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Original Article

EGFR-216G/T polymorphism as a predictor of clinical outcomes in advanced non-small cell lung cancer patients treated with EGFR-TKIs: a meta-analysis

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Abstract: Epidermal growth factor receptor gene -216G/T polymorphism has been implicated to be associated with clinical outcomes in advanced non-small cell lung cancer (NSCLC) patients treated with EGFR-TKIs. However, the results are inconsistent due to the limitation of sample size. Based on previous studies; we conducted this meta-analysis aiming to provide more reliable results. A total of 6 studies, including 955 advanced NSCLC patients treated with EGFR-TKIs, were included. Review Manager 5.3 was used to perform the statistical analysis. The response rate (RR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) were included in our outcomes. Our study suggested that TT+GT genotypes showed association with higher response rates (GT+TT vs. GG RR = 2.08; 95% CI = 1.53-2.82; P<0.00001), higher disease control rate (GT+TT vs. GG RR = 1.23; 95% CI = 1.08-1.40; P = 0.002), longer progression-free survival (GT+TT vs. GG HR = 0.80; 95% CI = 0.67-0.95; P = 0.009) and longer overall survival (GT+TT vs. GG HR = 0.80; 95% CI = 0.66-0.96; P = 0.01) than GG homozygote. The combined results based on all included studies suggested that EGFR-216G/T polymorphism could be a potential biomarker for EGFR-TKIs in advanced NSCLC patients.

Keywords: EGFR, 216G/T, polymorphism, NSCLC, EGFR-TKIs

Introduction

Lung cancer is the main cause of cancer-related death around the world, especially in China [1]. Non-small cell lung cancer (NSCLC) which include squamous cell carcinoma, adenocarcinoma, and large cell carcinoma sub-types accounts for about 85% of all lung cancers [2]. Unfortunately, approximately 80% of NSCLC patients are diagnosed as advanced stages of the disease [3]. Chemotherapy is still the main treatment option. First line chemotherapy for advanced NSCLC consists of two drugs-based on the platinum group. However, traditional cytotoxic chemotherapies are associated with significant toxicity and less effective with a low 5-year survival rate ranging from 1% to 14% at advanced stages of NSCLC [4]. The median progression-free survival time (PFS) for chemotherapy-treated advanced NSCLC patients is 4-6 months and median overall survival (OS) is 10-12 months [5].

Tyrosine kinase inhibitors (TKIs), targeted drugs of epidermal growth factor receptor (EGFR), have shown antitumor activity in 4% to 27% of unselected NSCLC patients [6]. In selected populations harboring EGFR-activating mutations, the response rates ranged from 60% to 82% [7, 8]. Moreover, advanced NSCLC patients harboring EGFR mutations with EGFR-TKIs therapy could achieved a progression-free survival of 10 months and an overall survival of 24 months, suggesting that TKIs could be a better choice than chemotherapy in selected patients [9].

It has been reported that EGFR gene expression and activity is related with clinical outcomes in EGFR-TKI treated NSCLC patients [10-12]. EGFR single nucleotide polymorphisms 216G/T (rs712829) were found in the essential promoter area, which is located in a Sp1 recognition site [13, 14]. As an important binding site of the transcription factor SP1, 216G/T can influence the activation of EGFR promoter [15, 16]. Both vivo and vitro experiments have demonstrated that -216G/T polymorphism could influence EGFR expression and activity. The replacement of G by T at position 216 increases the promoter activity by 30% [17]. Numerous

studies have been performed to find whether -216G/T polymorphisms of the epidermal growth factor receptor gene were associated with clinical outcomes in advanced NSCLC patients treated with EGFR-TKIs [18-23].

As we know, a single study could have low power to find overall effects and meta-analysis revealing the association between EGFR-216G>T polymorphism and clinical outcomes of advanced NSCLC patients with EGFR-TKIs therapy has not been conducted. We carried out this meta-analysis aiming to show that EGFR-216G>T polymorphisms may be a potential biomarker for EGFR-TKIs therapeutic strategies.

Methods

Search strategy

All published literatures up to November 30, 2015 was systematically searched via electronic databases. We searched Embase, PubMed, Cochrane Library and CNKI, using the terms "epidermal growth factor receptor" or "EGFR", "polymorphism" or "variation", "Non-small cell lung cancer" or "NSCLC", "EGFR-TKIs" or "Erlotinib" or "Gefitinib" or "Icotinib". We only searched studies conducted on human subjects and no studies were excluded due to the languages. Furthermore, we perused the reference of the literatures found as candidate articles. All retrieved literatures were merged using Endnote X7 and duplicates were removed.

Inclusion and exclusion criteria

The inclusion criteria for this meta-analysis were: 1) patients with histopathologically confirmed advanced NSCLC; 2) EGFR-TKIs based therapies; 3) comparisons of clinical Outcomes among different genotypes of EGFR-216G>T polymorphism. 4) studies that contained sufficient data for meta-analysis. The exclusion criteria: 1) repeated or overlapping studies; 2) cell lines studies in vitro; 3) no usable data reported.

Data abstraction

We abstracted the following data from the included studies: the author's name, journal and year of publication, study population, ethnicity, previous treatment, the dosage and duration of TKIs treatment, available genotype and treatment outcomes.

Quality assessment

We assessed the quality of the study in descriptive and qualitative methods rather than a quantitative one, which were greatly consistent with REMARK guidelines [24].

Statistical analysis

The response rate (RR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) were included in our outcomes. Disease control rate (DCR) was defined as the rate of complete response rate (CR), partial response rate (PR) and stable disease rate (SD), while response rate (RR) only include complete response rate and partial response rate. PFS is calculated from the first day of EGFR-TKI treatment until tumor progression. Overall survival (OS) was defined as the time from the start date of TKI therapy to the date of death or final follow-up. The risk ratio (RR) with 95% CI was used to evaluate the relationship between these polymorphisms and response rate, disease control rate. Hazard ratios (HRs) with their 95% CIs were calculated to give an effective value for the quantitative aggregation of survival results.

A total of three genetic models GG, GT and TT were considered in this meta-analysis. The genetic model used to evaluate pooled RR or HR of the above three polymorphisms was GT+TT versus GG homozygote. Statistical pooling was conducted using the Review Manager5.3. Q test was used to check the Heterogeneity assumption with a conservative $P<0.1$ for the Q test threshold to indicate heterogeneity. If there is no heterogeneity between studies, we used fixed effects model; In case of heterogeneity, a random-effect model was performed. Publication bias was assessed using funnel plot .The significance of the pooled RR or HR was determined by the Z-test, and $P<0.05$ was considered statistically significant.

If necessary statistical date were not given clearly in an article, we assessed them from available data by the methods introduced by Tierney [25].

Results

The initial search identified 358 results from the PubMed, Embase, Cochrane Library and CNKI. Among them, 63 were duplicates. After

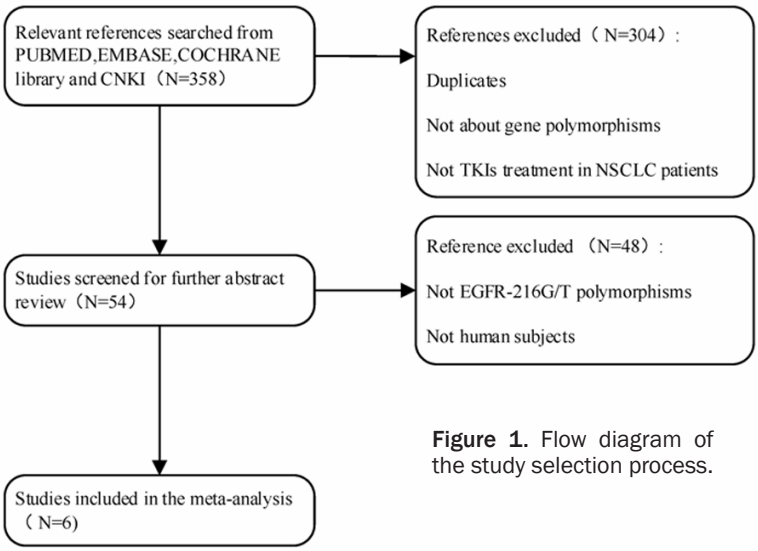

Figure 1. Flow diagram of the study selection process.

reading the titles or abstract, 241 articles were excluded because they did not relate to the correlation between gene polymorphism and clinical outcomes of non-small-cell lung cancer patients treated with EGFR-TKI. 54 studies were screened for further full text review and 48 studies were excluded because they did not include polymorphisms of interest or not human subjects. The selection process is summarized in the flow diagram shown in **Figure 1**. Finally, six studies were included in the final analysis according to the inclusion criteria [18-23]. The Main parameters for all eligible studies were presented in **Table 1**.

EGFR-216G/T polymorphism and clinical response to EGFR-TKIs in advanced NSCLC

There are four studies concerning the predictive value of EGFR-216G/T with respect to the sensitivity of advanced NSCLC to TKIs based treatment, which include 535 individuals. The data suggested that the GT+TT genotypes were more associated with better response rate than the GG homozygote (GT+TT vs. GG RR = 2.08; 95% CI = 1.53-2.82; P<0.00001) (**Figure 2**). In regard to disease control rate, GT+TT genotypes were also associated with better rate than GG genotype (GT+TT vs. GG RR = 1.23; 95% CI = 1.08-1.40; P = 0.002) (**Figure 3**). The combined results suggested that advanced NSCLC patients harboring T allele of EGFR-216G/T polymorphism inclined to have a better clinical response with EGFR-TKI treatment.

EGFR-216G/T polymorphism and PFS

Five studies focus on the association between EGFR-216G/T polymorphism and progression-free survival. 859 individuals were covered in this combined analysis. Pooled analysis indicated that TT+GT genotypes showed association with longer progression-free survival (GT+TT vs. GG HR = 0.80; 95% CI = 0.67-0.95; P = 0.009) than GG homozygote (**Figure 4**).

EGFR-216G/T polymorphism and OS

Data on overall survival (OS) was available in four of the studies. Among them, Jung's research which did not include the TT genotype was excluded because of the great heterogeneity (I^2 = 70%) it introduced. The pooled HR for the remaining three research was 0.80 (95% CI 0.66-0.96) (**Figure 5**), indicating that TT+GT genotypes showed association with longer overall survival than GG homozygote. More high quality studies are wanted to evaluate the relationship between EGFR-216G>T polymorphism and the overall survival.

Publication bias

For all comparisons, the funnel plots did not indicate any significant publication bias (**Figure 6**).

Discussion

In clinical practice, EGFR-TKIs have shown great efficacy in advanced NSCLC patients [26]. However, the clinical outcomes of TKIs treatment are considerable variability [27]. It is reported that EGFR-TKIs are more effective in some clinical features, such as adenocarcinoma subtypes, female sex, non-smoker status, and Asian populations [28]. Currently, the most accurate predictor of EGFR-TKIs could be EGFR mutation. Somatic mutations in exons 18-21 of the EGFR gene have been identified to account for the increased sensitivity to TKIs [29]. Unfortunately, only 10-30% of NSCLC patients possess an EGFR gene mutation. Worse still,

Table 1. Main characteristics for all eligible studies

Study	Year	Ethnicity	Patients included, n	Clinical stage	TKIs
Liu et al.	2008	Caucasian	92	IIIB or IV	Gefitinib
Giovannetti et al.	2010	Caucasian	96	IIIB or IV	Gefitinib
Jung et al.	2012	Asian	71	III or IV	Gefitinib or erlotinib
Li et al.	2014	Asian	135	III or IV	Erlotinib
Winther et al.	2015	Caucasian	331	IV	Erlotinib
Zhang et al.	2015	Asian	230	IIIB or IV	Erlotinib or gefitinib or icotinib

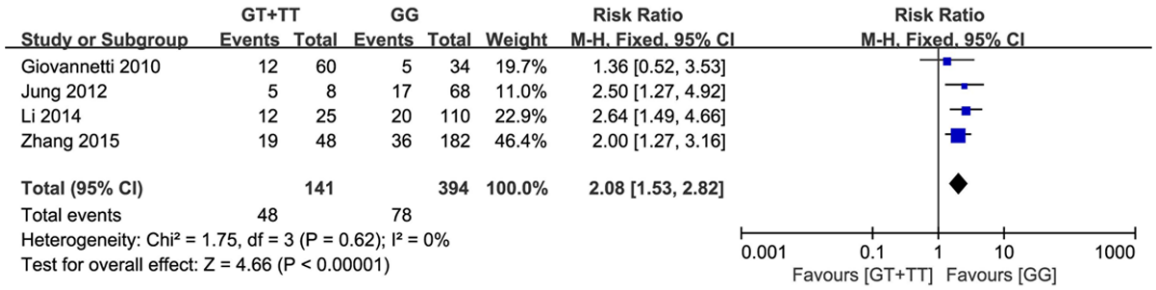

Figure 2. Association between EGFR-216G/T polymorphism and response rate of TKIs.

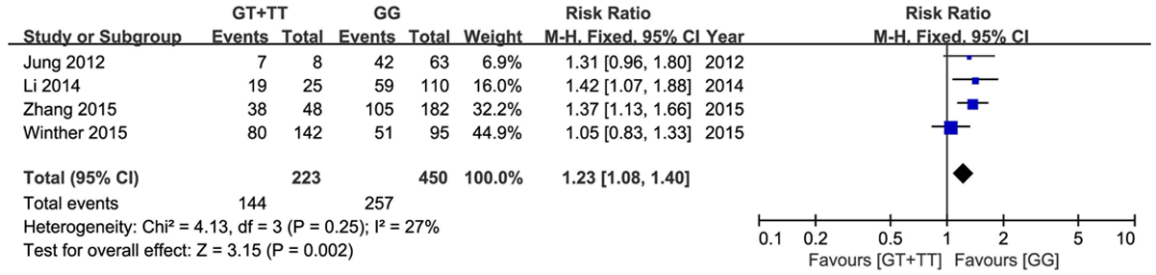

Figure 3. Association between EGFR-216G/T polymorphism and disease control rate of TKIs.

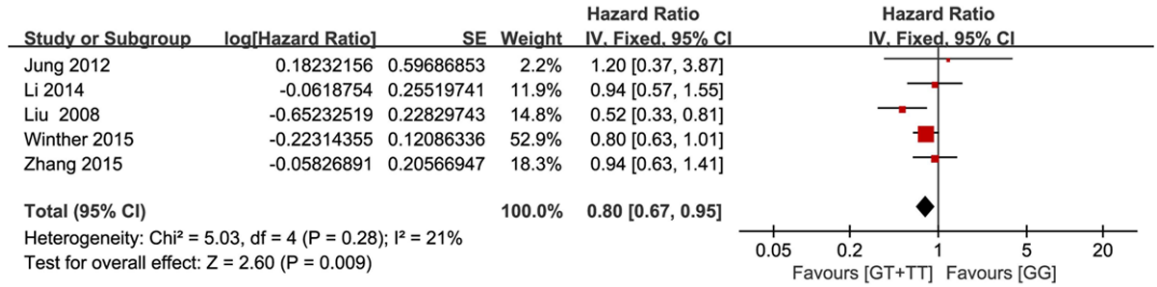

Figure 4. Forest plots of PFS associated with EGFR-216G/T polymorphism.

detecting the EGFR mutation status is not easy because of limited amount of tumor tissue. More clinical and molecular biomarkers affecting the sensitivity and resistance to EGFR inhibitors are demanded.

As an attractive target for treatment and prevention of cancer, EGFR overexpression has been associated with adverse disease stage, prognosis, survival, and response to chemotherapy in NSCLC. Interestingly, a growing number of studies showed that EGFR genetic polymorphisms could be potential predictive biomarkers of TKIs treatment [30-33]. The most studied EGFR polymorphisms include an intron 1 CA simple sequence repeat (CA-SSR) [30, 31,

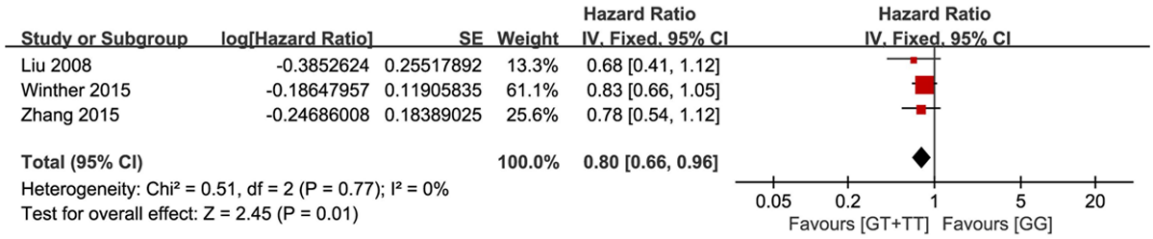

Figure 5. Forest plots of OS associated with EGFR-216G/T polymorphism.

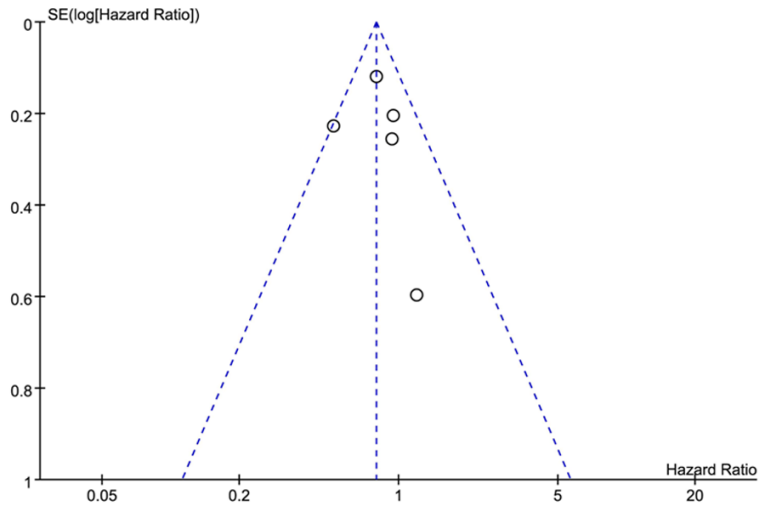

Figure 6. Funnel plot for publication bias.

33], rs2293347G/A [32, 34], -216G>T [18-23] and so on. As minimally invasive biomarkers, gene polymorphisms identified will be very helpful in clinics.

216G>T is a promoter SNP located in the binding site for the transcription factor Sp1, which could increase EGFR expression and activity in vitro and vivo. 216G/T is not linked to other polymorphisms in this region, reflecting that 216G/T might have independent function. Furthermore, it has been shown that -216G/T may be associated with inherited susceptibility to cancers, as well as other common diseases [35]. Some studies evaluating the association between EGFR-216G>T polymorphisms and efficacy of TKIs treatment have been published. In addition, EGFR-216G>T was also associated with the appearance of skin rash due to the EGFR inhibitors [36]. However, owing to the limitation of sample size, these studies can't get consistent conclusions. Meta-analysis can pool the result of every single study and overcome the restrictions of sample size.

Our study suggests that TT+GT genotypes showed more association with higher response rates (GT+TT vs. GG RR = 2.08; 95% CI = 1.53-2.82; P<0.00001), higher disease control rate (GT+TT vs. GG RR = 1.23; 95% CI = 1.08-1.40; P = 0.002), longer progression-free survival (GT+TT vs. GG HR = 0.80; 95% CI = 0.67-0.95; P = 0.009) and longer overall survival (GT+TT vs. GG HR = 0.80; 95% CI = 0.66-0.96; P = 0.01) than GG homozygote.

Several limitations should be noted in our study. Firstly, some studies had small sample sizes and different previous treatments. Secondly, meta-analysis based on a limited number of studies might reduce the chance of detecting publication bias. Thirdly, we did not pay attention to the potential interactions between -216G/T and other single nucleotide polymorphisms. Moreover, our result was not adjusted by other factors like gender, age, smoking status, EGFR mutation status, histological type and so on.

In conclusion, this meta-analysis showed that EGFR-216G/T polymorphism might be a potential biomarker for EGFR-TKIs in advanced non-small-cell lung cancer patients.

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Disclosure of conflict of interest

None.

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