SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
BREAST CANCER
PROGNOSTIC MARKERS

Received
18 February 2014

Accepted
4 August 2014

Published
1 September 2014

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Prognostic role of *PIK3CA* mutations and their association with hormone receptor expression in breast cancer: a meta-analysis

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The phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) gene is frequently mutated in breast cancer (BCa). Sex hormone receptors (HRs), including estrogen receptor (ER) and progesterone receptor (PR) play pivotal roles in BCa. In this study, we evaluated the association between *PIK3CA* mutations and ER/PR expression and the prognostic role of *PIK3CA* mutations in BCa patients, and in particular, HR-positive BCa. Thirty-two studies involving 5719 cases of BCa obtained from database searches were examined. *PIK3CA* gene mutations correlated significantly with ER/PR expression ($p < 0.00001$) and relapse-free survival (RFS) (hazard ratio [HR] 0.76, 95% confidence interval [CI] 0.59–0.98, $p = 0.03$) but not overall survival (OS) (HR 1.14, 95%CI 0.72–1.82, $p = 0.57$) in unsorted BCa patients. *PIK3CA* mutations were not associated with OS (HR 1.06, 95%CI 0.67–1.67, $p = 0.81$) or RFS (HR 0.86, 95%CI 0.53–1.40, $p = 0.55$) in HR-positive BCa patients. In conclusion, *PIK3CA* mutations were significantly related to ER/PR expression and RFS in unsorted BCa patients. However, the clinical implications of *PIK3CA* mutations may vary according to different mutant exons. And *PIK3CA* mutations alone may have limited prognostic value for HR-positive BCa patients.

Breast cancer (BCa) is one of the most common cancers among women, with more than 1,300,000 new cases and about 450,000 deaths reported each year worldwide[1]. This highly heterogeneous disease is divided into subgroups on the basis of molecular signatures, clinicopathologic features, and responses to therapy. Hormone receptors (HRs), including estrogen receptors (ERs) and progesterone receptors (PRs) are the most important markers of BCa. Most BCa cases are HR-positive (HR+), and ER-positive (ER+) BCa accounts for up to 80% of BCa cases among women 45 years and older[2,3]. Endocrine therapy is regarded as the cornerstone of ER+ BCa treatment. However, because of de novo or acquired resistance to endocrine therapy, prognosis is still poor for many ER+ BCa patients. Therefore, finding new effective treatment methods for ER+ BCa patients resistant to endocrine therapy is imperative.

After the *TP53* gene, the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) gene is the most frequently mutated gene in BCa. Phosphatidylinositol 3-kinase (PI3K) is composed of an 85-kD (p85) and a 110-kD (p110) subunit. When coupled to activated tyrosine kinases via p85 (the adaptor subunit), p110 (the catalytic subunit) phosphorylates the 3-hydroxy group of inositol phospholipids. Gain-of-function mutations in *PIK3CA* have been found in different types of cancers including BCa. The mutations result in PI3K activation independent of upstream signaling and constitutive activation of the downstream AKT pathway and may contribute to oncogenesis[4]. The frequency of *PIK3CA* mutations in BCa cases ranges from 16.4 to 45%[5]. There are 3 mutation "hotspots" in the *PIK3CA* gene: E542K, E545K at exon 9 (helix domain) and H1047R at exon 20 (kinase domain). The 3 hotspots represent almost 80% of *PIK3CA* mutations and lead to constitutive PI3K activity by different mechanisms[6].

Aberrant activation of the PI3K pathway is thought to contribute significantly to endocrine therapy resistance in patients with ER+ BCa[7]. There is evidence showing that endocrine therapy combined with p110 inhibitors is an effective treatment for ER+ BCa cases, including those with *PIK3CA* mutations[8]. The synthetic lethal interaction is a promising approach that needs further studies. Testing of several p110 inhibitors is underway in phase II clinical trials. Therefore, evaluation of the relationship between HRs and *PIK3CA* mutations in BCa is neces-

sary. It is also of great clinical interest to determine whether *PIK3CA* mutations are prognostic factors in HR+ BCa patients.

Results

Search results and description of eligible studies. A total of 1903 potentially relevant citations were retrieved. After exclusion of non-human studies, reviews, and duplicates, two authors independently perused the titles and abstracts of the articles. After screenings, 68 articles were chosen for further full-text review. Ultimately, 32 eligible studies were included in our meta-analysis[5,9–39] (Figure 1).

The 32 eligible studies were published from 2004 to 2014 and involved 5719 cases. Data from the studies were grouped as follows: group A evaluated the relationship between *PIK3CA* mutations and ER (26 studies) or PR (20 studies) expression in BCa patients, group B (12 studies) and group C (8 studies) evaluated the relationship between *PIK3CA* mutations and the outcomes of all BCa patients and HR+ BCa patients, respectively. In the 32 selected studies, the percentage of patients with *PIK3CA* mutations ranged from 7.1% to 44.6%, and the percentage of ER+ patients ranged from 48.1% to 84.0%. For PR, the percentage ranged from 41.4% to 64.8%. In the B and C groups, the median follow-up time ranged from 50 to 153.6 months.

ER and PR expression and *PIK3CA* gene mutations in BCa patients. The relationship between *PIK3CA* gene mutations and ER expression was investigated in 4754 patients from 26 selected studies (Group A, the ER arm) using a fixed-effect model (Table 1). There was a significant association between *PIK3CA* gene mutations and ER expression in the patients in this group (odds ratio [OR] 1.92, 95%CI 1.65–2.23; $P < 0.00001$; Figure 2). Then we performed a separate analysis for PR expression in 3507 patients from 20 studies (Group A, the PR arm) using a fixed-effect model (Table 1), and found that PR expression was also significantly associated with *PIK3CA* mutations (OR 1.88, 95% CI 1.61–2.20; $P < 0.00001$) (Figure 3). Direct sequencing was the most frequently used method for detecting mutations in the selected studies. We introduced subgroups and found that direct sequencing and the other mutation detection methods produced similar results ($p = 0.13$).

***PIK3CA* gene mutations and prognosis in all BCa patients.** Analyses were conducted to evaluate the relationship between *PIK3CA* gene mutations and prognosis as defined by overall survival (OS) and relapse-free survival (RFS) in all BCa patients (group B) (Table 2). Because of significant heterogeneity among the group B studies for OS ($P = 0.008$; $I^2 = 66\%$), a random-effect model was used to assess OS correlations. However, because there

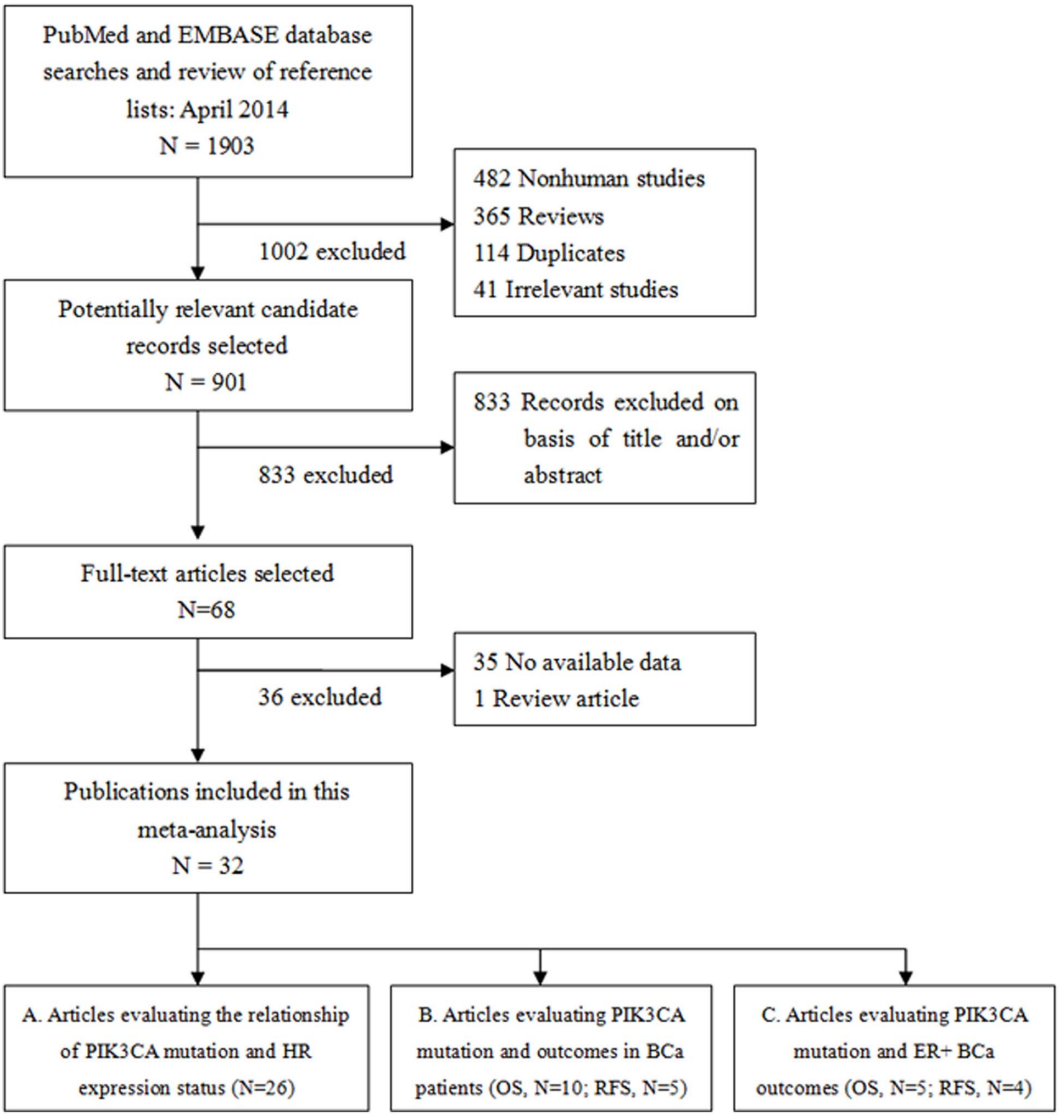

Figure 1 | **Summary flowchart of the literature search.**

Table 1 | Main characteristics of studies that evaluated the relationship of *PIK3CA* mutations and ER/PR status in breast cancer patients

First author	Year of publication	Country	Design	Mean age(years)	No.of ER positive patients (%)	No.of PR positive patients (%)	No.of *PIK3CA* mutant patients (%)	Sequenced *PIK3CA*	Mutation analysis methods
Bachman KE	2004	USA	HB	NR	28 (68.3)	23(57.5)	9 (22.0)	exon 1,9 and 20	DS
Benvenuti S	2008	Italy	HB	NR	95 (76.0)	79(64.8)	28(16.0)	exon 9 and 20	DS
Bozhanov SS	2010	Bulgaria	HB	NR	81 (55.9)	81(56.3)	45 (31.3)	exon 9 and 20	DS
Cizkova M	2012	France	HB	61.6 (31–91)	335 (74.1)	258(57.1)	151 (33.4)	exon 9 and 20	DS
Dunlap J	2010	USA	HB	NR	66 (81.5)	42(51.9)	12 (14.8)	exon 7,9 and 20	DS
Li H	2010	China	HB	51 (33–80)	137 (83.0)	100(60.6)	43 (26.1)	exon 9 and 20	DS
Liang X	2006	Singapore	HB	NR	37 (48.1)	41(53.2)	31 (38.8)	exon 9 and 20	DS
Liedtke C	2008	USA	HB (stage II–III)	51 (28–73)	78 (55.7)	58(41.4)	23 (16.4)	exon 1,9 and 20	DS
Lin CH	2011	China(Taiwan)	HB	NR (less than 35 y)	81 (69.8)	67(57.8)	22 (19.0)	exon 9 and 20	DS
López-Knowles E	2010	Australia	HB	54*	113 (70.2)	96(58.9)	12 (7.1)	exon 9 and 20	DS
Mangone FR	2012	Brazil	HB	55 (26–85)	53 (61.6)	37(46.3)	22 (30.6)	exon 9 and 20	DS
Maruyama N	2007	Japan	HB	NR	124 (66.0)	114(61.0)	54(28.7)	exon 1, 2, 4, 7, 9, 13, 18, and 20	DS
Michelucci A	2009	Italy	HB	43.5 (32–61)	98 (76.0)	88(61.5)	63 (35.8)	exon 9 and 20	DS
Saal LH	2005	USA	HB	59 (24–89)	162 (55.5)	142(51.4)	77 (26.4)	exon 1, 2, 4, 5, 7, 9,12,13,18, 20	DS
Sanchez CG	2011	USA	HB	53.4 (32–80)	32 (62.7)	NR	16 (31.4)	exon 9 and 20 (HS)	DS
Barbareschi M	2007	Italy	HB	62 (17–89)	137 (84.0)	98(60.1)	45 (27.6)	exon 9 and 20	SSCP + DS
Buttitta F	2006	Italy	HB	57.2*	124 (68.9)	106(58.9)	46 (25.6)	exon 1–20	SSCP + DS
Campbell IG	2004	Australia	HB	NR	32 (62.7)	NR	22(43.1)	exon 1–20	SSCP + DHPLC
Dupont Jensen J	2011	Denmark	HB	57 (32–87)	78 (77.2)	NR	45 (44.6)	exon 9 and 20 (HS)	SNaPshot/DxS
Harlé A	2013	France	HB	NR	113 (79.0)	88(61.5)	26(18.2)	exon 9 and 20 (HS)	PCR-ARMS
Jensen JD	2012	Denmark	HB (HER2+)	NR	118 (49.4)	NR	61 (25.7)	exon 9 and 20	PA
Kalinsky K	2009	USA	HB	NR	366 (62.0)	314(57.8)	192 (32.5)	exon 1–20	SM + SS
Li SY	2006	Australia	HB	59 (18–93)	168 (68.9)	156(63.9)	88 (35.2)	exon 7,9 and 20	F-SSCP
Loi S	2013	Finnish	HB	NR	475 (69.1)	NR	174 (25.3)	exons 1, 2, 4, 9, 13, 18, 20	SM
Pérez-Tenorio G	2007	Sweden	HB	NR	188 (70.4)	NR	65(24.3)	exon 9 and 20	SSCP + DS
Santarpia M	2008	Italy/Spain	HB	58 (32–85)	44 (74.6)	33(55.9)	17 (27.9)	exon 9 and 20 (HS)	AD

NR, not reported; HB, hospital based group; HS, hotspots mutation; AD, allelic discrimination; DHPLC, denaturing high performance liquid chromatography; DS, direct sequencing; SNaPshot, SNaPshot genotyping assay; DxS, DxS PI3K mutation test kit; F-SSCP, Fluorescent Single-Strand Conformation Polymorphism; PA, pyrosequencing assay; PCR-Amplification Refractory Mutation System (PCR-ARMS); SM, Sequenom MassARRAY; SS,Sanger sequencing.
*means that the ranges of age were not reported in the studies.

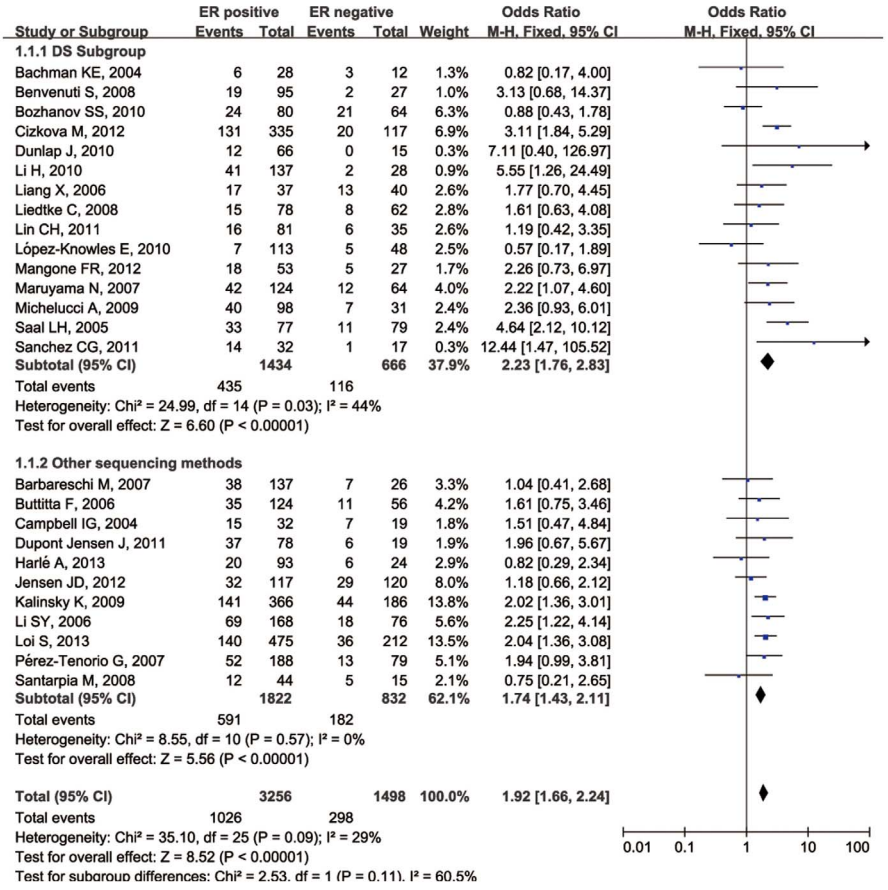

Figure 2 | Forest plot with OR evaluating the relationship between *PIK3CA* mutation and ER expression status.

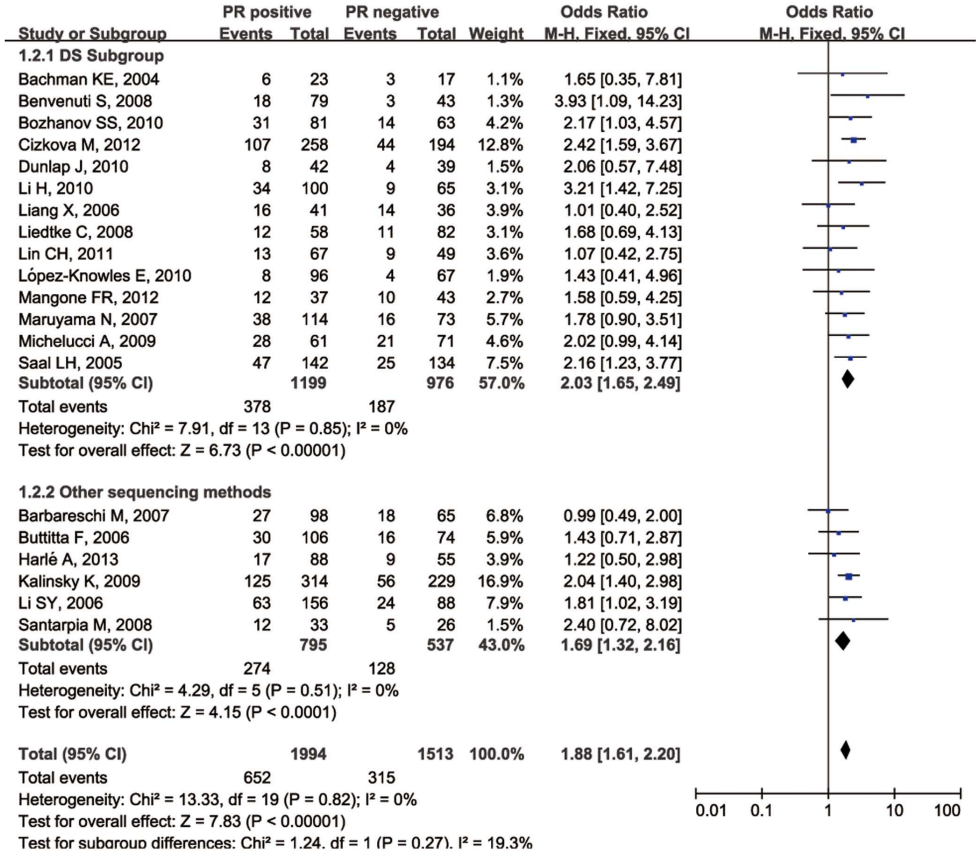

Figure 3 | Forest plot with OR evaluating the relationship between *PIK3CA* mutation and PR expression status.

Table 2 | Main characteristics of studies that evaluated the relationships of *PIK3CA* mutations and the OS/RFS in breast cancer patients

First author	Year of publication	Country	Design	Treatment	No.of *PIK3CA* mutant patients (%)	Sequenced *PIK3CA*	Mutation analysis methods	Median follow-up time (months, range)	Outcomes
Bozhanov SS	2010	Bulgaria	HB	H, C, RT	45 (31.3)	exon 9 and 20	DS	69 (11–96)	OS
Jensen JD	2012	Denmark	HB(Her2+)	H, C, T	61 (25.7)	exon 9 and 20	PA	67*	OS
Kalinsky K	2009	USA	HB	NR	192 (32.5)	exon 1–20	SM + SS	153.6*	OS, RFS
Lai YL	2008	China (Taiwan)	HB	H, C, RT	39 (25.7)	exon 4, 7, 9 and 20	DS	78 (1.3–113.2)	OS
Li SY	2006	Australia	HB	H, C	88 (35.2)	exon 7,9 and 20	F-SSCP	50 (2–78)	OS
Loi S	2013	Finnish	HB	H, C, T	174 (25.3)	exons 1, 2, 4, 9, 13, 18, 20	SM	62*	OS, RFS
Sanchez CG	2011	USA	HB	NR	16 (31.4)	exon 9 and 20 (HS)	DS	51.7 (0.9–256.7)	OS
Lin CH	2011	China (Taiwan)	HB	H, C	22 (19.0)	exon 9 and 20	DS	62.7*	OS
Mangone FR	2012	Brazil	HB	NR	22 (30.6)	exon 9 and 20	DS	63.3 (25–78)	OS
Gonzalez-Angulo AM	2009	USA	HB	H, C	78 (22.5)	exon 9 and 20	SM	50.4 (9.6–110.4)	OS, RFS
Maruyama N	2007	Japan	HB	H, C	54 (28.7)	exon 1, 2, 4, 7, 9, 13, 18, 20	DS	64 (38–88)	RFS
Pérez-Tenorio G	2007	Sweden	HB	H, C, RT	65 (24.1)	exon 9 and 20	SSCP + DS	132*	RFS

*means that the ranges of age or months were not reported in the studies.
C, Chemotherapy; T: Trastuzumab; H, Hormonal therapy; RT, Radiotherapy.

was no inter-study heterogeneity among the group B studies for RFS ($P = 0.93$; $I^2 = 0\%$), a fixed-effect model was used to assess RFS correlations. For OS, 7 studies involving 2105 patients were analyzed and no significant association between *PIK3CA* mutations and OS was found (HR 1.14, 95% CI 0.72–1.82; $P = 0.57$) (Figure 4). We also performed analysis for different exons. For exon 9 mutations, a significant worse OS was found (HR 1.42, 95% CI 1.02–1.99; $P = 0.04$). In addition, for exon 20, the results of OS did not reach a significant level (HR 1.63, 95% CI 0.93–2.85; $P = 0.09$) (Figure 4). For RFS, 5 studies involving 1913 patients were analyzed, and a significant relationship between *PIK3CA* gene mutations and prolonged RFS was observed (hazard ratio 0.76, 95% CI 0.59–0.98; $P = 0.03$) (Fig. 5).

***PIK3CA* gene mutations and prognosis in HR+ BCa patients.** The relationship between PIK3CA mutations and prognosis in HR+ BCa was evaluated in 8 studies involving 1021 patients, 5 studies (644 patients) for OS and 4 studies (534 patients) for RFS (group C) (Table 3). On the basis of the available data, kinase domain mutation is the priority for inclusion and analysis. No inter-study heterogeneity was found for OS ($P = 0.38$; $I^2 = 4\%$) or RFS ($P = 0.73$; $I^2 = 0\%$). *PIK3CA* gene mutations were not significantly associated with OS (hazard ratio 1.06, 95% CI 0.67–1.67; $P = 0.81$) (Fig. 6a) or RFS (hazard ratio 0.86, 95% CI 0.53–1.40; $P = 0.55$) (Fig. 6b) in HR+ BCa patients.

Publication bias. Publication bias was not investigated when the number of studies was less than 10 because of the low sensitivity of qualitative and quantitative tests[40]. When the number of studies was more than 10, bias was assessed by Begg's funnel plots. No evidence of obvious asymmetry was found in this analysis by visual evaluation (data not shown).

Discussion

Recently, several studies evaluating the prognosis of BCa patients suggest that *PIK3CA* mutations are "good mutations". Our meta-analysis shows that *PIK3CA* gene mutations are significantly associated with both ER and PR expression, which are believed to be favorable clinicopathologic features of BCa. Furthermore, in unsorted BCa patients with *PIK3CA* mutations, RFS was significantly improved.

There are some possible explanations for the puzzling favorable effects of *PIK3CA* mutations. First, signaling pathways downstream of PI3K may not be active in some BCa patients with *PIK3CA* mutations. Loi et al. found that *PIK3CA* mutations were associated with relatively low mTORC1 signaling and that some AKT-regulated genes were repressed in BCa patients with *PIK3CA* mutations[31]. Second, dysregulated gene expression resulting from *PIK3CA* mutations may be advantageous. Cizkova showed that the Wnt pathway was dysregulated and WNT5A was overexpressed in ER+ BCa patients with *PIK3CA* mutations[41]. Interestingly, WNT5A expression has been associated with favorable outcomes in patients with invasive breast tumors[42]. Third, *PIK3CA*, like many other oncogenes, may induce senescence, resulting in a less aggressive phenotype after cell transformation[43,44].

Despite of this, there was only an insignificant connection between *PIK3CA* mutations and OS. The improvement in RFS but not OS may suggest a BCa specific effect of *PIK3CA* mutations. However, considering specific exons, the effects seemed weak or even contradictory. In the future, more studies focusing on specific exons mutations, including the non-hotspot mutations of *PIK3CA*, are warranted.

Whether PIK3CA mutations contribute to endocrine therapy resistance remains unclear and intriguing. Another important finding of this study was that *PIK3CA* mutations did not affect either OS or RFS in HR+ BCa patients. In most of the studies selected for our

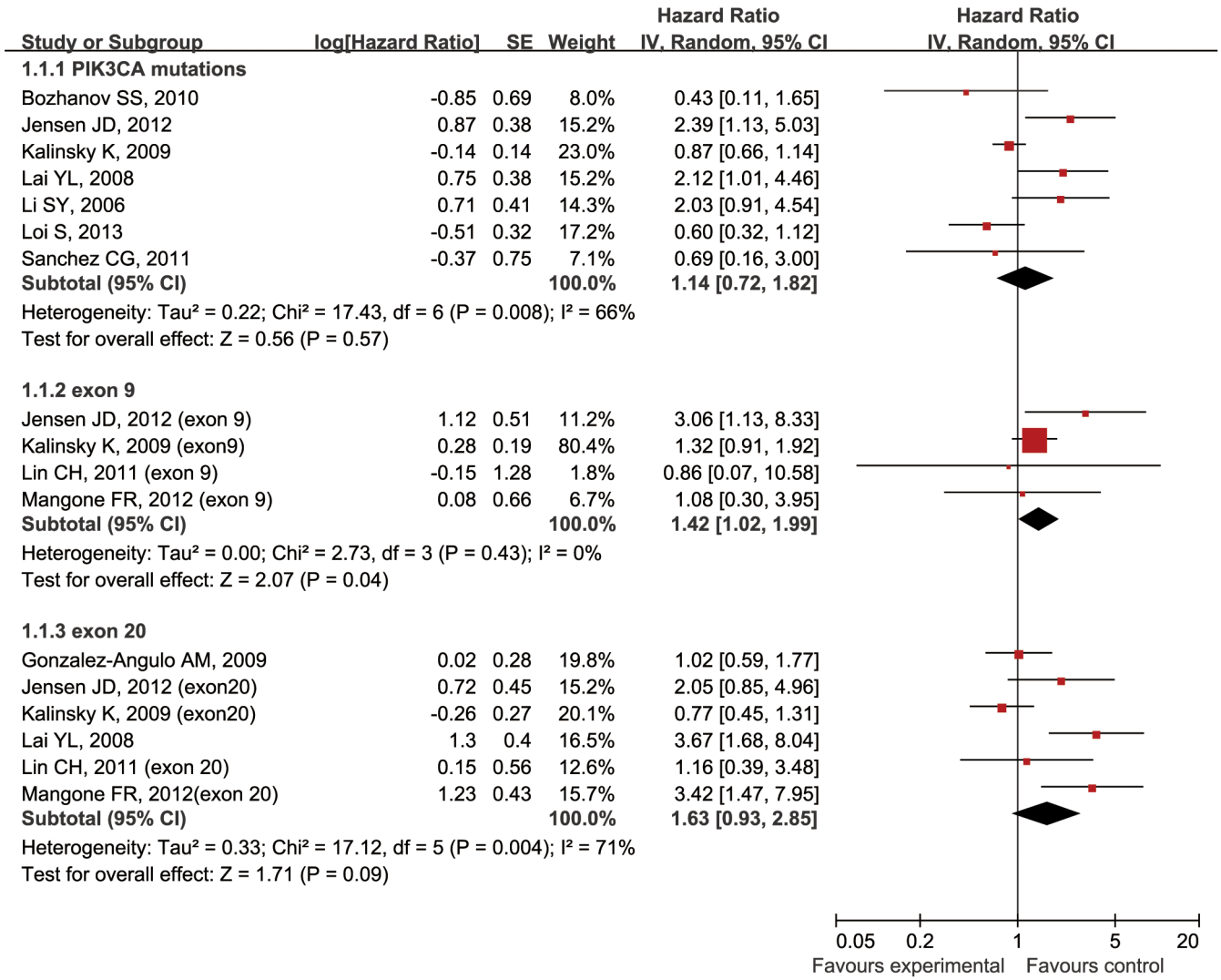

Figure 4 | **Forest plots of the analysis on the HR of OS in BCa patients.** Subgroups are introduced for evaluating exon 9 or 20 mutations.

analysis, hormone treatment was the standard therapy method. However, *PIK3CA* mutations may have only limited prognostic value with respect to hormone therapy responsiveness. Ellis et al. showed that the *PIK3CA* kinase domain mutations were inversely correlated with the clinical response to neoadjuvant endocrine treatment in BCa patients and was not associated with proliferation, as determined by immunostaining for Ki-67[20]. In patients who did not receive tamoxifen, as Beelen et al. showed, PIK3CA mutation was not a prognostic marker, either.

It also should be noted that there is some dissociation between *PIK3CA* mutations and activation of signaling pathways downstream of PI3K. In some phase I clinical trials, *PIK3CA* mutations were not strongly related to responses produced by PI3K inhibitors[17]. In our study, *PIK3CA* mutations were associated with favorable pro-

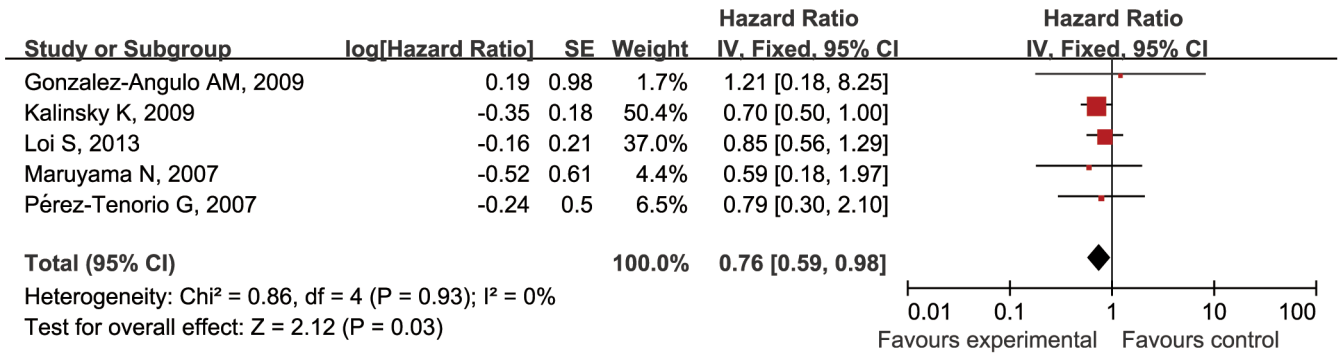

Figure 5 | **Forest plot of the analysis on the HR of RFS in BCa patients.**

Table 3 | Main characteristics of studies that evaluated the relationships of *PIK3CA* mutations and the OS/RFS in HR+ breast cancer patients

First author	Year of publication	Country	Design	Treatment	No.of *PIK3CA* mutant patients (%)	Sequenced *PIK3CA*	Mutation analysis methods	Median follow-up time (months, range)	Outcome type
Bozhanov SS	2010	Bulgaria	HB	H, C, RT	24(30.0)	exon 9 and 20	DS	69 (11–96)	OS
Cuorvo LV	2014	Italy	HB	H, C, T	50(20.3)	exon 9 and 20	HRM + PA	97 (8–140)	OS*
Li SY	2006	Australia	HB	H, C	69(41.1)	exon 7, 9 and 20	F-SSCP	50 (2–78)	OS
Sanchez CG	2011	USA	HB	NR	13(48.1)	exon 9 and 20 (HS)	DS	51.7 (0.9–256.7)	OS
Stemke-Hale K	2008	Spain, Netherlands and USA	HB	H	80(34.5)	23 known mutations	MS	NR	OS, RFS
Beelen K	2014	Netherlands	HB	Control arm	28(25.2)	exon 9 and 20 (HS)	SM	93.6	RFS
Ellis MJ	2010	Multicentre	HB	H	45(29.4)	exon 9 and 20	DS	NR	RFS*
Maruyama N	2007	Japanese	HB	H, C	54(28.7)	exon 1, 2, 4, 7, 9, 13, 18, and 20	DS	64 (38–88)	RFS

C, Chemotherapy; T: Trastuzumab; H, Hormonal therapy; RT, Radiotherapy; HRM, high resolution melting analysis.
*only exon 20 mutations were analyzed.

gnostic factors such as ER and PR expression, but are unlikely to be the single pivotal determinant of favorable responses to endocrine treatment. The gene signature associated with *PIK3CA* mutations was indicative of better clinical outcomes in ER+/HER2− BCa patients[45]. Perhaps its gene signature is more important than the *PIK3CA* mutation itself in respect to prognosis. Studies determining whether *PIK3CA* mutations are beneficial to tamoxifen-treated HR+ BCa patients with other molecular features such as PTEN loss or *AKT1* mutations are warranted.

There were some limitations to our study. First, we only analyzed available data in the literature. Second, because of significant heterogeneity, we used the random effect model, which is not as reliable as the fixed-effects model, in some analyses. Third, we only included articles that were published in English, and language bias might exist. Fourth, data extracted from the literature may not be as reliable as data generated directly. Fifth, several related studies of high quality were not included in our analysis because ideal unified prognosis parameters were lacking. Finally, the inclusion criteria and treatment procedures were not strictly unified in the studies used for our analysis. These differences are also a potential source of heterogeneity. Therefore, a cautious interpretation of our findings is warranted given possible bias in our meta-analysis.

In summary, our results show that *PIK3CA* mutations are significantly related to the ER and PR expression status of BCa patients. They also correlated with improved RFS in unsorted BCa patients, but not with OS or RFS in HR+ BCa patients. As a potential biomarker, *PIK3CA* mutations were not prognostic for HR+ BCa patients or, most notably, ER+ BCa patients. Future studies are needed that collectively explore the possible roles of *PIK3CA* mutations, the activation of signaling pathways downstream of PI3K, and other important biomarkers such as the genes encoding the components of the PI3K/AKT/mTOR pathway.

Methods

Literature search and eligibility criteria. We searched PubMed and Embase databases up to April 2014 for English-language titles or abstracts that included the words "phosphoinositide-3-kinase", "*PIK3CA*", "mutation", "breast cancer", or "breast neoplasms". We also screened the references of the retrieved articles and relevant reviews for additional articles. A published article was included if it (1) evaluated the association between *PIK3CA* mutations and ER or PR expression in BCa patients or the association between *PIK3CA* mutations and BCa prognosis; (2) had sufficient data for estimating an OR with a 95% CI or a HR with a 95% CI; and (3) evaluated OS, RFS, or other survival index. The exclusion criteria were as follows: (1) letters, reviews, conference abstracts, and case reports; and (2) articles that did not provide sufficient information such as a HR for OS or had data that could not be extracted.

Data extraction and quality assessment. Two authors independently screened all publications by title or abstract for inclusion in our study. Discrepancies were resolved by group discussion, and data were extracted from eligible publications. The following information was collected: name of the first author, year of publication, source of patients, study design, mean age of the patients, percentage of ER+ and PR+ patients, percentage of patients with *PIK3CA* mutations, the region of the sequenced *PIK3CA* mutations, mutation analysis methods, outcome of BCa patients, and median follow-up time (months, range). The studies were assessed for quality according to the Newcastle-Ottawa quality assessment scale, and articles with 5 stars or more qualified for our study[46].

Statistical analysis. An OR with a 95% CI was used to assess the strength of the association between *PIK3CA* mutations and ER or PR expression status. The primary end points were RFS and OS. A HR and a 95% CI were used to estimate the impact of *PIK3CA* mutations on RFS and OS. When a HR and a 95% CI were not given in the article, estimated values were derived indirectly from Kaplan-Meier curves using the methods described by Tierney et al.[47]. Kaplan-Meier curves were read by an Engauge Digitizer, version 4.1 (http://digitizer.sourceforge.net/), and the data from the curves were entered in the spreadsheet appended to Tierney's report[47]. A combined HR > 1 implied a worse survival for groups of patients with *PIK3CA* mutations. Cochran Q and I^2 statistic values were used to assess heterogeneity among the studies. For the Q statistic, a P value < 0.10 was considered statistically significant for heterogeneity[48], and the random effects model was calculated according to the DerSimonian-Laird method[49].Otherwise, the fixed-effects model (Mantel-Haenszel method) was used. $I^2 < 50\%$ was considered acceptable. If significant heterogeneity was found, a random-effects model was used for meta-analysis. Statistical analyses were performed using

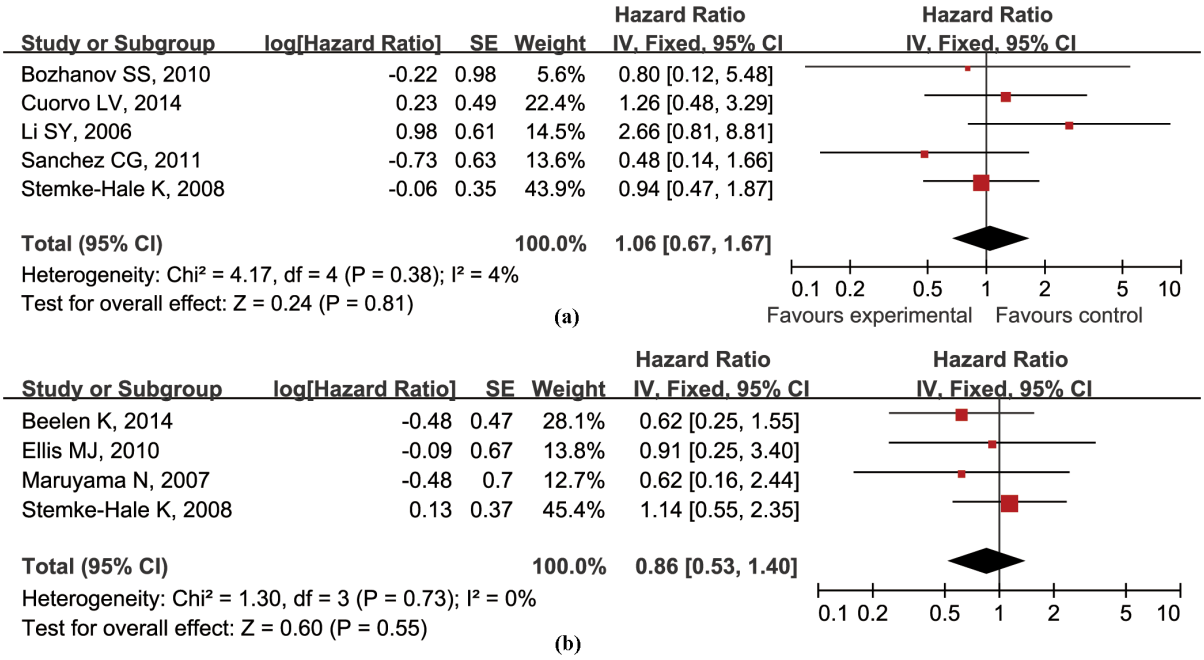

Figure 6 | Forest plots of the analysis on the hazard ratio of OS (a) and RFS (b) in HR+ BCa patients.

Review Manager 5.0 software (http://www.cochrane.org). A significant two-way *P* value for comparison was defined as $P < 0.05$.

Ethical Standards. We declare that the experiments comply with the current laws of China.

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Acknowledgments

This work was supported by Funding of Guang'an men Hospital (Grant No. 2011S244).

Author contributions

B.P. carried out the search of the Embase and Pubmed database, performed the statistical analysis by Revman, participated in the design of the study and drafted the manuscript. S.C. carried out the search of the Embase and Pubmed database and performed the statistical analysis by Revman. S.P.S. performed the data collection and extraction and helped to draft the manuscript. C.A. participated in the design of the study and made the language polishing. Z.Y.L. performed the data collection, extraction and arrangement. X.F. performed the data collection and arrangement. G.J.L. conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Pang, B. *et al.* Prognostic role of *PIK3CA* mutations and their association with hormone receptor expression in breast cancer: a meta-analysis. *Sci. Rep.* **4**, 6255; DOI:10.1038/srep06255 (2014).