Systematic Review and Meta-Analysis

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Effect of *BRCA* germline mutations on breast cancer prognosis

A systematic review and meta-analysis

Zora Baretta, MD[a], Simone Mocellin, MD, PhD[b,c], Elena Goldin, MD[b], Olufunmilayo I. Olopade, MD[d], Dezheng Huo, MD, PhD[e,*]

Abstract

Background: The contribution of *BRCA* germline mutational status to breast cancer patients' prognosis is unclear. We aimed to systematically review and perform meta-analysis of the available evidence of effects of *BRCA* germline mutations on multiple survival outcomes of breast cancer patients as a whole and in specific subgroups of interest, including those with triple negative breast cancer, those with Ashkenazi Jewish ancestry, and patients with stage I–III disease.

Methods: Sixty studies met all inclusion criteria and were considered for this meta-analysis. These studies involved 105,220 breast cancer patients, whose 3588 (3.4%) were BRCA mutations carriers. The associations between *BRCA* genes mutational status and overall survival (OS), breast cancer-specific survival (BCSS), recurrence-free survival (RFS), and distant metastasis-free survival (DMFS) were evaluated using random-effect models.

Results: *BRCA1* mutation carriers have worse OS than *BRCA*-negative/sporadic cases (hazard ratio, HR 1.30, 95% CI: 1.11–1.52) and worse BCSS than sporadic/*BRCA*-negative cases among patients with stage I–III breast cancer (HR 1.45, 95% CI: 1.01–2.07). *BRCA2* mutation carriers have worse BCSS than sporadic/*BRCA*-negative cases (HR 1.29, 95% CI: 1.03–1.62), although they have similar OS. Among triple negative breast cancer, *BRCA1/2* mutations carriers had better OS than *BRCA*-negative counterpart (HR 0.49, 95% CI: 0.26–0.92). Among Ashkenazi Jewish women, *BRCA1/2* mutations carriers presented higher risk of death from breast cancer (HR 1.44, 95% CI: 1.05–1.97) and of distant metastases (HR 1.82, 95% CI: 1.05–3.16) than sporadic/*BRCA*-negative patients.

Conclusion: Our results support the evaluation of *BRCA* mutational status in patients with high risk of harboring *BRCA* germline mutations to better define the prognosis of breast cancer in these patients.

Abbreviations: BCSS = breast cancer specific survival, CI = confidence interval, DMFS = distant metastasis free survival, HR = hazard ratio, OS = overall survival, PICOS = population, intervention, comparison, outcome, REMARK = reporting recommendations for tumor MARKer prognostic studies, RFS = recurrence free survival.

Keywords: BRCA germline mutations, breast cancer, meta-analysis, prognosis, survival, systematic review

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[a] U.O.C. di Oncologia ULSS5 Ovest Vicentino, Ospedale di Montecchio, Montecchio Maggiore (VI), [b] Department of Surgery, Oncology and Gastroenterology, University of Padova, [c] Istituto Oncologico Veneto, IOV-IRCCS, Padova, Italy, [d] Center for Clinical Cancer Genetics & Global Health, Department of Medicine, University of Chicago, Chicago, IL, [e] Department of Public Health Sciences, University of Chicago, Chicago, IL.

[*] Correspondence: Dezheng Huo, Department of Public Health Sciences, University of Chicago, Chicago, IL (e-mail: dhuo@health.bsd.uchicago.edu).

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1. Introduction

Breast cancer is the most common cancer and the leading cause of deaths from cancer in women worldwide.[1] Approximately 5% to 10% of breast cancer cases are hereditary, and up to 25% of the hereditary breast cancers have been linked to germline mutations of specific genes.[2] The most studied genes are *BRCA1* and *BRCA2*, whose highly penetrant mutations are associated with the Hereditary Breast/Ovarian cancer Syndrome, an autosomal-dominant inherited trait predisposing women to both breast and ovarian cancer.[3,4] Women with *BRCA* mutations have a lifetime risk of developing breast cancer and ovarian cancer of 45% to 75% and 18% to 40%, respectively.[5–7]

BRCA-related breast cancer is characterized by more aggressive phenotype than sporadic breast cancer, with *BRCA1*-related breast cancer being more frequently high grade and triple negative, and *BRCA2*-related breast cancer being on average of higher histological grade than sporadic cases.[5,8–10] Thus, it has been hypothesized that *BRCA*-associated breast cancer has a different prognosis as compared to the sporadic counterpart. However, clinical findings regarding the prognostic role of *BRCA* mutational status are controversial. Few studies have reported better survival outcomes for patients with *BRCA*-associated breast cancer as compared with control groups,[11–14] while other studies have reported worse prognosis or no difference. This

controversy is partially due to the relatively small sample size in many studies because *BRCA1/2* mutation carriers are rare in the breast cancer population. For oncologists, it would be important to know whether *BRCA* mutational status is a reliable prognostic factor to be used for risk stratification and thus considered in the therapeutic management of hereditary breast cancer cases.

The aim of the present work is to systematically review and meta-analyze the available evidence regarding the effects of *BRCA* germline mutations on multiple survival outcomes of patients with breast cancer as a whole and in specific subgroups of interest, including those with triple negative breast cancer, those with Ashkenazi Jewish ancestry, and patients with stage I–III disease.

2. Methods

Literature search, study design, and data analysis were performed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (see Supplemental Contents—PRISMA checklist, http://links.lww.com/MD/B316).[15] Ethical approval was not necessary for this study because this study does not involve patients. The PICOS (Population, Intervention, Comparison, Outcome) worksheet was used to identify the main question of the meta-analysis and define the targets of the search strategy (see in Supplemental Contents–PICOS worksheet, which describes type of population, intervention, type of comparison, and outcomes considered in the meta-analysis, http://links.lww.com/MD/B315). Finally, the REMARK (Reporting recommendations for tumor MARKer prognostic studies) checklist was used to evaluate the quality of studies included in the meta-analysis;[16] for each study, a quality score was calculated based on the number of recommendations met by the study over the total 20 items, assigning 1 point to each met recommendation.

2.1. Literature search and study selection

We used PubMed database to search articles published until August 2016, which evaluated the impact of *BRCA* mutational status on breast cancer prognostic outcomes. To this aim, we used the following search string "*BRCA* AND breast cancer survival*." Moreover, we screened the references of all original articles as well as those cited in reviews articles focusing on this topic, in order to maximize the likelihood of identifying all relevant articles. The 2 key inclusion criteria were as follows: (1) the study compared the survival of *BRCA*-positive women affected by breast cancer with that of women with sporadic/*BRCA*-negative breast cancer; (2) the article reported survival outcomes as hazard ratios (HRs) or Kaplan–Meier survival curves. Screening of eligible records and selection of articles to be included in the meta-analysis were independently performed by 2 reviewers (ZB and EG). Disagreements were resolved by discussion and consensus.

2.2. Endpoints and data extraction

Endpoints of the meta-analysis were overall survival (OS), breast cancer-specific survival (BCSS), recurrence-free survival (RFS), and distant metastasis-free survival (DMFS). For each included study, we retrieved the following information: type of genetic test, type of biospecimen used to perform the genetic test (blood/paraffin blocks of primary tumor), number of the *BRCA*-positive patients, number of the reference group patients (sporadic or *BRCA*-negative), median age of patients in the study and reference groups, Ashkenazi Jewish ancestry (yes/no), triple negative subtype (yes/no), pathological stage of the breast cancer, HRs and their 95% confidence interval (CI), factors considered in multivariate analysis (see Supplemental Contents–Master database, which reports the PUBMED identification number to retrieve the studies involved in the meta-analysis, and data for each study, http://links.lww.com/MD/B317). Extraction of data was done by ZB and EG.

2.3. Statistical analysis

Hazard ratios and their 95% CIs were used as measures of the association between *BRCA* mutations and patients' survival. The random effects model described by DerSimonia and Laird was used to calculate the summary HR and 95% CI.[17] Three main analyses were performed based on the mutational status in the experimental group: (1) in *BRCA1* mutated patients; (2) in *BRCA2* mutated patients; (3) in *BRCA1/2* mutated patients. In the latter analysis, HRs were calculated considering data from *BRCA1*-studies, *BRCA2*-studies, and *BRCA1/2*-studies. Patients with sporadic breast cancer (without being tested for *BRCA* mutational status) or *BRCA* mutation tested negative patients represented the reference group. An $HR > 1$ indicated a poorer outcome for the experimental group (i.e., *BRCA*-positive subjects).

Between-study heterogeneity was quantified by the *I*-square statistic (25% low heterogeneity, 25–50% medium, >50% high).[18] In order to investigate potential sources of heterogeneity, we performed the following pre-specified subgroup analyses: first, we focused our analysis on the studies where all control patients were *BRCA* tested negative; second, we conducted separate analyses for studies including and excluding patients with distant metastatic disease (TNM stage IV); third, we focused on studies including only patients with triple negative breast cancer; fourth, we investigated the role of *BRCA* mutational status in breast cancer patients with Ashkenazi Jewish ancestry as this is a population with high prevalence of *BRCA* mutations.[19–21] Finally, mixed effects meta-regression was utilized to investigate whether between-study heterogeneity is correlated with study quality, which was the ranking score from the REMARK checklist (range: 0–20) and year of study publication; both were considered as continuous covariates in the meta-regression.

HRs and 95% CIs were extracted from articles, when available; when unreported, they were extrapolated from Kaplan–Meier survival curves adopting a hierarchical series of steps as per Parmar et al.[22] If both univariate and multivariate analyses were available, HRs from the latter were considered. However, the pooled estimates and heterogeneity analysis according to univariate and multivariate analyses are available (see Supplemental Contents—Table S1, http://links.lww.com/MD/B313, which reports pooled estimates and heterogeneity analysis according to univariate and multivariate analyses). Small study effects (which includes publication bias) was evaluated by visual assessment of funnel plot symmetry and formally investigated by using Egger's test.[23] The test was performed only when at least 10 studies were available.

The level of significance was set at 5% with the exception of Egger's test, for which a 10% level was chosen due to the low power for characterizing this test. Analyses were conducted using Review Manager 5.2 (Cochrane Collaboration) and Stata 14.1 (StataCorp, College Station, TX).

Table 1

Number of studies and patients involved in the meta-analysis.

		All studies (N = 60)			Studies with tested patients (N = 42)		
	Outcome	N° studies	Control group patients*	BRCA+ patients	N° studies	BRCA– patients	BRCA+ patients
BRCA1	OS	27	89,627	1636	17	9974	926
	BCSS	16	6732	885	14	5901	646
	RFS	6	2366	327	4	2098	262
	DMFS	11	4337	745	9	3457	429
BRCA2	OS	12	83,733	457	6	4732	197
	BCSS	10	8527	598	8	7554	459
	RFS	3	217	61	0	–	–
	DMFS	3	2430	202	1	1555	71
BRCA 1/2	OS	8	2498	326	5	500	203
	BCSS	2	507	99	1	277	28
	RFS	8	702	338	6	444	260
	DMFS	2	447	60	2	447	60

BCSS = breast cancer-specific survival, DMFS = distant metastasis-free survival, OS = overall survival, RFS = recurrence-free survival.
* Control group includes BRCA-negative patients or sporadic cases.

3. Results

3.1. Characteristics of identified studies

Using the above-mentioned search strategy, 1330 records were identified from the PUBMED database (Flow Diagram, http://links.lww.com/MD/B314). Three additional records were retrieved from review articles.[24–26] Two duplicate articles were excluded leaving 1331 records to be screened. After abstract reading, 82 articles were retrieved for full text evaluation, which led to 60 articles that met all inclusion criteria and represented the data source for the following qualitative and quantitative analyses (see Supplemental Contents—Master database, which reports the PUBMED identification number to retrieve the studies involved in the meta-analysis, and data for each study, http://links.lww.com/MD/B317).

Overall, 105,220 breast cancer patients were available within the included studies, 3588 (3.4%) being *BRCA* mutations carriers. The median number of *BRCA* carriers involved in the studies was 39.5 (range 5–326). Table 1 lists the number of studies, the number of *BRCA* carriers and control group patients according to gene (*BRCA1*, *BRCA2*, and *BRCA1/2*) and endpoints (OS, BCSS, RFS, DMFS) in all studies, and separately in studies where the entire population was tested for *BRCA* mutations.

Forty-two (70%) studies, involving 21,977 patients, performed the genetic test in the entire population of the study, allowing the comparison between *BRCA* carriers and true *BRCA*-negative subjects. Six studies (n = 1748) were focused on triple negative patients and 12 on Ashkenazi Jewish women (n = 4161). As regards breast cancer stage, 28 (47%) studies excluded stage IV, 25 (42%) included stage IV, and 2 (3%) were focused on metastatic disease; however, this information was not reported in 5 (8%) studies. The genetic test was performed using blood sample in 29 (48%) studies, DNA extracted from formalin blocks in 13 (22%) studies, or both in 4 (7%) studies; yet this information was not specified in 14 (23%) studies.

3.2. Prognostic role of BRCA1 gene mutations

Twenty-seven studies were included in the meta-analysis for OS; in 17 (63%) studies, the genetic test was performed in the entire sample. Meta-analysis showed a significant higher risk of dying for *BRCA1* carriers as compared to the control group (HR 1.30, 95% CI: 1.11–1.52; *P*-value = 0.001, Table 2 and Fig. 1). This result was confirmed when the analysis was restricted to studies including only tested patients (HR 1.46, 95% CI:

Table 2

Pooled estimates and between study-heterogeneity analysis in all studies and in the studies that considered only tested patients.

		All studies (N = 60)				Studies with tested patients† (N = 42)			
	Outcome	Studies	HR (CI 95%)*	*P*	I^2	Studies	HR (CI 95%)*	*P*	I^2
BRCA1	OS	27	1.30 (1.11–1.52)	0.001	37%	17	1.46 (1.12–1.91)	0.006	47%
	BCSS	16	1.17 (0.91–1.49)	0.22	45%	14	1.22 (0.91–1.63)	0.17	48%
	RFS	6	0.98 (0.68–1.41)	0.90	59%	4	1.08 (0.72–1.63)	0.70	50%
	DMFS	11	1.06 (0.78–1.46)	0.70	55%	9	1.11 (0.71–1.73)	0.66	57%
BRCA2	OS	12	0.98 (0.76–1.25)	0.85	42%	6	1.10 (0.82–1.47)	0.54	0%
	BCSS	10	1.29 (1.03–1.62)	0.03	24%	8	1.34 (1.04–1.73)	0.02	23%
	RFS	3	1.41 (0.41–4.85)	0.59	66%	0	–	–	–
	DMFS	3	0.78 (0.59–1.02)	0.07	0%	1	1.00 (0.62–1.61)	1.00	–
BRCA1/2	OS	8	1.11 (0.68–1.80)	0.68	49%	5	0.85 (0.44–1.65)	0.64	54%
	BCSS	2	1.22 (0.42–3.49)	0.71	58%	1	2.08 (0.79–5.46)	0.14	–
	RFS	8	1.04 (0.67–1.60)	0.87	41%	6	0.90 (0.52–1.57)	0.71	49%
	DMFS	2	1.81 (1.03–3.17)	0.04	0%	2	1.81 (1.03–3.17)	0.04	0%
Overall	OS	37	1.19 (1.04–1.35)	0.009	41%	22	1.28 (1.06–1.53)	0.009	42%
	BCSS	22	1.22 (1.04–1.44)	0.02	36%	18	1.29 (1.07–1.57)	0.009	38%
	RFS	17	1.04 (0.80–1.35)	0.78	48%	10	1.01 (0.74–1.38)	0.96	44%
	DMFS	13	1.03 (0.82–1.31)	0.78	53%	11	1.19 (0.85–1.65)	0.31	51%

BCSS = breast cancer-specific survival, CI = confidence interval, DMFS = distant metastasis-free survival, HR = hazard ratio, I^2 = between study-heterogeneity index, OS = overall survival, RFS = recurrence-free survival.
* When both univariate and multivariate HRs were reported, multivariate HR was chosen.
† In this analysis, the reference group is BRCA-negative patients.

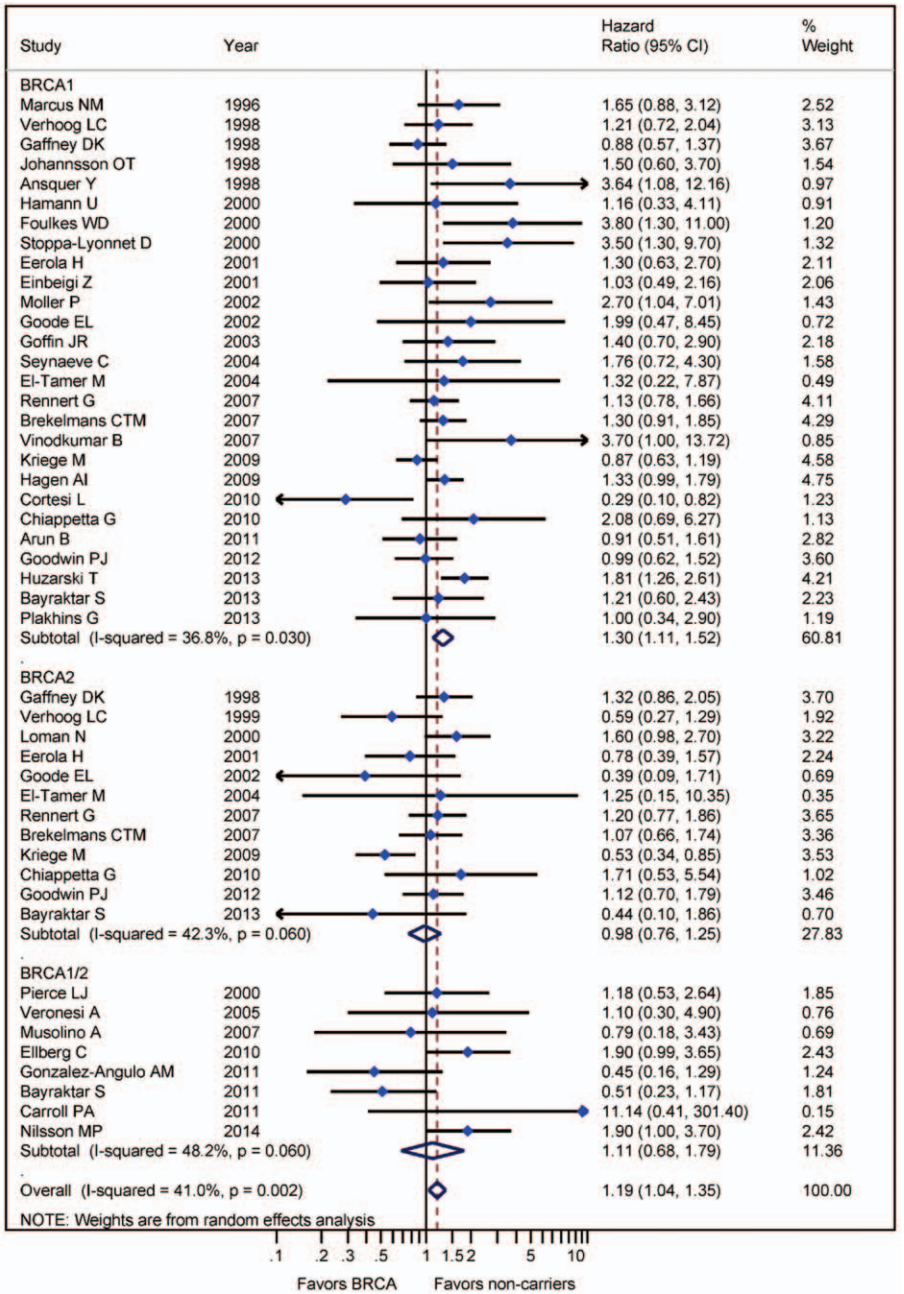

Figure 1. Forrest plot of overall survival by BRCA mutational status.

1.12–1.91; *P*-value = 0.006) as well as to studies excluding stage IV (HR 1.55, 95% CI: 1.24–1.95; *P*-value = 0.0001, Table 3). In all cases, between-study heterogeneity was moderate to low.

Meta-analysis for BCSS, RFS, and DMFS included 16, 6, and 11 studies, respectively (Table 1). No difference was found in these clinical outcomes between *BRCA1* mutation carriers and control group (Table 2, Figs. 2–4). Heterogeneity was high for RFS and DMFS in both general, and restricted to studies with all tested patients analyses. Interestingly, the risk of dying from breast cancer was significantly higher for *BRCA1* cases than control group when the studies including stage IV cases were excluded from the analysis (HR 1.45, 95% CI: 1.01–2.07; *P*-value = 0.045, Table 3).

The mean REMARK score was 15 (range 9–19) for OS studies, 16 (range 13–19) for BCSS studies, 14.5 (range 12–17) for RFS studies, and 17.5 (range 13–19) for DMFS studies. Meta-regression analysis did not demonstrate any association between REMARK score and all considered survival outcomes, as well as between year of publication and *BRCA1* effect on survival, with the exception of DMFS (HR decreased by 7% per year, 95 percent CI: 0–13%; *P* = 0.049. See Supplemental Contents—Figure S1, http://links.lww.com/MD/B313, which illustrates the relationship between HRs of DMFS for BRCA1 mutational status and publication years).

3.3. Prognostic role of BRCA2 gene mutations

The prognostic role of *BRCA2* gene mutations in terms of OS, BCSS, RFS, and DMFS was evaluated in 12, 10, 3, and 3 studies, respectively (Table 1). In 6 OS-studies and in 8 BCSS-studies, all

Table 3

Pooled estimates and heterogeneity analysis according to disease stage.

		Studies including stage IV (N = 25)				Studies excluding stage IV (N = 28)			
	Outcome	Studies	HR (CI 95%)*	P	I^2	Studies	HR (CI 95%)*	P	I^2
BRCA1	OS	11	1.15 (0.92–1.42)	0.21	28%	11	1.55 (1.24–1.95)	0.0001	20%
	BCSS	8	0.94 (0.67–1.32)	0.74	38%	8	1.45 (1.01–2.07)	0.045	49%
	RFS	3	0.80 (0.48–1.33)	0.38	64%	3	1.27 (0.69–2.33)	0.44	59%
BRCA2	OS	9	1.12 (0.93–1.36)	0.24	2%	0	–	–	–
	BCSS	8	1.28 (0.93–1.76)	0.13	37%	1	1.90 (0.59–6.11)	0.28	–
	RFS	2	0.82 (0.43–1.54)	0.54	0%	1	4.10 (1.30–13.00)	0.016	–
BRCA1/2	OS	3	1.55 (0.89–2.68)	0.12	0%	5	0.99 (0.49–2.02)	0.98	63%
	BCSS	0	–	–	–	2	1.22 (0.42–3.49)	0.72	58%
	RFS	4	1.40 (0.73–2.69)	0.32	0%	3	0.66 (0.28–1.60)	0.36	75%
Overall	OS	16	1.15 (1.01–1.32)	0.04	11%	16	1.42 (1.10–1.82)	0.006	43%
	BCSS	11	1.11 (0.88–1.39)	0.37	37%	10	1.42 (1.05–1.92)	0.02	39%
	RFS	9	0.90 (0.69–1.17)	0.43	6%	7	1.12 (0.68–1.85)	0.65	70%

BCSS = breast cancer specific survival, CI = confidence interval, DMFS = distant metastasis-free survival, HR = hazard ratio, I^2 = between study-heterogeneity index, OS = overall survival, RFS = recurrence-free survival.

* When both univariate and multivariate HRs were reported, multivariate HR was chosen.

patients received the genetic test; however, all RFS studies and 2 DMFS studies compared the *BRCA2* mutation carriers with sporadic nontested cases. The meta-analysis of these data showed a worse BCSS for *BRCA2* carriers as compared to control group in both general population (HR 1.29, 95% CI: 1.03–1.62; $P = 0.03$. Table 2 and Fig. 2) and among tested patients (1.34, 95% CI: 1.04–1.73; $P = 0.02$. Table 2), with low heterogeneity being observed. No difference was found between the *BRCA2* mutation carriers and the control groups for OS, RFS, or DMFS. Very few studies for *BRCA2* excluded stage IV breast cancer (Table 3).

The mean REMARK score was 15.6 (range 12–19) for OS studies, 16.3 (range 14–18) for BCSS studies, 13.6 (range 13–15) for RFS studies, and 18.3 (range 17–19) for DMFS studies. Meta-regression analysis did not demonstrate any association between REMARK score and all considered survival outcomes, as well as

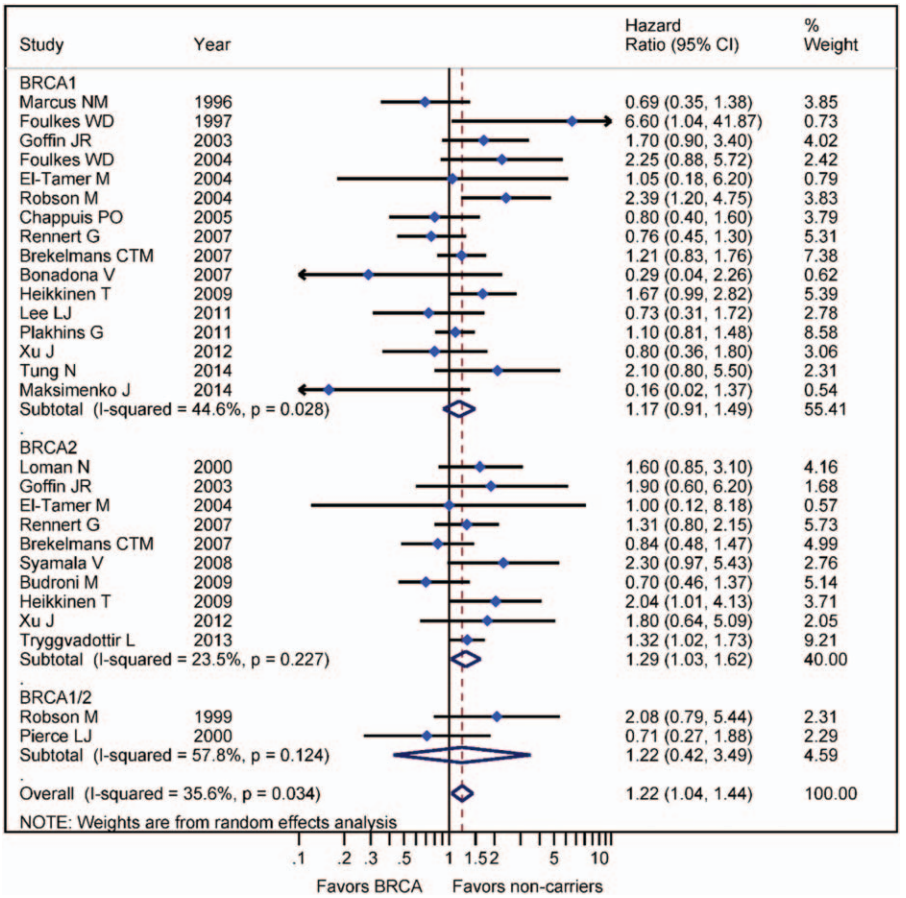

Figure 2. Forrest plot of breast cancer-specific survival by BRCA mutational status.

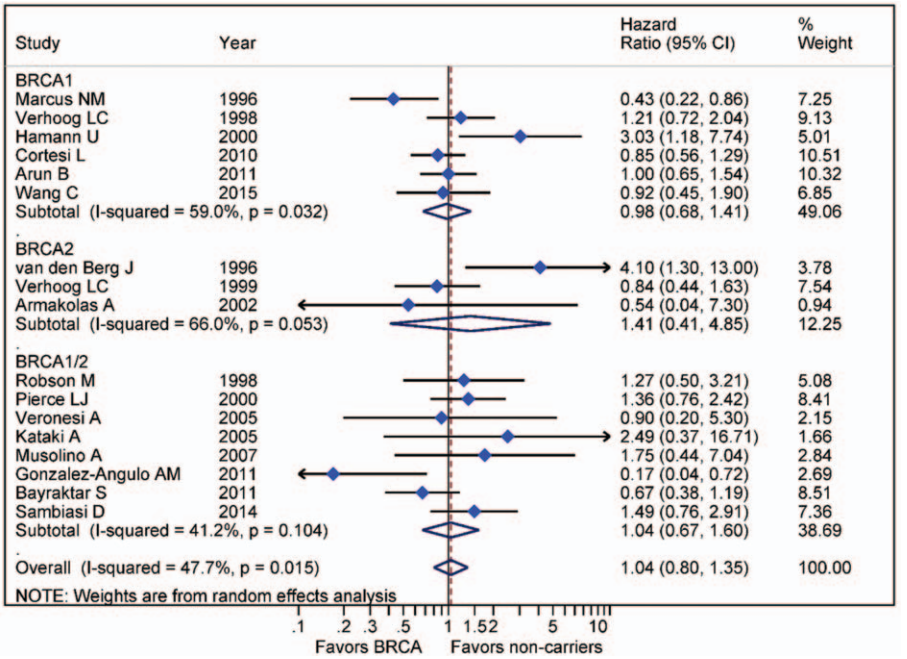

Figure 3. Forrest plot of recurrence-free survival by BRCA mutational status.

between year of publication and *BRCA2* effect on survival (data not shown).

3.4. Prognostic role of BRCA genes mutations

In order to reduce potential bias due to stringent inclusion criteria, we conducted meta-analysis that accounted for data of *BRCA1*-studies, *BRCA2*-studies and studies that pooled *BRCA1* and *BRCA2* carriers (*BRCA1/2*-studies). *BRCA* carriers were associated with worse OS (HR 1.19, 95% CI: 1.04–1.35; $P=0.009$. Table 2 and Fig. 1) and BCSS (HR 1.22, 95% CI: 1.04–1.44; $P=0.02$ Table 2 and Fig. 2). These results were also confirmed when the analysis was restricted to studies including only tested patients (Table 2) as well as to studies excluding stage IV (Table 3). RFS and DMFS of *BRCA* carriers were not significantly different of those of control group. Meta-regression analysis did not show any association between REMARK score or year of publication for the considered survival outcomes (data not shown).

3.5. Prognostic role of BRCA mutations in triple negative patients

Six studies (n = 1748) focused on triple negative patients: 4 out of these 6 studies evaluated the prognostic role of *BRCA1*

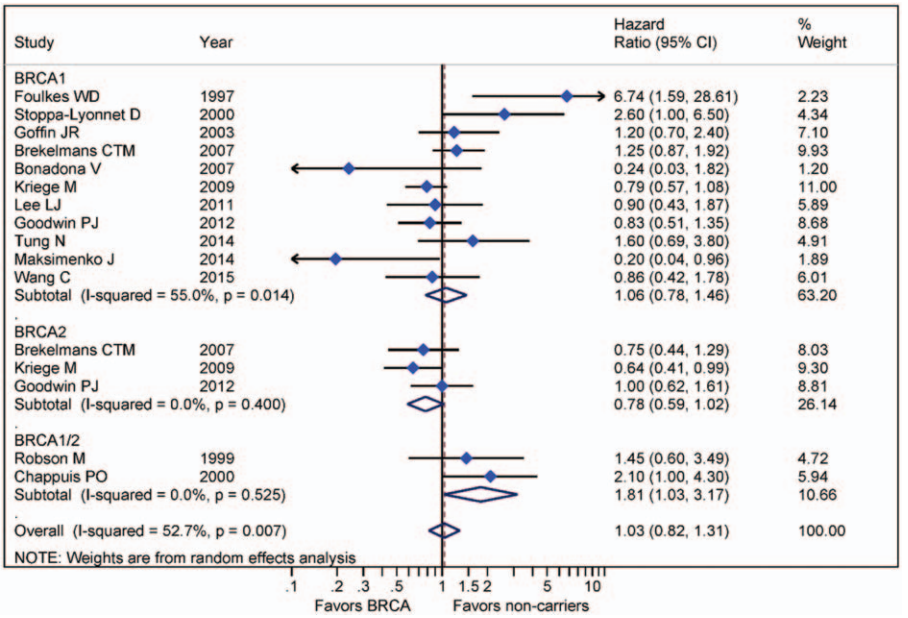

Figure 4. Forrest plot of distant metastasis-free survival by BRCA mutational status.

Table 4

Pooled estimates and heterogeneity analysis in Ashkenazi Jewish and triple negative breast cancer patients.

		Studies focusing on Ashkenazi Jewish (N = 12)				Studies focusing on triple negative BC (N = 6)			
	Outcome	Studies	HR (CI 95%)*	P	I^2	Studies	HR (CI 95%)*	P	I^2
BRCA1	OS	4	1.46 (0.91–2.34)	0.12	32%	0	–		–
	BCSS	7	1.45 (0.90–2.35)	0.13	57%	3	0.84 (0.27–2.62)	0.76	64%
	RFS	0	–		–	1	0.92 (0.45–1.90)	0.82	–
	DMFS	2	2.50 (0.47–13.31)	0.28	78%	4	0.87 (0.48–1.57)	0.65	43%
BRCA2	OS	2	1.20 (0.78–1.85)	0.42	0%	0	–	–	–
	BCSS	3	1.37 (0.88–2.13)	0.17	0%	0	–	–	–
	RFS	0	–	–	–	0	–	–	–
	DMFS	0	–	–	–	0	–	–	–
BRCA1/2	OS	0	–	–	–	2	0.49 (0.26–0.92)	0.03	0%
	BCSS	1	2.08 (0.79–5.44)	0.14	–	0	–		–
	RFS	1	1.27 (0.50–3.21)	0.61	–	2	0.40 (0.11–1.47)	0.17	67%
	DMFS	2	1.81 (1.03–3.17)	0.04	0%	0	–	–	–
Overall	OS	4	1.27 (0.99–1.64)	0.06	0%	2	0.49 (0.26–0.92)	0.03	0%
	BCSS	8	1.44 (1.05–1.97)	0.02	34%	–	–	–	–
	RFS	1	1.27 (0.50–3.21)	–	–	3	0.60 (0.30–1.19)	0.14	53%
	DMFS	4	1.82 (1.05–3.16)	0.03	42%	–			

BCSS = breast cancer-specific survival, CI = confidence interval, DMFS = distant metastasis-free survival, HR = hazard ratio, I^2 = between study-heterogeneity index, OS = overall survival, RFS = recurrence-free survival.

* When both univariate and multivariate HRs were reported, multivariate HR was chosen.

mutational status and 2 studies investigated the prognostic role of *BRCA1/2* mutational status. In all 6 studies, the genetic test was offered to entire study population. BCSS and DMFS of *BRCA1* mutation carriers did not differ from those of *BRCA1* negative triple-negative breast cancer patients (Table 4). *BRCA1/2* carriers had better OS than *BRCA*-negative triple-negative breast cancer patients (HR 0.49, 95% CI: 0.26–0.92; *P*-value = 0.03) but there were only 2 studies. The risk of recurrence was not statistically different between the *BRCA* carriers and *BRCA*-negative breast cancer cases (HR 0.60, CI: 0.30–1.19; *P*-value = 0.14).

3.6. Prognostic role of BRCA mutations in Ashkenazi Jewish women

Twelve studies focused on Ashkenazi Jewish women (n = 4161). Of these, 9 studies evaluated the prognostic role of *BRCA1* mutational status, 3 the prognostic role of *BRCA2* and 3 the prognostic role of *BRCA1/2* (Table 4). Taking into account data derived from all studies, *BRCA* mutation carriers showed a trend for worse OS (HR 1.27, 95% CI: 0.99–1.64; *P*-value = 0.06) and a higher risk of death from breast cancer (HR 1.44, 95% CI: 1.05–1.97; *P*-value = 0.02), and of distant metastasis (HR 1.82, 95% CI: 1.05–3.16; *P*-value = 0.03) than control group.

3.7. Publication bias

We found no evidence of publication bias in the 9 meta-analyses including at least 10 studies (See Supplemental Contents—Figures S2–S5, http://links.lww.com/MD/B313, which show funnel plots for publication bias). The only exception was represented by the meta-analysis of 27 studies investigating the relationship between *BRCA1* mutation status and overall survival, where we found some evidence that small studies were more likely to have larger effect (Egger's test *P*-value = 0.081).

4. Discussion

The prognostic role of BRCA germline mutational status in breast cancer patients is unclear. In the present meta-analysis, we collected and analyzed the largest series of patients so far reported in this field of investigation. Meta-analysis of the available evidence supports 4 main conclusions: (i) *BRCA1* carriers have worse OS than sporadic/*BRCA*-negative breast cancer cases; (ii) BRCA1 carriers have worse OS and BCSS than sporadic/*BRCA*-negative breast cancer cases among women with early stage breast cancer; (iii) BRCA2 carriers have worse BCSS than sporadic/*BRCA*-negative breast cancer cases; (iv) among Ashkenazi Jewish breast cancer patients, *BRCA* mutation carriers have higher risk of death from breast cancer and distant metastasis as compared with sporadic/*BRCA*-negative women.

The present analysis is based on data derived from a number of subjects (105,220 patients and 3588 *BRCA* mutation carriers) higher than those used in the previous 3 meta-analyses.[27–29] Moreover, the present work provides readers with a more comprehensive analysis of the association between *BRCA* mutational status and breast cancer prognosis compared with previous meta-analyses. First, we considered 4 clinical outcomes separately to investigate the relationship between *BRCA* mutational status and all prognostic outcomes: risk of death from any cause, death from breast cancer, any recurrence, and occurrence of distant metastasis. This kind of analysis was performed only by 1 previous meta-analysis,[29] whereas the other 2 meta-analysis evaluated only OS and RFS.[27,28] Second, we performed 4 pre-specified subgroup analyses never reported before. In particular, we think that the analysis focusing only in the studies where the entire study population was tested for *BRCA* mutations, allowed us to better and clearly define the relationship between *BRCA* mutational status and the outcomes in breast cancer women, because the experimental group of *BRCA* carriers was compared with true *BRCA*-negative patients.

We found that *BRCA1* mutation carriers had a 30% higher risk of dying than *BRCA1* negative/sporadic cases, which confirmed the results of 2 previous meta-analysis studies,[27,28] although our meta-analysis included many more studies. Of note, we found that the association between *BRCA1* and OS was stronger when excluding studies with sporadic breast cancer cases (e.g., nontested for BRCA mutations), which eliminated misclassification bias (HR = 1.46). Furthermore, the association between *BRCA1* and OS was stronger in studies excluding stage IV disease (HR = 1.55) than in studies that included stage IV disease (HR = 1.15). As regards BCSS, we did not find a statistically significant association between BRCA1 and the risk of death from breast cancer, with moderate heterogeneity across the studies. Interestingly, we found that the heterogeneity can be at least in part explained by study design: in fact, among the 8 studies including patients with stage IV disease, no evidence of association between *BRCA1* and BCSS was observed (HR = 0.94), whereas among the 8 studies excluding patients with state IV disease, we found that *BRCA1* was associated with 45% increased risk of dying from breast cancer (HR = 1.45). Thus, our findings suggest that the future prospective studies, which aim to define the role of *BRCA1* mutational status on breast cancer outcomes, should exclude patients with stage IV breast cancer, and offer the genetic test to entire study population allowing comparison between *BRCA* carriers and tested BRCA-negative cases.

With regard to BRCA2, we did not find a significant association between the mutational status of *BRCA2* and OS, which is consistent with the results of previous meta-analyses.[27–29] However, we demonstrated a significant association between *BRCA2* mutation and worse BCSS (HR = 1.29), a finding even stronger after excluding studies with sporadic breast cancer cases (HR = 1.34). Only van den Broek et al[29] evaluated the association between *BRCA2* and BCSS and concluded that *BRCA2* had worse BCSS compared with the control group (HR 1.57; CI: 1.29–1.86), but this analysis included only 2 studies, judged as high-quality studies according to a scoring system developed by the authors, without demonstrating that the quality of studies was an effect modifier. We evaluated the quality of studies using the REMARK checklist, a well-accepted tool to define the quality of studies evaluating a tumor marker. The REMARK score of the 10 studies considered in our analysis of the association between *BRCA2* and BCSS was high, with a mean score of 16.3 points out of 20. Moreover, we did not find any evidence that this association depended on the REMARK score. Therefore, we believe that our meta-analysis of 10 studies provided a more reliable estimate of the relationship between BRCA status and prognosis than just 2 studies considered by van den Broek's meta-analysis.

With the aim of comprehensively cover the topic of the prognostic role of BRCA mutations in breast cancer patients, we reported also the results derived from the analysis evaluating the combination of data from *BRCA1*-studies, *BRCA2*-studies, and *BRCA1/2* studies. However, considering that breast cancer has different characteristics in *BRCA1* and *BRCA2* carriers, and taking into account the different prognostic role of *BRCA1* and *BRCA2* mutations (as demonstrated by the analysis of data of *BRCA1*-studies and *BRCA2*-studies taken separately), we suggest that the future studies should differentiate *BRCA1* from *BRCA2* carriers. Moreover, an important question still unanswered is whether the prognosis of breast cancer in BRCA1 carriers is different from that in BRCA2 carriers, which warrants further investigation.

Another novel and interesting results of our work came from the meta-analyses performed in women with triple negative breast cancer and of Ashkenazi Jewish ancestry, 2 populations with high probability of being *BRCA* carriers. Concerning the analyses on women with triple negative breast cancer, we found that the presence of *BRCA* mutations correlates with better OS. However, this information derives from data of only 2 studies, where *BRCA1/2* carriers were compared with noncarriers women, suggesting the need of more studies in this subgroup of patients. In contrast, a sufficient number of studies have evaluated the prognostic role of *BRCA* mutations in Ashkenazi Jewish women, demonstrating a higher risk of dying from breast cancer as well as of developing distant metastasis of *BRCA* carriers compared with sporadic/*BRCA*-negative cases.

Although multivariable analyses have been used in many of the studies to adjust for age at diagnosis, tumor stage, estrogen receptor status, and other clinical factors, our meta-analysis still found that *BRCA* mutation carriers had worse survival outcomes than noncarriers, suggesting that the aggressive nature of breast cancer in mutation carriers may not be fully characterized by known clinical and pathological factors. It is also possible that other causes of deaths, in particular ovarian cancer, account for the decreased OS, especially for *BRCA1* carriers because lifetime risk of ovarian cancer is higher for *BRCA1* than for *BRCA2* mutation carriers.[5–7] As regard the effect of clinical and pathological factors as modifiers of survival in *BRCA* carriers, Templeton et al evaluated the interaction between hormonal receptor, age at diagnosis, and survival in patients with *BRCA1* and/or *BRCA2* mutations.[30] These authors identified only an inverse association between estrogen receptor status and OS in *BRCA1* carriers, concluding that the estrogen receptor expression is a modifier of prognosis in *BRCA1* carriers. Future studies are required to evaluate the role of hormone receptor status in *BRCA* carriers.

The quality of studies included in the present meta-analysis was moderate to high, ranging the REMARK score between 9 and 19 for *BRCA1*-studies, and between 14 and 19 for *BRCA2*-studies. However, we acknowledge that our meta-analysis presents some limitations. First, all studies are retrospective, which increases the risk of selection bias. Second, in order to eliminate confounding factors, we used adjusted HR when both univariate and multivariate HR were reported; however, for studies reporting only univariate analysis, unadjusted HRs were considered. Third, most of studies did not report important treatment information related to *BRCA* mutation carriers, such as prophylactic procedures (e.g., prophylactic mastectomy, bilateral mastectomy, bilateral prophylactic oophorectomy, treatment with tamoxifen) and diagnosis of second cancers that could affect survival outcomes in these patients. Fourth, despite a thorough literature search, we might have overlooked 1 or more publications on this topic; however, no publication bias was found in the meta-analyses.

The controversy on the prognostic value of *BRCA1/2* mutation in breast cancer patients is often related to the small sample size of single existing studies, as demonstrated by the fact that more than half of the 60 eligible studies included fewer than 40 carriers. Our work overcomes this limit and allows us to suggest that *BRCA1* and *BRCA2* mutation status has a significant prognostic value in early stage breast cancer, which supports *BRCA* mutation testing in patients with high risk of harboring *BRCA* germline mutations in order to better define the prognosis of these patients. Clearly, further perspective studies with larger sample size and unified study design and analysis method are desirable, especially in subgroups such as triple-negative breast cancer patients.

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