RESEARCH ARTICLE

Association of CYP2C19 Polymorphisms with Survival of Breast Cancer Patients Using Tamoxifen: Results of a Meta-analysis

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Abstract

Background: Previous studies accessing the association of CYP2C19 with outcomes of patients using tamoxifen for breast cancer have yielded conflicting results. The aim of this meta-analysis is to obtain a more precise estimate of effects of CYP2C19 polymorphisms and to clarify their effects on survival of the breast cancer patients using tamoxifen. Materials and Methods: A systematic search of PubMed and Embase was performed, comparing patients with or without CYP2C19*2 and CYP2C19*17, relevant articles searched for. The following outcomes were included from the eligible studies: disease-free survival (DFS) and overall survival (OS), expressed by hazard ratios (HR) with corresponding 95% confidence interval (CI). Subgroup analysis by genotypes was also performed. Pooled estimates were calculated using random-effect model in accordance to the heterogeneity. Results: Six studies met the inclusion criteria. The integrated OR on the association between CYP2C19 and DFS, calculated by the random-effect model, was 0.54 (95%CI=0.34-0.84, *p*=0.013). Subgroup analysis showed that both CYP2C19*2 and CYP2C19*17 were associated with increased survival. The pooled results of two studies for OS were OR=0.46 (95%CI=0.21-1.01, *p*=0.233). Conclusions: This meta-analysis suggests that the CYP2C19*2 and CYP2C19*17 genotypes are associated with increased survival in breast cancer patients using tamoxifen.

Keywords: Breast cancer - CYP2C19*2 - CYP2C19*17 - survival - tamoxifen - meta-analysis

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Introduction

Breast cancer is a well-known important disease which is health-related, and its incidence is still rising (Darakhshan et al., 2013; Donnelly et al., 2013). Over 14% of female death is due to breast cancer, which is the leading cause of death in women (Parkin et al., 2005; Ibrahim et al., 2012). However, approximately 30% – 50% of estrogen receptor (ER)-positive breast cancer patients do not respond to tamoxifen treatment (Osborne et al., 1998).

Tamoxifen is the most commonly prescribed and widely used treatment for the prevention of ER-positive breast cancer (Fisher et al., 2005; Colleoni et al., 2006). It is metabolized by the pathway: the pro-drug, tamoxifen, is converted into N-desmethyl tamoxifen, 4-hydroxy-tamoxifen (4-HT) and the 4-hydroxy-N-desmethyltamoxifen (endoxifen), which is the result of the oxidation of the N-desmethyl tamoxifen, 4-hydroxy-tamoxifen (4-HT) (Desta et al., 2004). The evidence suggests that the metabolites 4-hydroxy-tamoxifen (4-HT) and 4-hydroxy-N-desmethyltamoxifen (endoxifen) are the active metabolites. They have at least 100-fold higher potency, in terms of binding to ER and suppression of breast cancer cell proliferation (Stearns et al., 2003; Desta et al., 2004; Johnson et al., 2004).

CYP2C19 is an important enzyme, by converting tamoxifen into 4-HT. CYP2C19 was divided into five groups: *2/*2 were homozygous for CYP2C19*2; *1/*2 were heterozygous for CYP2C19*2. CYP2C19*1 (homozygous wild type allele), CYP2C19*2/*17 and *1/*17 were heterozygous for CYP2C19*17. In addition, *17/ *17 were homozygous for CYP2C19*17 (Jennifer et al., 2010). Two genotypes of CYP2C19 have been analyzed in this study. The most common defect, CYP2C19*2 has a minor allele frequency of 13% in healthy Caucasians (Tamminga et al., 2001). The second defect, CYP2C19*17 is ultra-active, which has around 20% minor allele frequency (Li-Wan-Po et al., 2010).

To date, we explored potential effect outcomes of the association between CYP2C19*2, CYP2C19*17, tamoxifen and survival of breast cancer. However, the results were often conflicted and based on small sample size of the cohorts. Therefore, we conducted this meta-analysis to explore a possible estimation of the associations.

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Materials and Methods

Search strategy

Eligible articles were identified by a search of PubMed and Embase database using the following keywords: "breast cancer", "tamoxifen", "CYP2C19" and the last search updated on February 18, 2013. There was no limitation to the language restrictions. Furthermore, to find the additional studies, we also used the referenced lists of all the relevant studies.

Selection criteria

Studies were included if they met the following criteria for further meta-analysis: (1) patients who received tamoxifen with breast cancer; or (2) evaluated the function of CYP2C19*2 or CYP2C19*17; and (3) assessed disease-free (DFS), recurrence-free (RFS) or OS.

Data extraction

Two investigators (Lan Bai and Juan He) independently performed data according to the included studies. The following information was extracted from each study: first author's surname, year of publication, study location, participants, genotypes compared, and the data of disease-free survival (DFS) and overall survival (OS).

Assessment of study quality

Study quality was assessed by the Newcastle-Ottawa Scale (Stang et al., 2010).

Statistical analysis

Summary hazard ratios (HRs) with their 95% confidence interval (CI) were used to assess the association between the CYP2C19 genotype and the survival in breast cancer patients. It was the dichotomous data. The I^2 statistic and the *p* value were used to assess between-study statistical heterogeneity (Higgins et al., 2003). The data of OR were computed using the random effects model. DFS and OS were defined as the primary outcomes and OR as secondary outcome. For evaluating the influence of the results, one-way was performed to evaluate the sensitivity analyses with the influence of individual study on pooled estimates. We performed subgroup analysis by genotypes. The potential publication bias was tested using Egger's funnel plot with pseudo 95% confidence limits. All meta-analyses were conducted with STATA version 12.0 (StataCorp LP, College Station, TX, USA).

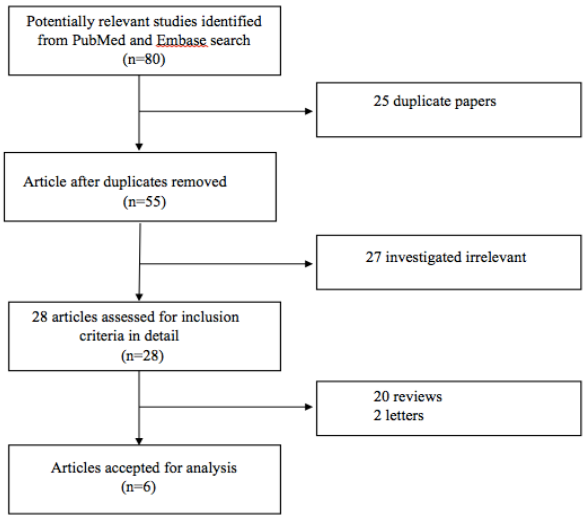

Figure 1. Flow Diagram of the Study Selection Process

Results

Search results and study characteristics

The initial search strategy identified 27 studies in PubMed and 53 articles in Embase, respectively (Figure 1), of which 74 were excluded (25 were duplicate papers, 27 investigated irrelevant outcomes, 20 were reviews, 2 were letters). A total of 6 studies (Werner et al., 2007; Masatsugu et al., 2008; Rikje et al., 2010; Ann et al., 2011; Ron et al., 2011; Beelen et al., 2013) were employed in this meta-analysis (Table 1). Of these, two studies (Werner et al., 2007; Rikje et al., 2010) were eligible for OS, and five studies (Werner et al., 2007; Masatsugu et al., 2008; Ann et al., 2011; Ron et al., 2011; Beelen et al., 2013;) for DFS. We extracted the OS and DFS from one study (Werner et al., 2007). Characteristics of these studies were summarized in Table 1.

Of all the above, six studies were published as full papers (Werner et al., 2007; Masatsugu et al., 2008; Rikje et al., 2010; Ann et al., 2011; Ron et al., 2011; Beelen et al., 2013). Sufficient data were available according to the studies. Therefore, there was no need to contact specific authors.

There was significant between-study heterogeneity (I^2=68.3%, *p*=0.013, Figure 2) for the analysis of five studies in DFS. Furthermore, there was moderate heterogeneity of OS (I^2=29.7%, *p*=0.233, Figure 3). Overall, we did not reveal the evidence of obvious asymmetry from the shapes of the funnel plots.

Association between CYP2C19 genotype and DFS

The study HRs ranged from 0.20 to 0.93 in five studies (Werner et al., 2007; Masatsugu et al., 2008; Ann et al., 2011; Ron et al., 2011; Beelen et al., 2013). Pooled data did

Table 1. Characteristics of Studies Included in Meta-Analysis

Trial	Year	Study Location	No. of patients	Study quality	Genotypes compared	DFS		OS	
						HR	95%CI	HR	95%CI
Beelen	2013	Netherlands	99	good	wt vs homo*2 or hete*2	0.26	0.12-0.55	NS	NS
Moyer	2011	USA	190	fair	*wt/*17 and *17/*17	0.93	0.64-1.37	NS	NS
Schroth	2007	Germany	206	good	*wt/*wt vs *wt/*17 *17/*17	0.58	0.32-1.01	0.61	0.29-1.26
Ruiter	2010	Netherlands	80	fair	*wt vs *2/*2	NS	NS	0.26	0.08-0.87
Okishiro	2009	Switzerland	132	poor	*wt/*wt vs wt/*2,wt/*3	0.2	0.13-1.55	NS	NS
Schaik	2011	Caucasian	80	fair	*wt/*17 and *17/*17	0.66	0.46-0.95	NS	NS

*DFS disease-free survival - OS overall survival - HR hazard ratio - wt wild-type - NS not specified - Homo homozygous - Hete heterozygous

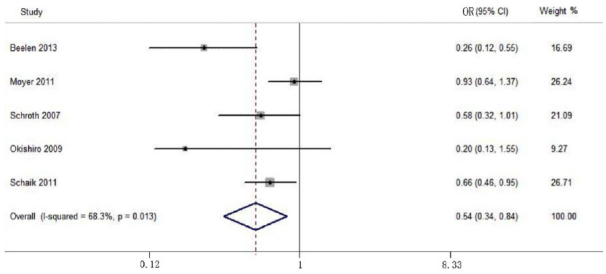

Figure 2. Forest Plot of Meta-Analysis of Disease-Free Survival

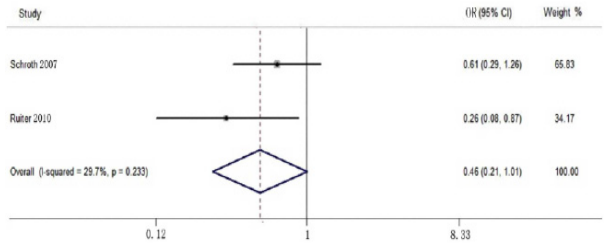

Figure 3. Forest Plot of Meta-Analysis of Overall Survival

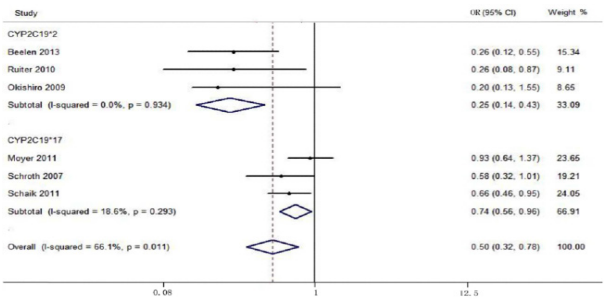

Figure 4. Forest Plot of Meta-Analysis of CYP2C19*2, CYP2C19*17 and Survival

not show a significant trend toward an increase survival of disease OR of 0.54 (95% CI=0.34 - 0.84, Figure 2), with a moderate heterogeneity (I^2=68.3%, p=0.013, Figure 2).

Association between CYP2C19 genotype and OS

We pooled two studies (Werner et al., 2007; Rikje et al., 2010) for this analysis. The data of HRs ranged from 0.26 to 0.61 in the studies. Pooling of data from two studies did not show toward to increase survival of disease with an OR of 0.46 (95% CI=0.21 - 1.01, Figure 3). With a moderate heterogeneity (I^2=29.7%, p=0.233).

Association between CYP2C19 genotype and survival

There was no evidence of significant heterogeneity for the total (95% CI) of the studies (P=0.011). Pooling of data from CYP2C19*2 showed trend toward an increase survival of disease with HR of 0.25 (95% CI=0.14 - 0.43, Figure 4). No heterogeneity was found among the different studies. (I^2=0%, p=0.934). The data from CYP2C19*17 showed trend toward an increase survival of disease with HR of 0.74 (95% CI=0.56 - 0.96, Figure 4). The data suggested a low heterogeneity (I^2=18.6%, p=0.293).

Discussion

The overall results of this meta-analysis focused on the function of CYP2C19 genotype for survival of the breast cancer patients using tamoxifen. No meta-analysis has been performed, and our meta-analysis is the first one on this association. In addition, a sensitivity analysis was performed by removing one study at a time to reflect the influence after excluding the individual study. Estimates changed quite little, suggesting that the result is stable in this meta-analysis.

The exact biological mechanism of the function of CYP2C19 is understood. Previous studies suggest that CYP2C19 is involved in the conversion of tamoxifen to its active metabolites (Crewe et al., 2002; Boocock et al., 2002; Stearns et al., 2003; Desta et al., 2004; Jin et al., 2005). 4-HT has estrogen receptor more markedly affinity than tamoxifen (Johnson et al., 2004). Furthermore, prior studies suggest that 4-HT is metabolized by CYP2C19, which is the main metabolized product. These findings (Ruiter et al., 2009; Justenhoven et al., 2009; Lu et al., 2012) about the common associations of CYP2C19 and tamoxifen suggest that CYP2C19 genotypes play an important role in women suffering from breast cancer. It is well documented that the patients who are post-menopausal ER positive would get more benefit from receiving tamoxifen than pre-menopausal ones (Sukasem et al., 2012).

In this meta-analysis, significant between-study heterogeneity was detected (I^2 = 68.3%, p = 0.013) for the analysis of five studies in DFS. Furthermore, there was moderate heterogeneity of OS (I^2 = 29.7%, p = 0.233). The results can be influenced by the ways of the experiment of the studies, the groups of the population, environmental exposures and sample size. As a measure of heterogeneity between these confounding factors or some unknown factors. Due to limited number of studies and sufficient information heterogeneity, our ability was limited to detect heterogeneity by subgroup analysis. Heterogeneity was assessed by I^2 statistic among studies, which is defined for low (< 25%), moderate (~ 50%), and high (> 75%) heterogeneity (Higgins et al., 2003). It is possible that other limitations of these studies may partially contribute to the heterogeneity. For this reason, the random-effect model (DerSimonian Laird method) was conducted when there exists a significant I^2 >50%. In our meta-analysis, Funnel plot and Egger's test were used to test the publication bias. The results suggest that the publication bias affects on the results in this study and the results of our meta-analysis are not relatively stable.

Our meta-analysis suggested that the CYP2C19 genotype was associated with DFS, but not associated with OS. This result indicated that the CYP2C19 genotype is associated with the survival in breast cancer patients, which are consistent with previous studies. Zafra-Ceres et al. (Zafra-Ceres et al., 2013) demonstrated that CYP2C19*2 can serve as a predictive factor for survival in breast cancer patients treated with tamoxifen in the Spanish population (Schaik et al., 2011). Ruiter et al. (Ruiter et al., 2010) also suggests that CYP2C19 genotype may possibly be a predictive factor for survival in breast cancer patients after using tamoxifen. In the current study, we also performed subgroup analyses based on genotype, to further evaluate the value of CYP2C19 genotype for survival in breast cancer patients who received tamoxifen.

It has even been suggested that CYP2C19 genotype may possibly be a predictive factor for survival in breast cancer patients who used tamoxifen.

Limitations of the Meta-analysis

Other potential limitations should be taken into account in this meta-analysis. First, the selection bias were not avoided since our study was reported in English and identified in PubMed and Embase. Second, the studies of the meta-analysis were often small-scale, and patient selection bias may be possible. Third, publication bias was not investigated. Although this bias can be estimated using funnel plot in STATA. Thus, the likelihood of bias from published studies should be minimized.

In summary, this meta-analysis explores the association between CYP2C19 genotypes and the survival of breast cancer patients. Our results supporting the association of the CYP2C19*2 and CYP2C19*17 genotypes may possibly be a predictive factor for survival in breast cancer women. Previous Meta-analysis have not found a similar association between them. Because of the between-study heterogeneity in small size of cohorts, further larger scale studies should focus on investigating. There is an evidence suggesting that CYP2C19 genotypes might be useful in predicting tamoxifen efficacy in post-menopausal women. The ER positive postmenopausal patients with CYP2C19*17 variant will benefit from tamoxifen. Our meta-analysis supplies the way of the potential effect outcomes of the association between CYP2C19*2, CYP2C19*17, tamoxifen and survival of breast cancer patients. Hopefully, further studies can verify the results to clinical relevance in a larger population.

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