or consequence	First author, year	Genotyping platform used	Genotype	Total number of patients, n	Patients with skin toxicity, n	OR[a]	95% CI[a]	z[a]	P-value[a]	(Refs.)
rs11568315	Intron variant, g.55020560_55020561AC[n]	Huang *et al*, 2009	PCR and direct sequencing	SS	7	5	Ref.		2.13	0.032	(23)
				SL	26	8	6.87	1.1734-40.2797	70	6	
				LL	19	4	5				
		Parmar *et al*, 2013	Real-Time PCR and 16-capillary electrophoresis or KASPar	SS	22	19	Ref.		1.23	0.217	(25)
				SL	58	41	2.27	0.6156-8.4150	30	7	
				LL	29	23	6				
		Giovannetti *et al*, 2010	TaqMan PCR	SS	27	18	Ref.		1.71	0.087	(26)
				SL-LL	60	28	2.28 57	0.8863-5.8947	00	2	
		Ma *et al*, 2017	Sequenom Massarray system	SS	6	6	NA	NA	NA	NA	(28)
				SL	20	14					
				LL	16	10					
		Kim *et al*, 2017	Sanger sequencing and Taqman PCR	SS	38	28	Ref.		0.15	0.874	(27)
				SL-LL	96	72	0.93	0.3961-2.1994	80	7	
		Liu *et al*, 2012	PCR and direct sequencing	LL-S-	NR	NR	0.60	0.2-1.9	NA	NA	(24)
rs712829	5' UTR variant, g.5031G>T, -216G>T	Huang *et al*, 2009	PCR and direct sequencing	GG	45	14	Ref.		0.61	0.540	(23)
				GT	5	1	0.60	0.1186-3.0567	20	6	
				TT	2	2	22				
		Parmar *et al*, 2013	Real-Time PCR and 16-capillary electrophoresis or KASPar	GG	49	33	Ref.		1.92	0.054	(25)
				GT	48	39	0.41	0.1670-1.0188	00	9	
				TT	12	11	25				
		Giovannetti *et al*, 2010	TaqMan PCR	GG	30	19	Ref.		1.41	0.158	(26)
				GT-TT	57	27	1.91 92	0.7752-4.7513	00	7	
		Kim *et al*, 2017	Sanger sequencing and Taqman PCR	GG	40	32	Ref.		0.91	0.358	(27)
				GT-TT	83	60	1.53	0.62-3.82	90	2	
		Liu *et al*, 2012	PCR and direct sequencing	GT-GG	NR	NR	2.00	0.5-8.3	NA	NA	(24)

Table II. Continued.

A, Patients with skin toxicity (n=880, 78.36%)

dbSNP-ID	Variant type, location, and/or consequence	First author, year	Genotyping platform used	Genotype	Total number of patients, n	Patients with skin toxicity, n	OR[a]	95% CI[a]	z[a]	P-value[a]	(Refs.)
rs712830	5' UTR variant, g.5056A>C, -191C>A	Parmar *et al*, 2013	Real-Time PCR and 16-capillary electrophoresis or KASPar	CC	79	59	Ref.		0.5800	0.5617	(25)
				CA	30	24	0.73	0.2637-2.0624			
				AA	0	0	75				
		Giovannetti *et al*, 2010	TaqMan PCR	CC	72	36	Ref.		1.1620	0.2451	(26)
				CA-AA	15	10	0.5	0.1554-1.6089			
		Kim *et al*, 2017	Sanger sequencing and Taqman PCR	CC	100	72	Ref.		1.4480	0.1477	(27)
				CA- AA	23	20	0.3857	0.1062-1.4007			
		Liu *et al*, 2012	PCR and direct sequencing	CA-CC	NR	NR	1.00	0.1-6.8	NA	NA	(24)
rs2227983[b]	Missense variant, (1562G>A, R497K)	Parmar *et al*, 2013	Real-Time PCR and 16-capillary electrophoresis or KASPar	GG	57	49	Ref.		2.4510	0.0142	(25)
				GA	47	29	3.24	1.2657-8.3073			
				AA	5	5	26				
		Giovannetti *et al*, 2010	TaqMan PCR	GG-GA	75	38	0.5869	0.1585-2.1734	0.7980	0.4250	(26)
				AA	11	7	Ref.				
		Huang *et al*, 2009	PCR and direct sequencing	GG	12	4	Ref.		0.1670	0.8671	(23)
				GA	28	8	1.12	0.2832-4.4695			
				AA	12	5	5				

B, Patients with diarrhea (n=233, 20.75%)

dbSNP-ID	Variant type, location, and/or consequence	First author, year	Genotyping platform used	Genotype	Total number of patients, n	Patients with diarrhea toxicity, n	OR[a]	95% CI[a]	z[a]	P-value[a]	(Refs.)
rs11568315	Intron variant, g.55020560_55020561AC[n]	Giovannetti *et al*, 2010	TaqMan assay	SS	26	13	Ref.		0.7610	0.4466	(26)
				SS-LL	49	20	1.45	0.5569-3.7751			
		Ma *et al*, 2017	Sequenom Massarray system	SS	6	2	Ref.		0.5060	0.6128	(28)
				SL	20	9	0.62	0.1012-3.8585			
				LL	16	7	5				

Table II. Continued.

B, Patients with diarrhea (n=233, 20.75%)

dbSNP-ID	Variant type, location, and/or consequence	First author, year	Genotyping platform used	Genotype	Total number of patients, n	Patients with diarrhea toxicity, n	OR[a]	95% CI[a]	z[a]	P-value[a]	(Refs.)
		Kim *et al*, 2017	Sanger sequencing and Taqman PCR	SS	38	15	Ref.		0.12	0.902	(27)
				SL-LL	96	39	0.95	0.4424-2.0535	20	5	
rs712829	5' UTR variant, g.5031G>T, -216G>T	Kim *et al*, 2017	Sanger sequencing and Taqman PCR	GG	40	18	Ref.		0.94	0.346	(27)
				GT-TT	83	30	1.44	0.6711-3.1131	10	6	
							55				
rs712830	5' UTR variant, g.5056A>C, -191C>A	Kim *et al*, 2017	Sanger sequencing and Taqman PCR	CC	100	37	Ref.		0.95	0.339	(27)
				CA-AA	23	11	0.64	0.2570-1.597	50	4	
rs2227983[b]	Missense variant, (1562G>A, R497K)	Giovannetti *et al*, 2010	TaqMan PCR	GG-GA	74	26	2.76	0.7163-10.7057	1.47	0.139	(26)
							92		60	8	
				AA	10	6	Ref.				

C, Patients with hepatotoxicity (n=10, 0.89%)

dbSNP-ID	Variant type, location, and/or consequence	Author, year	Genotyping platform used	Genotype	Total number of patients, n	Patients with hepatotoxicity, n	OR[a]	95% CI[a]	z[a]	P-value[a]	(Refs.)
rs1156	Intron variant, g.55020560_55020561AC[n]	Ma *et al*, 2017	Sequenom Massarray system	SS	6	1	Ref.		0.40	0.682	(28)
8315				SL	21	6	0.62	0.0640-6.0507	90	7	
				LL	16	3	22				

[a]Pre-calculated values according to available data for *EGFR* SNP genotypes, [b]rs11543848 was merged with rs2227983. CI, confidence interval; dbSNP-ID, single nucleotide polymorphism database identifier); rs11568315 SS, rs712829 GG, rs712830 CC, rs2227983 GG: wild-type homozygotes; rs11568315 SL, rs712829 GT, rs712830 CA, rs2227983 GA: heterozygotes; rs11568315 LL, rs712829 TT, rs712830 AA, rs2227983 AA: mutant homozygotes; NA, not applicable; Ref., reference value; NR, not reported; OR, odds ratio; EGFR, epidermal growth factor receptor; UTR, untranslated region.

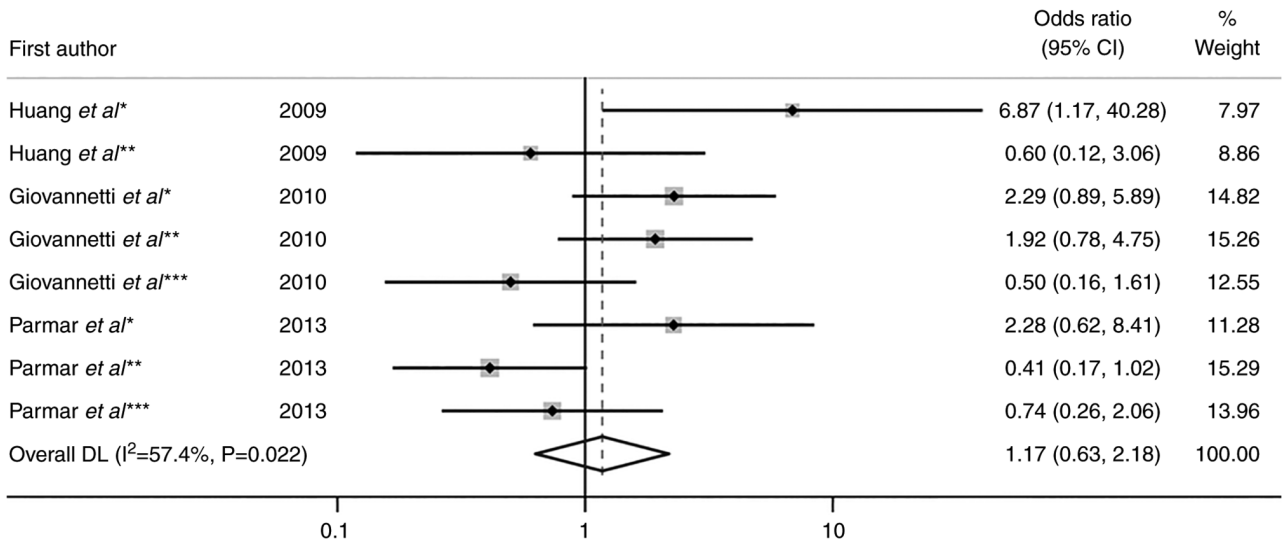

Figure 2. Forest plot of pooled odds ratio and 95% CI of three epidermal growth factor receptor single nucleotide polymorphisms relative to tyrosine kinase inhibitor-caused skin toxicity. *rs11568315 (CA repeat), **rs712829 (-216G>T), ***rs712830 (-191 C/A). Weights are from the random effects model. CI, confidence interval; DL, Der Simonian and Laird method.

Due to lack of data from other studies for comparison, one study was excluded from further analysis, although the study did identify rs884225, a 3'-untranslated region variant c.*774T>C associated with *EGFR* TKI toxicity (41). Similarly, data for the hepatotoxicity, as well as for four *EGFR* SNPs were not included (rs2075102, rs2293347, rs11977388 and rs4947492) (27). An RCT study reported pre-calculated ORs for three examined *EGFR* SNPs, but without precise numbers of patients per genotype (24), and was therefore included in the qualitative, but not the quantitative, analysis. Consequently, another RCT study was excluded from quantitative analysis (27) to avoid comparison between observational and RCT studies, and prevent potential heterogeneity. If zeros present in patient genotype numbers for rs2227983 and rs11568315 interfered with computation or if there were insufficient data for analysis, studies were excluded from quantitative synthesis (23-25,28). In the literature search, three other studies explored *EGFR* TKI toxicity, as well as *EGFR* SNPs, but failed to find any associations between them (42-44).

Side effects of EGFR-TKIs. There was notable inconsistency in the scientific reports describing the association between *EGFR* SNPs and TKI toxicity in patients with NSCLC. While some articles reported evidence of association with skin toxicity (23-26) or severe diarrhea (26), one article found no association with skin or gastrointestinal toxicities (27).

In patients treated with gefitinib, there was a significant association between SS genotype in CA repeat polymorphism and early G2/3 skin rash (P=0.031), meaning these patients were more likely to develop early G2/3 rash (23). Despite this, the *EGFR* polymorphisms -216G>T and R521K were not associated with early G2/3 rash (P=0.104 and P=0.720, respectively) (23). Another study on patients treated with erlotinib found a similar result for three *EGFR* polymorphisms and skin rash: -191C>A, -216G>T and CA repeats (P=1.00, P=0.13 and P=0.34, respectively) (24). Only the *EGFR* -216/-191GC haplotype was associated with the appearance of skin rash (P=0.029) (25). Nevertheless, the absence of association with skin rash was evidenced for the single *EGFR* SNPs -191C>A (P=0.62), -216G>T (P=0.147) and CA repeats (P=0.36) (25). Diarrhea was a less frequent toxicity and no significant association between any of the *EGFR* SNPs or haplotypes with diarrhea was observed (25). However, in another study, severe diarrhea occurred in patients with NSCLC treated with gefitinib, most frequently in carriers of -191C>A, -191A>A (P<0.0001) and -216G>G genotypes (P<0.01) (26). There was no significant association between *EGFR* CA repeat polymorphisms and skin or gastrointestinal toxicity, nor any association between *EGFR* polymorphism and skin toxicity (26).

Toxicity. The most common adverse effects associated with TKIs in treating advanced NSCLC were skin toxicity (78.36%) and diarrhea (20.75%; Table II). One study reported hepatotoxicity (0.89%) (27), but the study was excluded since there were no data from other studies for comparison. Among the studies available for the meta-analysis, gefitinib (250 mg/day) or erlotinib (150 mg/day) were predominant. For data available for genotypes relative to skin toxicity, the OR and 95% confidence interval (CI) were calculated and their effect was summarized in the quantitative synthesis (Fig. 2). This involved three *EGFR* SNPs (rs11568315, rs712829 and rs712830) obtained from three studies for skin toxicity (23,25,26). Of these, two examined rs11568315 and diarrhea (26,28). The pooled OR for skin toxicity and rs11568315, rs712829 and rs712830 was 1.17 (95% CI, 0.63-2.18; P=0.616) with moderate heterogeneity (I^2=57.4%; P=0.022; Fig. 2).

To test heterogeneity, random effect model and subgroup analyses were performed. Subgroup analysis for skin toxicity showed that the OR for rs11568315 was 2.72 (95% CI, 1.34-5.49; P=0.005) without heterogeneity (I^2=0.0%; P=0.533). A statistically significant result for skin toxicity (z=2.785 and P=0.005) were obtained under the dominant genetic model for rs11568315 (SS vs. SL + LL). OR for rs712829 was 0.81 (95% CI, 0.28-2.36; P=0.700) with moderate heterogeneity

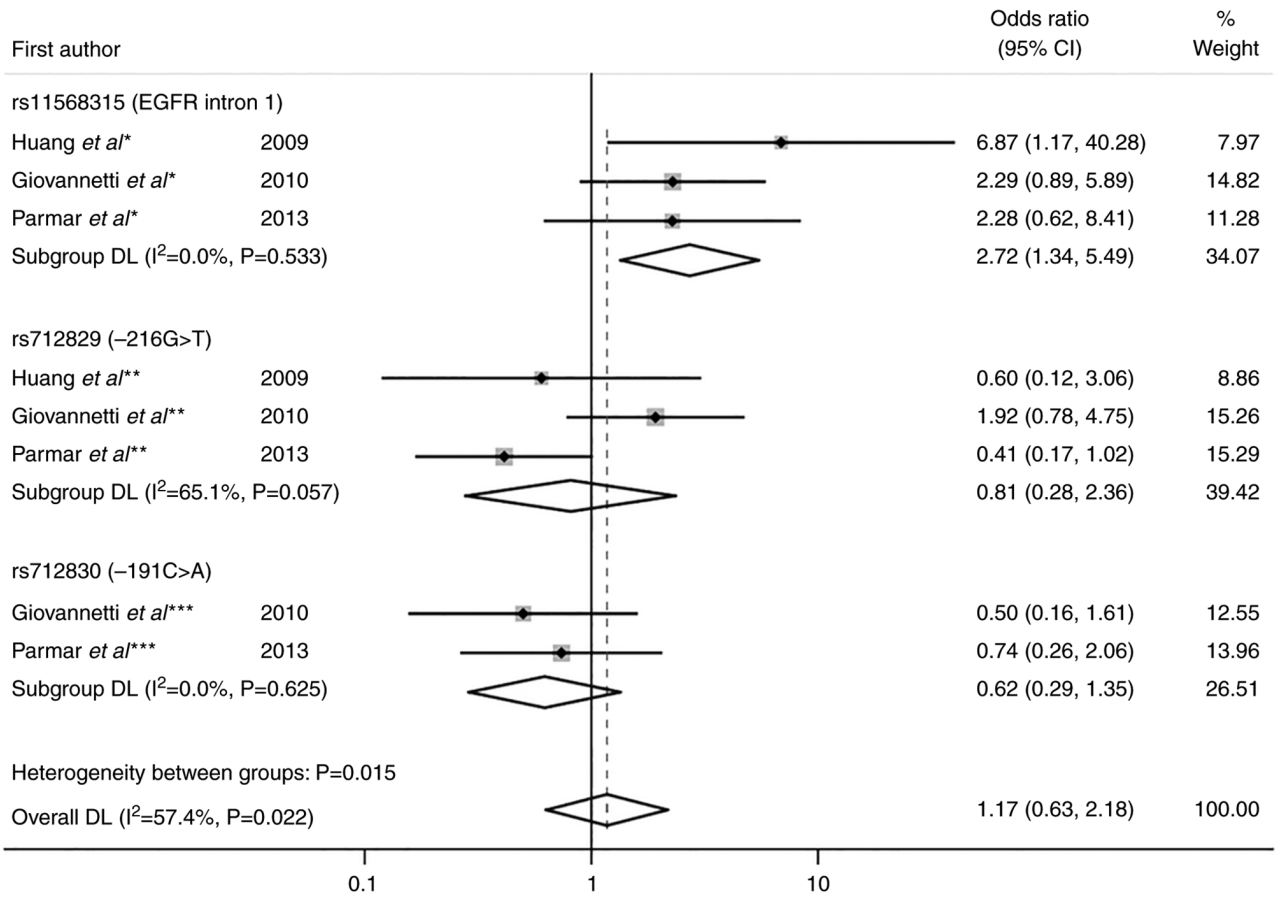

Figure 3. Forest plot of subgroup analysis of three *EGFR* SNPs relative to tyrosine kinase inhibitor-caused skin toxicity. *rs11568315 (CA repeat), **rs712829 (-216G>T), ***rs712830 (-191 C/A). Weights and subgroup heterogeneity test are from the random effects model. CI, confidence interval; DL, Der Simonian and Laird method; EGFR, epidermal growth factor receptor; SNP, single nucleotide polymorphism.

(I^2=65.1%; P=0.057) and OR for rs712830 was 0.62 (95% CI, 0.29-1.35; P=0.229) with no heterogeneity (I^2=0.0%, P=0.625; Fig. 3). Data for diarrhea was only available for rs11568315 (data not shown). It was tested in two studies using the fixed effects model (OR, 1.21; 95% CI, 0.52-2.82), with no evidence of heterogeneity (I^2=0.0%; P=0.422) and without statistically significant association (P=0.661) (26,28).

Publication bias and sensitivity analysis. The results of the sensitivity analysis regarding toxicity were relatively stable. The overall effective size was not affected by exclusion of each of the studies, even by a study with a smaller sample size (OR, 6.87; 95% CI, 1.17-2.28; Fig. 4A) (23). The funnel plot for *EGFR* SNPs and TKI skin toxicity in patients with NSCLC was roughly symmetric (Fig. 4B). Begg's funnel plot and Egger's regression test (P=0.545) were used to test the publication bias, but no significantly different results were obtained (Fig. 4C and D). Similarly, the funnel plot revealed no potential bias of rs11568315 (CA repeat) and TKI-caused diarrhea (data not shown). Galbraith's plot identified no source of heterogeneity relative to skin toxicity (data not shown).

Discussion

The present systematic review involved the analysis of two RCTs and four cohort studies to test the association of *EGFR* polymorphisms with the potential toxicity of TKI therapy regimens in patients with NSCLC. A total of 1,123 patients per genotype were observed with any TKI-associated toxicity. A total of four studies provided data for the meta-analysis (23,25,26,28), while six were involved in quality analysis (23-28). In the literature search, nine *EGFR* SNPs relative to TKI toxicity were identified: rs11568315, rs712829, rs712830, rs2227983, rs2075102, rs2293347, rs11977388, rs4947492 and rs884225 (23-28,41). Of these, enough data was available for three (rs11568315, rs712829 and rs712830) to be included in the meta-analysis.

Our recent meta-analysis showed that CA repeat polymorphism and -216G>T significantly affected survival in patients with NSCLC treated with TKI (17). In light of the inconsistency of previous reports (23-31), the present meta-analysis was performed to extend our previous findings and to analyze the effect of the *EGFR* polymorphisms and TKIs on NSCLC.

A number of studies in the present review founded an association between some *EGFR* polymorphisms and TKI-related skin toxicity (23,25,26) or diarrhea (26,27). Contradictory results were also detected in previous studies published before 2009 (45-47). The most common TKI adverse effects in the present meta-analysis were skin toxicity and diarrhea, which were 78.36% and 20.75%, respectively (concerning any grade of toxicity vs. no toxicity). They were separately analyzed in the meta-analysis. The pooled OR for three *EGFR* SNPs

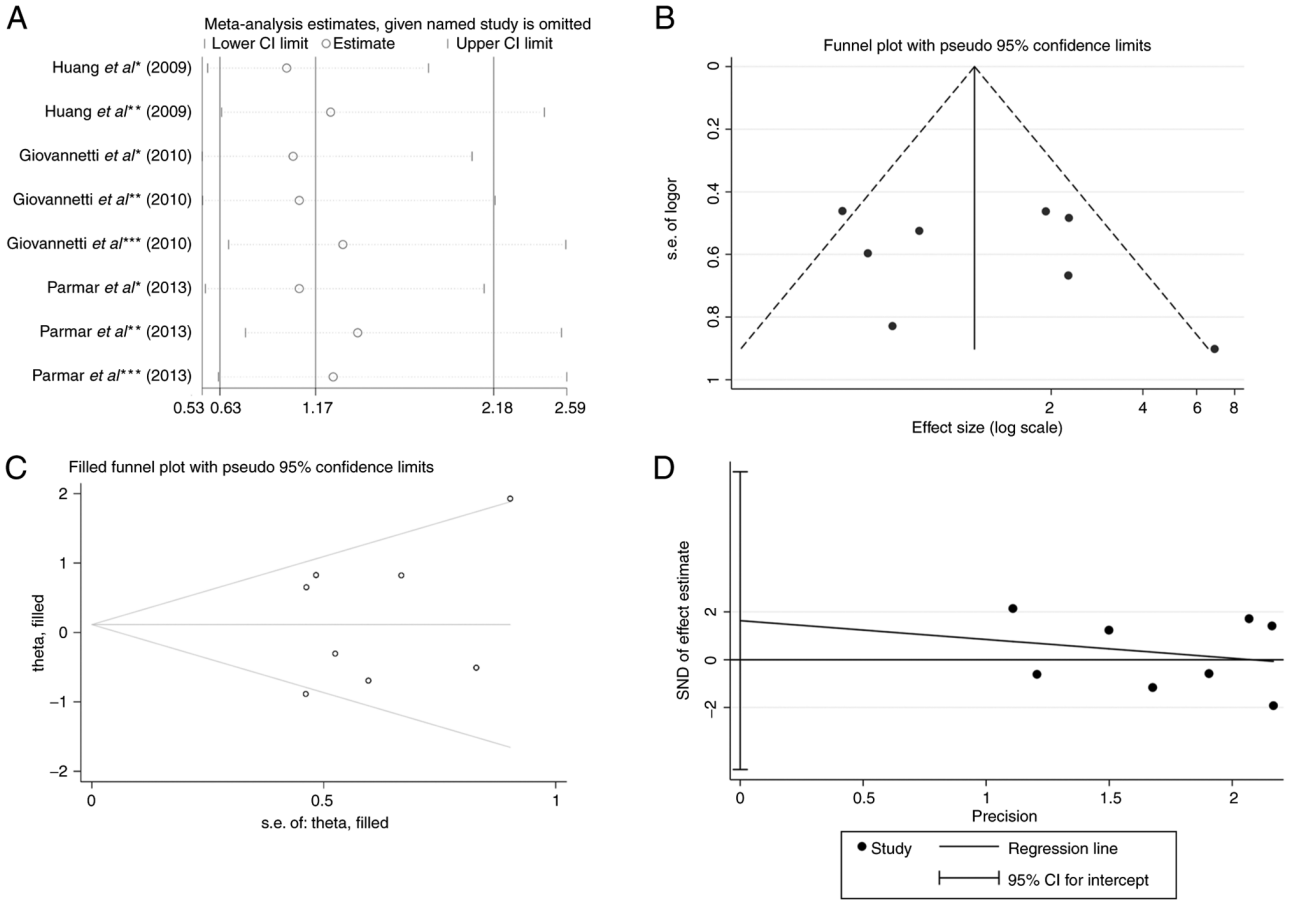

Figure 4. Funnel plots of potential bias of skin toxicity relative to *EGFR* single nucleotide polymorphisms and sensitivity analysis. (A) Individual studies were omitted but did not significantly modify the overall effect. (B) Funnel plot appeared to be roughly symmetrical. (C) Begg's funnel plot suggested no publication bias. (D) Egger's test (P=0.545) indicated that publication biases did not exist. *rs11568315 (CA repeat), **rs712829 (-216G>T), ***rs712830 (-191 C/A). CI, confidence interval; logor, log of odds ratio; theta, the effect estimate; s.e., standard error; SND, standard normal deviate.

(rs11568315, rs712829 and rs712830) was 1.17 (95% CI, 0.63-2.18) without a statistically significant overall effect on skin toxicity (P=0.616). In further analysis, a moderate overall heterogeneity (I^2=57.4%; P=0.022) was observed. To explore the heterogeneity further, a subgroup analysis was performed and the random effect model was applied. The subgroup analysis involved three *EGFR* SNPs (rs11568315, rs712829 and rs712830) concerning skin toxicity (23,25,26). The source of heterogeneity (I^2=65.1%; P=0.057) was likely due to the -216G>T (rs712829) polymorphism (26). The CIs were overlapping the line of no effect for all three studies, suggesting the result was not statistically significant. A total of two studies favored the GG genotype for -216G>T (rs712829) and skin toxicity (23,25), which contrasts the GT+TT genotype favored by Giovannetti *et al* (26). Most importantly, there was no heterogeneity for the other two SNPs examined (rs11568315, I^2=0.0%; P=0.533; rs712830, I^2=0.0%; P=0.625).

Chemotherapy is the first-line treatment for patients with NSCLC, but notable improvements in the response rate have been observed following the application of the TKIs gefitinib and erlotinib (6-8,48). However, resistance, as well as adverse effects, is common in this therapy regimen. Typical side effects of the drugs used in NSCLC treatment (for both monoclonal antibodies and small molecule TKIs) are skin rash and diarrhea (49,50). Since the EGFR is commonly affected by somatic mutations in altered neoplastic cells and the *EGFR* gene is highly polymorphic, the potential cause of those toxic manifestations of drugs may be *EGFR* genetic variability (11,13-16). SNPs or microsatellite tandem repeats are typically found in the *EGFR* promoter region and intron 1. These notably affect *EGFR* gene expression and may mediate response to TKI therapy. A CA single sequence repeat polymorphism (rs11568315) is located in *EGFR* intron 1 and it usually comprises 14-21 variable short tandem repeats. The shorter allele is associated with increased *EGFR* expression and carriers of this polymorphism are better responders to TKI therapy and have prolonged overall survival time (13,14,47,51-54).

Among side effects of TKI therapy, typical skin rash manifestations were in the form of papules and pustules on the scalp, face, neck and upper trunk. To the best of our knowledge, the mechanism of skin rash development has not yet been elucidated. One hypothesis is that there is a genetic susceptibility for rash development, where altered *EGFR* expression alters the TKI response (11,13-16,28). Another is that poor vascularization of the tumor tissue and drug concentrations at a level that does not inhibit tumor growth may cause a skin rash by over-saturation of EGFR (18,55). There is evidence of a significant association between skin rash and an improved outcome in patients with NSCLC (18,56). Skin rash has been reported to be a predictor of tumor response (25) and *EGFR* CA repeat

is a valuable predictor of early G2/3 rash (23). Previous studies have reported that lower number CA repeat carriers develop skin toxicity when treated with gefitinib (13,23,57), while other studies did not (24-28,45,47,53). In another study where patients with NSCLC were treated with erlotinib, SL allele length was associated with a higher risk of diarrhea (46). In the present meta-analysis, the pooled OR values for CA repeats (rs11568315) and skin toxicity were 2.72 (95% CI, 1.34-5.49). A significant association with skin toxicity was evident under the dominant genetic model. Namely, heterozygote and long alleles (SL + LL) or prevalently long CA repeat carriers were more likely to develop TKI-related skin toxicity (P=0.005). However, it is probable that short CA carriers would be less likely develop skin rash. There was no association between CA repeats and diarrhea (P=0.661).

The other well-examined SNPs, -191C>A (rs712830) and -216G>T (rs712829) polymorphisms, are located in the *EGFR* promoter region and are associated with enhanced *EGFR* mRNA expression (14,53,58). A previous meta-analysis revealed that any genotype with T allele for -216G>T showed an association with higher response and disease control rates and longer PFS and OS times than GG homozygote carriers (59). Another meta-analysis elucidated that the -216G>T polymorphism significantly affected OS and PFS times in patients with NSCLC treated with gefitinib or erlotinib (17). Both of the aforementioned polymorphisms are reported to be in linkage disequilibrium (D'=1.0) (25). Considering their association with toxicities, a study reported the haplotypes showing association with the appearance of skin rash (25). Other studies reported that the T allele of -216G>T was significantly associated with high-risk of TKI-induced skin rash (24) or diarrhea (14). An association between -216G>T and -191C>A with grade >1 diarrhea has also been reported (26). Contrary to these findings, the present meta-analysis observed no significant association for *EGFR* SNPs -216G>T and -191C>A with skin toxicity (P=0.700 and P=0.229, respectively), which agreed with the findings from previous studies (23,24,27,45).

The advantage of the present meta-analysis over previous meta-analyses is the examination of commonly used TKIs (such as gefitinib and erlotinib) and their toxicity, while other studies involved a single therapeutic agent (60,61). Other analyses investigated *EGFR* mutations, not *EGFR* polymorphisms (29,30) or their association with toxicity (59), or they only investigated toxicity in relation to radiotherapy (31). The present meta-analysis has certain limitations. Firstly, some studies included in the analysis had small sample sizes so consistent conclusions could not be obtained, as with the RCTs that have larger sample sizes. A total of two RCTs were excluded from the meta-analysis (24,27), since one study alone did not provide enough data to be tested. In particular, exact numbers of patients with NSCLC with each *EGFR* SNP genotype were not reported and the study presented only pre-calculated data for OR (24). The aforementioned RCTs obtained low NOS scores in the quality analysis, whereas the other studies included in the present meta-analysis had relatively good scores. Also, the results of the present meta-analysis were not adjusted for other factors (i.e., demographic factors), although the majority of the studies did not report ethnicity for the examined subjects. Potential bias in the results may be due to the absence of a consensus in the literature of an exact number of CA repeats when reporting short vs. long alleles. The linkage disequilibrium between examined SNPs was not taken into account and selection bias may be present.

In conclusion, the results of the present meta-analysis revealed that out of nine *EGFR* SNPs related to TKI side effects, rs11568315, rs712829 and rs712830 were associated with skin toxicity. NSCLC carriers of long CA repeats (rs11568315, SL + LL) were more likely to develop TKI-associated skin toxicity than short CA repeats (rs11568315, SS). To establish clear inter-individual benefits of TKI therapy, future RCTs that include a broader genetic panel are required to determine genetic susceptibility to TKI-induced toxicity in patients with NSCLC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Conceptualization and supervision of the study were conducted by VJ. The selection of papers, formal analysis, investigation and writing were conducted by JO. The acquisition of data was conducted by JT. All authors have read and approved the final manuscript. JO, JT and VJ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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