JBUON 2017; 22(5): 1209-1216
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Clinicopathological and prognostic significance of cyclin D1 amplification in patients with breast cancer: a meta-analysis

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Summary

Purpose: *Cyclin D1 plays a critical role in tumorigenesis and the regulation of the G1/S transition in the cell cycle. The relationship between cyclin D1 amplification and clinicopathological parameters in patients with breast cancer remains controversial and its impact on survival outcome is not completely clear. We conducted a meta-analysis to investigate the associations between cyclin D1 gene amplification and certain clinicopathological characteristics and the prognosis in breast cancer.*

Methods: *Literature search of PubMed (up to August 3, 2016) was performed. We used Stata 12.0 (Stata Corporation, Texas, US) to analyze the correlations between cyclin D1 amplification and clinicopathological features and the prognostic indicator relapse free survival (RFS) and overall survival (OS) in patients with breast cancer. Publication bias analysis and sensitivity analysis were performed.*

Results: *A total of 9,238 breast cancer patients from 21 studies were included. The pooled odds ratios (ORs) indicated that cyclin D1 amplification was significantly associated with estrogen receptor (ER), progesterone receptor (PR), histological grade and lymph node status, but not associated with human epidermal growth factor receptor-2 (HER2) and tumor size. The combined hazard ratios (HRs) for RFS and OS showed that patients with cyclin D1 amplification displayed a 1.31-fold higher risk of recurrence (HR =1.31, 95% confidence interval (95% CI):1.02-1.60, p<0.01), and a risk of mortality 1.22-fold higher times greater than those without cyclin D1 amplification (HR=1.22, 95% CI:0.99-1.44, p<0.01), respectively.*

Conclusion: *Our meta-analysis indicated that cyclin D1 amplification is significantly associated with established clinicopathological variables and can be used as a poor prognostic indicator for patients with breast cancer.*

Key words: *CCND1, estrogen receptor, histological grade, progesterone receptor, overall survival, relapse free survival*

Introduction

Breast cancer is one of the most common cancers in females. The incidence of breast cancer ranks first both in the United States and China and is expected to account for 29% and 15% respectively of all new cancers in females [1,2]. Although recent advances in multidisciplinary therapies have improved treatment outcomes, breast cancer is still one of the leading causes of cancer death among women. Therefore, a deep understanding of the molecular mechanisms underlying the pathogenesis of breast cancer and finding a marker of novel targeted drugs to predict therapeutic effect is essential.

The cyclin D1 gene, named CCND1 or PRAD1, located on chromosome 11q13, encodes the cell-cycle regulatory protein cyclin D1. Cyclin D1/cyclin D-dependent kinases 4 (CDK4) play a critical role in the regulation of the G1/S transition through regulation of the phosphorylation of the retinoblastoma protein (pRb) resulting in the release of transcription factors such as E2F-1 that then allow the transition from G1 to S phase and progression of the

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Received: 13/03/2017; Accepted: 27/03/2017

cell cycle [3]. Oncogene amplification is a common event for driving tumorigenesis. Cyclin D1 amplification is one of key alterations in the carcinogenetic process of breast cancer [4]. A previous study in breast cancer has reported that amplification of cyclin D1 and inactivation of RB are present in a significant fraction of breast carcinomas [5]. Furthermore, cyclin D1 gene appears to be a promising candidate therapeutic target in breast cancer. Recent studies [6,7] have shown that the cyclin D/CDK4/6 pathway has been identified as an attractive target in breast cancer. However, the clinical and prognostic significance of cyclin D1 gene status in breast cancer is not completely clear.

There are inconsistent data regarding the cyclin D1 amplification and clinicopathological features and prognosis. Several studies have shown that cyclin D1 amplification was not significantly associated with any clinicopathological characteristics [8-14], whereas other studies reported that the cyclin D1 amplification status was positively correlated with some clinicopathological parameters such as ER, PR, HER2, histological grade and lymph node status [15-23]. Whether discrepancy in these data was due to limited sample size or genuine heterogeneity is still unknown. To address the controversial issues, a meta-analysis was carried out to determine the associations between cyclin D1 amplification and clinicopathological parameters and survival outcome.

Methods

Search strategy

Eligible studies were identified to determine the associations between the cyclin D1 amplification and clinicopathological variables by searching the electronic literature of PubMed up to August 3, 2016, with the following terms: ("breast cancer" or "breast neoplasms" or "breast tumor") and ("cyclin D1"or "CCND1" amplification). Two authors independently completed the tasks, and if there were any disagreements, they were resolved by discussions among the other authors.

Inclusion and exclusion criteria

Eligible studies included had to meet the following criteria: (1) research should be evaluated in the primary breast cancer; (2) data should provide details about the sample size, the correlations between cyclin D1 amplification and at least 1 of 5 clinicopathological features or prognosis of breast cancer; (3) if there were multiple articles based on similar patients, only the largest or most recently published article was included. Studies were excluded from the meta-analysis when they (1) were reviews, letters, meta-analysis studies, comments, conference abstracts, or editorial articles; (2) not studies on humans; (3) duplicated data; (4) not published in English.

Data extraction

For each study, the following characteristics were extracted: the last name of the first author, year of publication, country, sample size, method of detecting the cyclin D1 amplification, cut-off value of definition of cyclin D1 amplification and clinicopathological parameters and survival data. In line with the clinically established cut-off value used for hormone receptor assessment, ER and PR statuses were considered positive if at least 10% of neoplastic cells showed clear nuclear staining. For HER-2 staining we used a scoring system according to ASCO/CAP 2007 guidelines [24]. Grading of tumors was done according to modified Bloom-Richardson Grading System and tumor size according to the American Joint Committee on Cancer (AJCC) TNM staging system [25]. If the HR or standard errors (SE) were not reported in included studies, we calculated or estimated the HR from available data or Kaplan-Meier curves using the methods reported by Tierney et al. [26].

Statistics

Statistical analyses were performed using the Stata 12.0 (Stata Corporation, Texas, US). The pooled ORs and HRs together with 95% CIs for dichotomous outcomes were calculated to assess the strength of the associations between the cyclin D1 amplification and clinicopathological variables and the impact on prognosis. All of the p values were two-sided, and $p<0.05$ was considered to be statistically significant.

Publication bias was investigated through funnel plots and tested using Egger's regression asymmetry test. We considered that publication bias was present if the intercept of the Egger's regression line deviated from zero with a two-sided p value <0.10.

Heterogeneity was tested using the x^2 test with significance being set at $p<0.10$. The total variation among studies was estimated by I^2. $I^2 > 50\%$ was considered to indicate significant heterogeneity. If there was heterogeneity among studies, we used a random effect model to pool the OR, otherwise, a fixed effect model was selected.

To analyze the potential sources of heterogeneity among studies that may be caused by geographical factors, the assay method and definition of cyclin D1 amplification, we also performed subgroup analysis.

A sensitivity study was performed to identify any individual study that significantly affected the overall estimates by omitting each study repeatedly and calculating the pooled estimates for the remaining studies.

Results

The basic characteristics of the eligible studies

A total of 9,238 patients were identified by the primary computerized literature search. After screening the titles and abstracts, 61 articles were further reviewed in detail. As indicated in the search flow diagram (Figure 1), 21 studies published from 1996 to 2016 were eligible for meta-

analysis. Cyclin D1 amplification was seen in 1367 of 9238 (14.8 %) patients with breast cancer. Their characteristics are summarized in Table 1. Most of the study populations were from Europe (n=15), and the remaining 6 studies were from Asia (n=4), North America (n=1) and Australia (n=1). Cyclin D1 amplification was evaluated by the method of fluorescence *in situ* hybridization (FISH) and non-FISH including chromogenic *in situ* hybridization (CISH), real-time quantitative polymerase chain reaction (PCR) and blotting hybridization. The definition of cyclin D1 amplification varied among the studies.

Cyclin D1 and clinicopathological features

No significant heterogeneity was observed in the analysis of cyclin D1 and any of the clinicopathological variables, except ER status (I^2=53.1%, p<0.01). Thus, the random-effects model was selected for ER status, while the fixed-effects model was selected for each of the remaining 5 clinicopathological features.

All included studies analyzed the association of cyclin D1 amplification with ER status of breast cancer. The combined OR revealed cyclin D1 amplification was significantly related to ER status (ER+ vs ER-, OR=1.95; 95% CI: 1.44-2.54, p<0.01; Table 2).

The association between cyclin D1 amplification and PR status was investigated in 15 studies and it was found that cyclin D1 amplification was different in PR+ and PR- patients with breast cancer (PR+ vs PR-, OR=1.49; 95% CI: 1.30-1.72; p<0.01; Table 2).

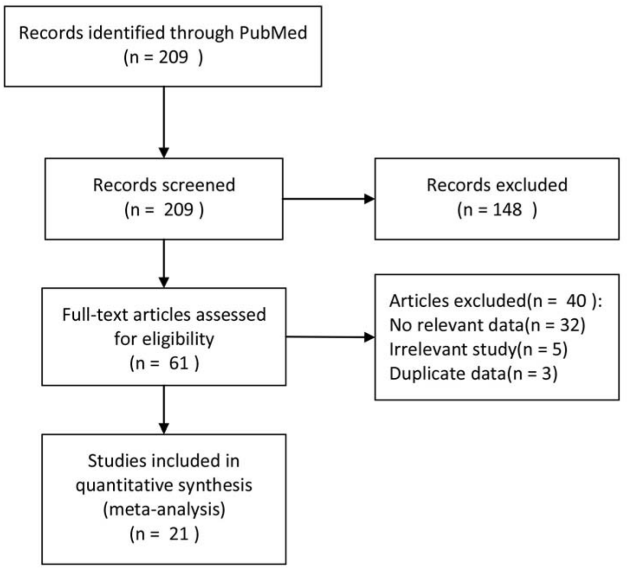

Figure 1. Flow chart for study selection.

Table 1. Characteristics of eligible studies

Study	*Year*	*Country*	*Cases*	*CyclinD1 amplification, n (%)*	*Method*	*Cut off*
Seshadri [8]	1996	Australia	1014	103 (10.2)	Slot-blot hybridization	2.0
Courjal [15]	1996	Italy	1164	146 (12.5)	Southern blotting	2.0
Barbareschi [9]	1997	Italy	53	13 (24.5)	Southern blotting	2.0
Bieche [16]	2002	France	134	15 (11.2)	Real-time PCR	2.5
Naidu [17]	2002	Malaysia	440	119 (27.0)	Differential PCR	1.5
Janssen [27]	2002	France	946	101 (10.7)	Southern blotting	2.0
Al-Kuraya [18]	2004	Switzerland	1785	358 (20.1)	FISH	2.0
Jirstrom [11]	2005	Sweden	280	44 (15.7)	FISH	2.0
Reis-Filho [12]	2006	UK	206	30 (14.6)	CISH	3.0
Mottolese [10]	2007	Italy	121	8 (6.6)	FISH	2.0
Bostner [19]	2007	Sweden	224	28 (12.5)	Real-time PCR	3.6
Elsheikh [20]	2008	UK	613	59 (9.6)	CISH	5.0
Kirkegaard [28]	2008	UK	115	17 (14.8)	FISH	2.0
Cho [13]	2008	Korea	95	13 (13.7)	CISH	5.0
Hadzisejdic [14]	2010	Croatia	112	15 (13.4)	FISH	2.0
Massidda [29]	2010	Italy	53	12 (22.6)	FISH	3.0
Bane [22]	2011	Canada	255	44 (17.3)	FISH	1.5
Mu [30]	2011	China	61	16 (26.2)	FISH	2.0
Quintayo [31]	2012	UK	1076	146 (13.6)	FISH	2.0
Burandt [21]	2016	Germany	133	28 (21.0)	FISH	2.0
Li [32]	2016	China	355	52 (14.6)	FISH	2.0

FISH: fluorescence in situ hybridization, CISH: chromogenic in situ hybridization, PCR: polymerase chain reaction

Six out of 19 studies evaluated the association of cyclin D1 amplification with HER2 status. The combined OR suggested that there was no relation between these two factors (HER2+ vs HER2-, OR=0.77; 95% CI: 0.53-1.11; p=0.16; Table 2).

Twelve studies examined the relationship between cyclin D1 amplification and histological grade. The outcome showed that cyclin D1 amplification was significantly different between grade I-II and grade III (grade I-II vs grade III, OR=0.83; 95% CI: 0.71-0.95; p=0.01; Table 2).

In 9 studies, the association of cyclin D1 amplification with tumor size was investigated. The combined OR revealed cyclin D1 amplification was not significantly related to tumor size (T1 vs T2-4, OR=0.92; 95% CI: 0.76-1.11; p=0.39; Table 2).

In 14 studies, the relationship of cyclin D1 amplification with lymph node metastasis was investigated and combined OR revealed cyclin D1 amplification was significantly related to lymph node metastasis (N- vs N+, OR=0.74; 95% CI:0.64-0.86; p<0.01; Table 2).

Cyclin D1 and prognosis

RFS

A total of 2,538 patients from 4 studies were recruited. The heterogeneity test for RFS indicated that a fixed effect model could be selected (I^2=0.0%, p=0.506). The meta-analysis showed that the pooled HR was 1.31 (95% CI:1.02-1.60) and statistical significance was identified in terms of the cyclin D1 amplification relative to the cyclin D1 non-amplification (p<0.01, Figure 2).

Table 2. Relationship between cyclinD1 amplification and the clinicopathological features

Clinicopathological features	*Statistical model and statistical methods*	*Heterogeneity*		*OR*	*95% CI*	*p value*
		p value	*I^2 (%)*			
ER+ *vs* ER-	Random-effects mode, D-L method	0.00	51.30	1.95	1.44-2.54	0.00
PR+ *vs* PR-	Fixed-effects mode, M-H method	0.12	30.00	1.49	1.30-1.72	0.00
HER2+ *vs* HER2-	Fixed-effects mode M-H method	0.18	33.90	0.77	0.53-1.11	0.16
Grade I-II *vs* Grade III	Fixed-effects mode, M-H method	0.60	0.00	0.83	0.71-0.95	0.01
T1 *vs* T2-4	Fixed-effects mode, M-H method	0.99	0.00	0.92	0.76-1.11	0.39
N- *vs* N+	Fixed-effects mode, M-H method	0.75	0.00	0.74	0.64-0.86	0.00

OR: odds ratio, CI: confidence interval, T: tumor size, N: lymph node, D-L method: Der Simonian Laird method [33]; M-H method: Mantel-Haenszel method [34]

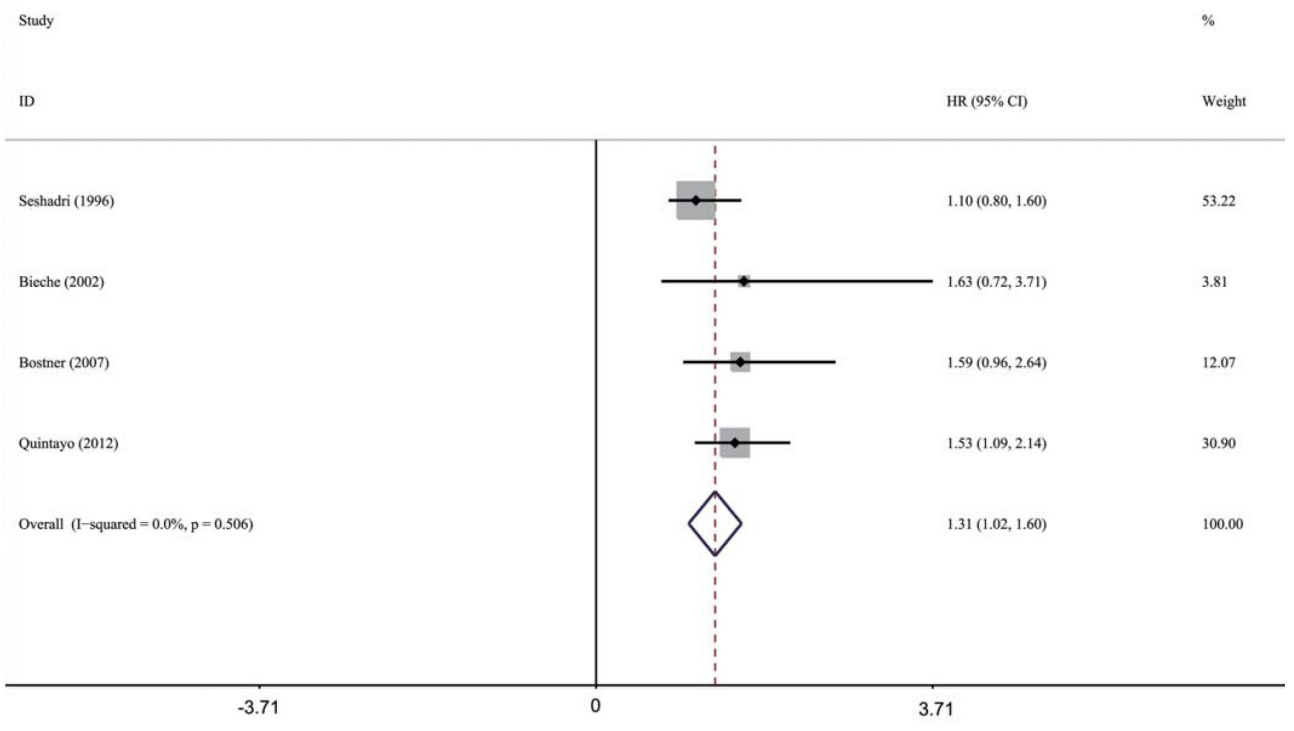

Figure 2. Forest plot of hazard ratio for relapse free survival of patients with breast cancer. The squares and horizontal lines correspond to the study- specific HR and 95% CI. The area of the squares reflects the study-specific weight (inverse of the variance). The diamonds represent the pooled HR and 95% CI. The solid vertical line is at the null value.

OS

A total of 3,786 patients from 6 studies regarding OS were incorporated into this meta-analysis. The heterogeneity test indicated that a fixed effect model could be selected (I^2=0.0 %, p=0.416). The pooled results showed that there was significant difference between the two groups (HR=1.22; 95% CI: 0.99 -1.44; p<0.01) (Figure 3).

Publication bias

Egger's regression asymmetry test was used to assess the publication bias of the studies. The result of Egger's test indicated no publication bias in this meta-analysis (p=0.43). The asymmetrical regression plot is shown in Figure 4.

Subgroup analysis

Subgroup analysis was used to evaluate the factors that might modify this association, such as continent, assay method and cut-off value. The outcomes of subgroup analysis (Table 3) indicated that these 3 factors could not explain the source of heterogeneity. None of the factors significantly affected the OR.

Sensitivity analysis

Sensitivity analysis was performed to evaluate the influence of each individual study on the pooled OR by sequential removal of individual studies. The results illustrated that our meta-analysis results were stable and reliable (Figure 5).

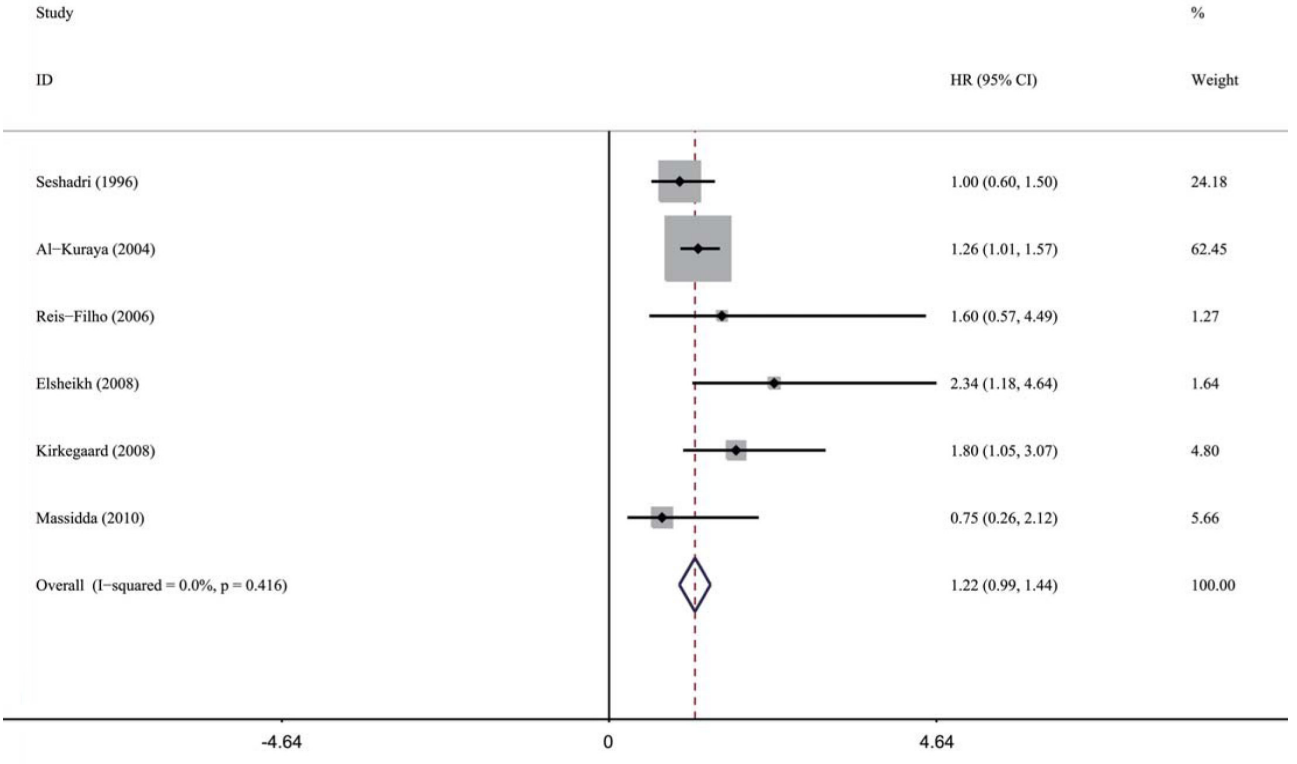

Figure 3. Forest plot of hazard ratio for overall survival of patients with breast cancer.

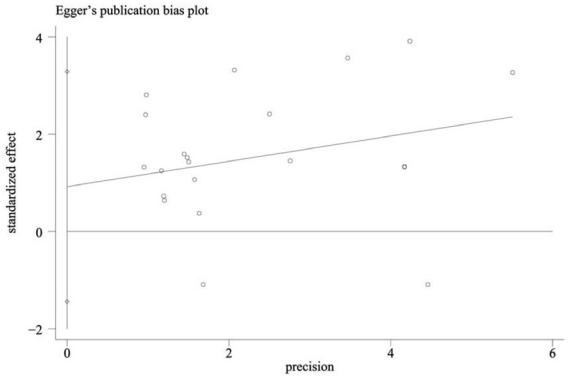

Figure 4. Asymmetrical regression plots to detect publication bias. Each point represents a separate study for the indicated association. For each study, the OR is plotted on a logarithmic scale against the precision (the reciprocal of the SE).

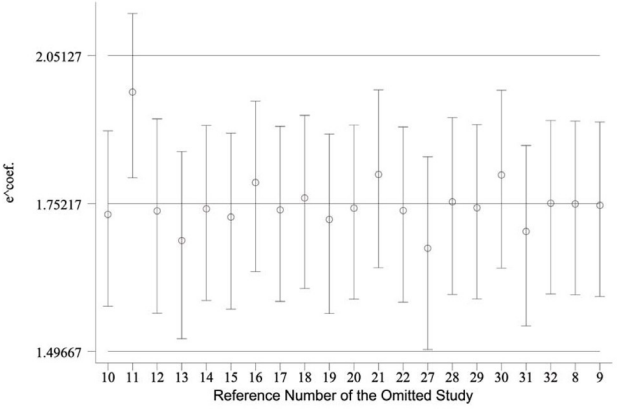

Figure 5. Sensitivity analysis graph. The pooled odds ratio and 95% CIs for each study are displayed on a logarithmic scale.

Table 3. Results of subgroup analysis

Subgroups	*Study*	*Heterogeneity*		*OR (95% CI)*	*p value*
		I^2 (%)	*p value*		
Continent					
Australia	1	NA	NA	1.37 (0.86-2.20)	0.185
Europe	15	5.7	0.388	2.13 (1.73-2.63)	0.000
Asia	4	81.5	0.001	1.52 (0.53-4.39)	0.441
North America	1	NA	NA	1.70 (0.83-3.45)	0.146
Overall	21	52.3	0.003	1.95 (1.50-2.55)	0.000
Method					
FISH	11	22.0	0.233	2.14 (1.57-2.93)	0.000
CISH	3	61.4	0.075	1.47 (0.55-3.92)	0.438
PCR	3	73.7	0.022	1.65 (0.46-5.90)	0.440
Blotting	4	37.3	0.188	2.07 (1.42-3.03)	0.000
Overall	21	52.3	0.003	1.95 (1.49-2.55)	0.000
Cut-off					
1.5	2	69.5	0.070	1.10 (0.52-2.30)	0.816
2	13	30.2	0.142	2.15 (1.66-2.77)	0.000
2.5	1	NA	NA	1.26 (0.38-4.17)	0.710
3	2	0.713	0.0	2.26 (0.83-6.16)	0.109
Other	3	77.7	0.011	2.16 (0.48-9.78)	0.317
Overall	21	52.3	0.003	1.95 (1.50-2.55)	0.000

Discussion

Cyclin D1 gene is a known oncogene and plays a critical role in tumorigenesis of breast cancer. There are disputed data in studies of breast cancer regarding the cyclin D1 amplification and clinicopathological characteristics. A total of 9,238 breast cancer patients were recruited from 21 relevant studies and the frequency of cyclin D1 amplification (14.7%) determined in this meta-analysis is in agreement with previous research [11-13].

Clinicopathological features have been reported to be associated with cyclin D1 amplification in breast carcinoma, but conflicting results were correlated with ER status and 8 studies failed to detect a connection [8,10,12,14,17,21,22]. Five studies reported significant association with histological grade [17,18,21-23], but 6 studies [8,12,14,16,20,29] could not find any positive relationship. While cyclin D1 amplification was found to be associated with lymph node status in previous studies [15,17], other studies could conversely not observe any link [16,19,20,22,23]. This meta-analysis indicated cyclin D1 amplification was significantly associated with ER and PR status, histological grade and lymph node metastasis. However, no statistically significant association was observed with HER2 status and tumor size. One of the studies found that amplification of cyclin D1 gene occurred more frequently in invasive ductal carcinomas, invasive lobular carcinomas and comedo ductal carcinoma *in situ* than in colloid carcinomas, medullary carcinomas and non-comedo ductal carcinoma *in situ* [17]. The inconsistency among studies may derive from different cut-off values [8] and the genetic alterations of cyclin D1-interacted genes, such as EGFR, BRCA-1 and BRCA-2 and histopathological type [17].

When we pooled the data together, no significant heterogeneity was observed in the analysis of cyclin D1 and any of the clinicopathological features, except the ER status. Furthermore, we carried out a subgroup analysis on ER-positive compared to ER-negative. The results in our study indicated that continent, testing method and definition of cyclin D1 could not explain the source of heterogeneity. The heterogeneity of studies could result from patients' age, menopause, specific histopathological type and genotype of breast cancer. We lacked sufficient raw data of the included trials. Should they be provided, it would be in favor of further analysis of heterogeneity. Sensitivity analysis demonstrated that the results of this meta-analysis were stable and reliable.

The meta-analysis of 6 studies evaluated cyclin D1 amplification as a prognosis factor and

revealed significant differences in RFS and OS between patients with and without cyclin D1 amplification. No significant heterogeneity was observed in the analysis.

Limitations of the present analysis should be acknowledged. First, the assay method and the definition of cyclin D1 amplification were various. Second, in the survival analysis, we calculated or estimated some of the HR from available data or Kaplan-Meier curves. Third, all the studies were restricted to papers published in English. Fourth, 15 of 21 included studies were performed in Europe and only 4 were carried out in Asia. We maintain that the present meta-analysis needs to be confirmed in a wider range of population.

Our meta-analysis suggested that cyclin D1 amplification was significantly associated with ER-positive, PR-positive, high grade and lymph node metastasis in patients with breast cancer. Amplification of cyclin D1 might be a prognostic biomarker for breast cancer. However, due to the above mentioned limitations, future studies evaluating the significance of cyclin D1 amplification on the clinicopathological characteristics and prognosis of breast cancer are strongly recommended.

Conflict of interests

The authors declare no confict of interests.

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