Clinical Cancer Research

Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients

Jie Sun[1], Hua Meng[1], Lu Yao[1], Meng Lv[2], Jian Bai[2], Jianguang Zhang[2], Lientu Wang[2], Tao Ouyang[1], Jinfeng Li[1], Tianfeng Wang[1], Zhaoqing Fan[1], Tie Fan[1], Benyao Lin[1], and Yuntao Xie[1]

Abstract

Purpose: The prevalence of mutations in cancer susceptibility genes such as *BRCA1* and *BRCA2* and other cancer susceptibility genes and their clinical relevance are largely unknown among a large series of unselected breast cancer patients in the Chinese population.

Experimental Design: A total of 8,085 consecutive unselected Chinese breast cancer patients were enrolled. Germline mutations in 46 cancer susceptibility genes were detected using a 62-gene panel.

Results: Pathogenic mutations were identified in 9.2% of patients among the 8,085 unselected breast cancer patients. Of these, 5.3% of patients carried a *BRCA1* or *BRCA2* mutation (1.8% in *BRCA1* and 3.5% in *BRCA2*), 2.9% carried other breast cancer susceptibility genes (BOCG) and 1.0% carried another cancer susceptibility genes. Triple-negative breast cancers had the highest prevalence of *BRCA1/2* mutations (11.2%) and other BOCG mutations (3.8%) among the four molecular subgroups, whereas ER^-/PR^-HER2^+ breast cancers had the lowest mutations in *BRCA1/2* (1.8%) and BOCG (1.6%). In addition, *BRCA1* mutation carriers had a significant worse disease-free survival [unadjusted hazard ratio (HR) 1.60; 95% confidence interval (CI) 1.10–2.34; $P = 0.014$] and disease-specific survival (unadjusted HR 1.96; 95% CI, 1.03–3.65; $P = 0.040$) than did non-carriers, whereas no significant difference in survival was found between *BRCA2* mutation carriers and non-carriers.

Conclusions: 9.2% of breast cancer patients carry a pathogenic mutation in cancer susceptibility genes in this large unselected series. Triple-negative breast cancers have the highest prevalence of mutations in *BRCA1 /2* and other breast cancer susceptibility genes among the four molecular subgroups, whereas ER^-/PR^-HER2^+ breast cancers had the lowest mutations in these genes. *Clin Cancer Res; 23(20); 6113–9. ©2017 AACR.*

Introduction

Next-generation sequencing provides a unique platform to rapidly screen germline mutations in multiple genes in a huge number of patients. Recently, germline mutations in *BRCA1/2* and other breast/ovarian cancer susceptibility genes detected by using multiple-gene panels assay have been reported in breast cancer patients, such as triple-negative breast cancer (TNBC) patients (1), early-onset or familial breast cancer patients (2–7), and those who are referred for *BRCA1/2* testing (8–10), and more recently in unselected breast cancer patients with a relatively small sample size ($n = 488$; ref. 11).

The prevalence of mutations in *BRCA1* and *BRCA2* or other cancer susceptibility genes and their phenotype and clinical relevance are not well investigated in a large unselected breast cancer series. It is largely unknown whether *BRCA1* and *BRCA2* mutation carriers are associated with poor survival. Some previous studies suggested that *BRCA1* mutation is associated with worse survival (12–18), others do not (19–24). More importantly, no studies so far are available for investigating the association between mutations in other breast cancer susceptibility genes and survival in large unselected breast cancer series. Another interesting issue is to explore whether molecular breast cancer subtypes based on estrogen receptor, progesterone receptor, and HER2 status could predict the prevalence of mutations in *BRCA1* and *BRCA2* and other breast cancer susceptibility genes.

In this study, we detected germline mutations in the 46 cancer susceptibility genes (including *BRCA1* and *BRCA2*) using a 62-gene panel in a consecutive unselected large cohort of 8,085 breast cancer patients. The 46 cancer susceptibility genes were selected based on their roles in the development of breast cancer and other cancers.

We investigated the frequency of germline mutation in *BRCA1* and *BRCA2* genes and other breast cancer susceptibility genes and another cancer susceptibility genes in the entire cohort and in subgroups according to breast cancer family history, age at diagnosis, or molecular subtype and further explored the associations between the mutation carriers and

[1]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Breast Center, Peking University Cancer Hospital & Institute, Beijing, P. R. China. [2]Berry Genomics, Building 9, No. 6 Court Jingshun East Road, Chaoyang District, Beijing, P. R. China.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

J. Sun and H. Meng contributed equally to this article.

Corresponding Author: Yuntao Xie, Peking University Cancer Hospital & Institute, Fucheng Road 52, Haidian District, Beijing 100142, China. Phone. 861088196362; Fax: 861088196362; E-mail: zlxyt2@bjmu.edu.cn

doi: 10.1158/1078-0432.CCR-16-3227

Translational Relevance

The prevalence of mutations in cancer susceptibility genes and clinical relevance are not well investigated in a large unselected breast cancer series. In this study, germline mutations in 46 cancer susceptibility genes were detected in 8,085 consecutive unselected Chinese breast cancer patients. We found that 9.2% of breast cancer patients carried a pathogenic mutation in this large unselected series, and of these, 5.3% of patients carried *BRCA1/2* mutations, 2.9% carried other breast cancer susceptibility genes, and 1.0% carried another cancer susceptibility genes. Triple-negative breast cancers had the highest prevalence of mutations in *BRCA1/2* genes (11.2%) and other breast cancer susceptibility genes (3.8%) among the four molecular subgroups, whereas ER^-/PR^-HER2^+ breast cancers had the lowest mutations in *BRCA1/2* (1.8%) and other breast cancer susceptibility genes (1.6%). Our finding may be useful to select the right breast cancer to receive multiple-gene panel testing.

clinicalpathological characteristics. Finally, we investigated whether mutations in *BRCA1* and *BRCA2* and other cancer susceptibility genes are associated with poorer survival compared with non-carriers in terms of disease-free survival (DFS) and disease-specific survival (DSS) in the entire cohort of 8,085 patients.

Materials and Methods

Study population

A total of 10,378 consecutive breast cancer patients were treated at the Breast Center of Peking University Cancer Hospital from October 2003 to May 2015. Among these, 8,085 cases have an enough genomic DNA quantity and quality drawn from blood samples to meet multi-gene panel analyses. The cohort of 8,085 breast cancer patients (91.7% were primary operable breast cancer with stage I to III disease) were unselected for age at diagnosis and family history of breast cancer. Family history of breast cancer is defined as the breast cancer patient had one or more breast and/or ovarian cancer patients in the first-, second-, or third-degree relatives; family history of any cancer is defined as the breast cancer patient had one or more cancer patients (any kind of cancers) in the first-, second-, or third-degree relatives.

Familial breast cancer is defined as breast cancer patients who had family history of breast and/or ovarian cancer; early-onset breast cancer (EBC) is defined as breast cancer patients who did not have family history of breast and/or ovarian cancer and were diagnosed at or before the age of 40 years; sporadic breast cancer (SBC) is defined as breast cancer patients who did not have a family history of breast and/or ovarian cancer and were diagnosed over the age of 40 years.

Tumor size was defined as the maximum tumor diameter measured by ultrasound at the time of diagnosis. The tumors were graded according to the modified Bloom–Richardson system. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status were determined using the breast tumor tissue obtained from a core-needle biopsy or taken from surgery. ER or PR immunostaining was considered positive when >1% of the tumor cells showed positive nuclear staining. HER2 positivity was defined as a score of 3+ via immunohistochemical staining or HER2 gene amplification via fluorescence *in situ* hybridization as described elsewhere (25). Informed written consent was obtained from all participants. The study was reviewed and approved by the Ethics Committee of Peking University Cancer Hospital (No. 2011KT12) and was performed in accordance with the Declaration of Helsinki.

Panel-based sequencing assay

Genomic DNA extracted from peripheral blood was captured target sequences using the SeqCap EZ hybridization and purification kit (Roche). Target sequencing was designed to cover all coding regions and intron–exon boundaries of the 62 cancer-gene panel (including 46 cancer susceptibility genes; Supplementary Table S1). Products were sequenced on a HiSeq 2500 (Illumina) to at least an average depth of 200-fold coverage, with a minimum of 30-fold required at every targeted position. Reads were aligned to the reference human genome GRCh37. Germline variations were called with GATK, Atlas2, and PlatyPus. Annotations were defined using ANNOVAR (http://annovar.openbioinformatics.org/en/latest/). Population allele frequencies were extracted from 1000 Genomes (http://www.1000genomes.org), dbSNP (version 138; http://www.ncbi.nlm.nih.gov/projects/SNP), and the Exome Variant Server (http://evs.gs.washington.edu/EVS).

Variant classification

In this study, we analyzed the genetic variants in 46 cancer susceptibility genes from the 62 cancer-gene panel. Only novel variants or variants with <1% population frequency in the 1000 Genomes were collected. The variant data were filtered to identify all protein-truncating mutations, including nonsense and frameshift mutations. For splice-site variants that have been verified by functional analyses from the literature were included. For synonymous, nonsynonymous, in-frame, and stop-loss variants, only variants classified as pathogenic or probably pathogenic by ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) were included in the analysis. The variant data were further assessed and classified according to American College of Medical Genetics and Genomics recommendations (26), with supporting data from function prediction software, public literature, and curated databases. Variants classified to be pathogenic or likely pathogenic were considered as pathogenic in this study. All the pathogenic mutations were validated by Sanger sequencing. If a patient carried more than one pathogenic mutation, mutation status was classified using the most damaging one when analyzed.

Statistical analyses

Categorical variables were compared between mutation carriers and non-carriers using the Chi-square test or the Fisher exact test, where appropriate. Continuous variables were tested with a *t* test. DFS was defined as from the time of diagnosis to first recurrence (local or distant), or contralateral breast cancer, or death from breast cancer (for patients without a recorded relapse) or date of last follow-up. DSS was defined as from the time of diagnosis to death from breast cancer or date of last follow-up. Survival was estimated using the Kaplan–Meier product-limit method and differences were tested for statistical significance using the log-rank test. A Cox proportional hazards model was used to determine whether a factor was associated with survival. Two-sided *P* values less than 0.05

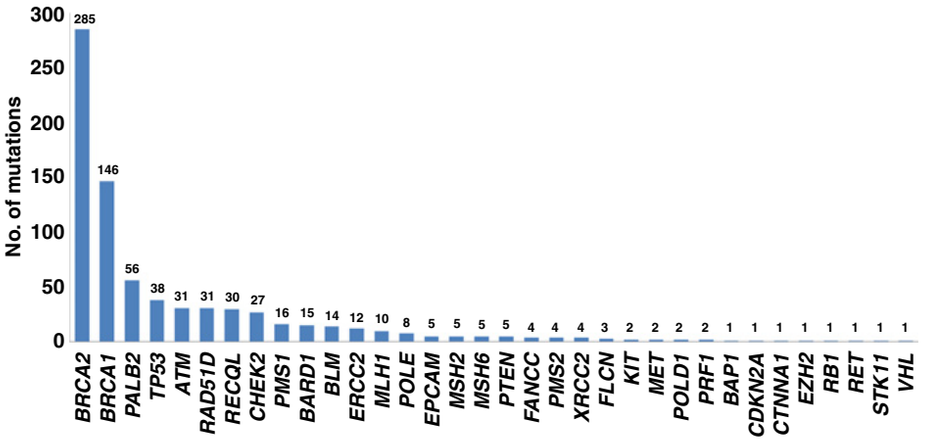

Figure 1.
Pathogenic mutations identified in 46 cancer susceptibility genes in 8,085 unselected breast cancer patients.

were considered to be statistically significant. All analyses were performed using SPSS 20.0 software.

Results

Prevalence of germline mutations in cancer susceptibility genes

Germline mutations in 46 cancer susceptibility genes were detected in a large cohort of 8,085 unselected breast cancer patients using the 62-gene panel. The clinicopathological characteristics of these patients are presented in Supplementary Table S2. A total of 770 pathogenic mutations were identified in 743 (9.2%) of the 8,085 unselected breast cancer patients (Fig. 1; Supplementary Table S3). Twenty-seven patients (0.3%) had more than one mutation (Supplementary Table S4). Biallelic mutations in *ATM* and *RAD51D* were found in two patients (Supplementary Table S4).

Four hundred and twenty-eight (5.3%) of the patients carried a germline mutation in *BRCA1* or *BRCA2* gene. Of these, 146 (1.8%) were in *BRCA1* and 285 were (3.5%) in *BRCA2*; three women carried deleterious mutations in both *BRCA1* and *BRCA2* genes. In addition, 237 (2.9%) patients carried a total of 256 pathogenic mutations in other breast and/or ovarian cancer susceptibility genes (BOCG) beyond *BRCA1* and *BRCA2* genes, with *PALB2* ($n = 56$), *TP53* ($n = 38$), *ATM* ($n = 31$), *RAD51D* ($n = 31$), *RECQL* ($n = 30$), and *CHEK2* ($n = 27$) ranked the most frequently mutated genes (Fig. 1).

Eighty-three (1.0%) patients carried pathogenic mutations in another cancer susceptibility genes in this cohort of 8,085 unselected breast cancer patients. Of these, 58 mutations were identified in gastrointestinal cancer susceptibility genes, in which 42 were in DNA mismatch repair (MMR) genes, 8 in *POLE*, 5 in *EPCAM*, 2 in *KIT*, and 1 in *CTNNA1*. The remaining 25 mutations were found in cancer susceptibility genes related to renal cancer ($n = 15$), hematologic tumors ($n = 6$), melanoma ($n = 3$), and thyroid tumors ($n = 1$; Supplementary Table S5). No mutations were found in *AKT1*, *PIK3CA*, *APC*, *CDH1*, *SMAD4*, *BMPR1A*, *AXIN2*, *MEN1*, *FH*, *CDK4*, and *EGFR* genes.

Germline mutations in cancer susceptibility genes in subgroups

BRCA1/2 mutation rates were 18.1% in familial breast cancer patients (FBC), 6.4% in EBC patients, and 3.3% in SBC patients, respectively (Table 1). The prevalence of *BRCA1/2* mutations in the FBC group was significantly higher than that of EBC and SBC subgroups. Overall, the *BRCA2* mutation rate was more pronounced than the *BRCA1* mutation rate (3.5% versus 1.8%) and also in each subgroup (Table 1). The mutation frequencies of other BOCG and another cancer susceptibility genes were 4.6% and 1.4% in FBC, 3.6% and 0.7% in EBC, and 2.5% and 1.0% in SBC, respectively (Table 1). Furthermore, the overall prevalence of pathogenic mutations in FBC, EBC, and SBC were 24.1%, 10.7%, and 6.8%, respectively (Table 1).

Next, we analyzed the pathogenic mutations in molecular subgroups based on ER, PR, and HER2 status (Table 2). The *BRCA1* mutation rate was significantly higher in ER^-/PR^-, $HER2^-$ (TNBC) than that of the other subgroups, representing 7.4% in the TNBC group (Table 2). *BRCA2* mutation rate was higher in ER^+ and/or PR^+, $HER2^-$ group than in other subgroups. Both *BRCA1* and *BRCA2* mutation rates were lower in ER^-/PR^-, and $HER2^+$ group than in other groups (Table 2). Furthermore, mutations in other BOCG were more frequent in the TNBC group (3.8%), and were less in the $ER^-/PR^-/HER2^+$ group (1.6%; Table 2). Mutations in another cancer susceptibility genes were not significantly different among the molecular subgroups (Table 2). Taken together, the overall prevalence of pathogenic mutations was the highest in the TNBC group (16.0%), following by ER^+ and/or PR^+, $HER2^-$ group (9.2%), ER^+ and/or $PR^+/HER2^+$ group (6.7%), and the $ER^-/PR^-/HER2^+$ group (4.7%; Table 2).

Table 1. Germline mutations in subgroups according to age at diagnosis and family history of breast cancer in this cohort of 8,085 unselected breast cancer patients

		Mutation cases (prevalence, %)				
	Patients, *N*	***BRCA1* (%)**	***BRCA2* (%)**	**Other BOCG (%)**	**Another mutation (%)**	**Total (%)**
All patients	8,085	146 (1.8)	282 (3.5)	237 (2.9)	78 (1.0)	743 (9.2)
FBC	805	59 (7.3)	87 (10.8)	37 (4.6)	11 (1.4)	194 (24.1)
EBC	1,317	31 (2.4)	53 (4.0)	48 (3.6)	9 (0.7)	141(10.7)
SBC	5,963	56 (0.9)	142 (2.4)	152 (2.5)	58 (1.0)	408 (6.8)

Abbreviations: Another mutation, mutations in another cancer susceptibility genes unrelated to breast cancer; BOCG, breast and/or ovarian cancer susceptibility genes; EBC, early-onset breast cancer (diagnosed at and before age 40); FBC, familial breast cancer; SBC, sporadic breast cancer.

Table 2. Germline mutations in molecular subgroups based on hormone receptor and HER2 status

		Mutation cases (prevalence, %)				
	Patients, *N*	***BRCA1* (%)**	***BRCA2* (%)**	**Other BOCG (%)**	**Another mutation (%)**	**Total (%)**
All patients	7,361	141 (1.9)	261 (3.5)	222 (3.0)	68 (0.9)	692 (9.4)
ER^+ and/or PR^+,$HER2^-$	4,438	47 (1.1)	190 (4.3)	134 (3.0)	39 (0.9)	410 (9.2)
ER^+ and/or PR^+,$HER2^+$	965	8 (0.8)	18 (1.9)	32 (3.3)	7 (0.7)	65 (6.7)
ER^-/PR^-,$HER2^+$	854	4 (0.5)	11 (1.3)	14 (1.6)	11 (1.3)	40 (4.7)
ER^-/PR^-,$HER2^-$	1,104	82 (7.4)	42 (3.8)	42 (3.8)	11 (1.0)	177 (16.0)

Abbreviations: Another mutation, mutations in another cancer susceptibility genes unrelated to breast cancer; BOCG, breast and/or ovarian cancer susceptibility genes; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; TNBC, triple-negative breast cancer.

Tumor characteristics and mutation status

The mean age at diagnosis of breast cancer was significantly younger for *BRCA1* carriers (44.8 years, $P < 0.001$), *BRCA2* carriers (47.8 years, $P < 0.001$), and other BOCG carriers (48.4 years, $P < 0.001$) when compared with non-carriers (51.4 years; Table 3). Both *BRCA1* and *BRCA2* mutation carriers and other BOCG mutation carriers were more likely to have a family history of cancer, especially a family history of breast and/or ovarian cancer (Table 3). Bilateral breast cancers were more frequent in *BRCA1* and *BRCA2* carriers compared with non-carriers (Table 3). *BRCA1* mutation carriers were significantly associated with high-grade tumor (*BRCA1* carriers versus non-carriers, 39.7% versus 15.3%, $P < 0.001$), and a triple-negative phenotype (58.2% versus 13.9%, $P < 0.001$; Table 3).

Survival

In this cohort of 8,085 unselected breast cancer patients, 114 patients with stage IV disease when administrated for treatment and 56 patients lost to follow-up were excluded for survival analysis, the remaining 7,915 (97.9%) primary operable breast cancer patients were then analyzed for survival. The median follow-up time of these 7,915 patients was 50 months (range, 1–152 months).

BRCA1 mutation carriers exhibited a significantly worse DFS and DSS than did non-carriers [DFS, unadjusted hazard ratio (HR) 1.60, 95% confidence interval (CI), 1.10–2.34, $P = 0.014$; DSS, unadjusted HR 1.94, 95% CI, 1.03–3.65, $P = 0.040$, respectively; Fig. 2; Supplementary Table S6]. Although *BRCA2* mutations showed a slightly poor DFS and DSS when compared with non-carriers, the differences did not reach significance (Fig. 2; Supplementary Table S6). Mutations in other BOCG or in another cancer susceptibility genes were not significantly associated with DFS and DSS. Furthermore, in multivariate analysis, *BRCA1* mutations did not show a poor survival after adjustment for age, tumor size, lymph node, tumor grade, ER, PR, HER2 status, and treatment; it did not reach a significance (Supplementary Table S7).

We then analyzed the associations between *BRCA1*, *BRCA2*, and other BOCG mutations and survival in molecular subgroups stratified by ER, PR, and HER2 status. However, no significant associations were found between mutation carriers and non-carriers in the subgroups (Supplementary Table S8).

Discussion

In this study, we found that 9.8% of patients carried at least one pathogenic mutation in the 46 cancer susceptibility genes in the 8,085 unselected breast cancer patients. To our knowledge, this is the most comprehensive study undertaken to investigate the germline mutations in multiple cancer susceptibility genes in a large consecutive unselected breast cancer series to date.

Our data showed that 5.3% of patients carried a *BRCA1* or *BRCA2* mutation in this large series. *BRCA1* and *BRCA2* mutation rates in unselected breast cancer patients detected by next-generation sequencing assay have been only reported in a few studies. The prevalence of *BRCA1/2* mutations in our current study is consistent with a recent report using multi-gene panel in a small series of 488 unselected breast cancer (6.1% in the entire cohort and 5.1% in non-Ashkenazi; ref. 11) and with the report from The Cancer Genome Atlas in 507 breast cancer patients (5.5%; ref. 27). Mutations in *BRCA2* were almost twice compared with mutations in *BRCA1* in our current large unselected series (3.5% vs. 1.8%), similar reports are also found in other Asian populations (28–31).

In addition, 2.9% of patients had a mutation in other breast cancer susceptibility genes in our large unselected series. Previous studies have reported the mutation rates in other breast cancer susceptibility genes using multi-gene panel assay in TNBC patients (3.7%; ref. 1) and in patients who are recommended for *BRCA1/2* testing based on current guidelines (mutation rate ranges from 2.9% to 3.9%; refs. 8, 9). Considering our patients are unselected, the prevalence of mutations in other breast cancer susceptibility genes in our series is remarkable. Our data suggested that the most frequently mutated genes in non-*BRCA1/2* breast cancer susceptibility genes were *PALB2*, *TP53*, *RAD51D*, and *ATM*, which are in agreement with most recent studies using a multiple-gene panel assay (1, 3, 5–7, 11, 32). However, the frequency of *CHEK2* mutation in our large unselected breast cancer series was lower when compared with the mutation rate in Caucasian women (9, 11, 33). The possible reason might be the frequency of *CHEK2* mutations depending on ethnic populations; for example, the *CHEK2* recurrent mutation, 1100delC, is frequently found in Caucasian women but not in Chinese women (33). In contrast, the frequency of *RAD51D* mutation in this study was higher than that of the reports in Caucasian women (7, 11); the p.K91fs mutation of the *RAD51D* gene that is very rare in Caucasian women was very common in our series, which may have a founder effect in Chinese women.

We also reported that 1.0% of patients carried mutations in another cancer susceptibility genes in this series, particularly in mismatch repair genes, where the mutations are closely linked to Lynch syndrome. Although the association between mutations in mismatch repair genes and breast cancer risk is not fully established, increasing evidence suggested that mutations in mismatch repair genes may not only confer to a high risk for colorectal cancer, but may also increase breast cancer risk (34–36).

Clinical factors such as early-onset age and family history of breast or ovarian cancer were significantly associated with mutations in *BRCA1/2* genes in our series. We also found that patients with a family history of breast cancer had a higher mutation rate in other breast cancer susceptibility genes than did SBC patients (4.6% vs. 2.5%). More importantly, germline mutation overall is

Table 3. Clinicopathological characteristics between mutation carriers and non-carriers in this cohort of 8,085 unselected breast cancer patients

Characteristics	Non-carriers ($n = 7342$) No. (%)	*BRCA1* carriers ($n = 146$) No. (%)	*BRCA2* carriers ($n = 282$) No. (%)	Other BOCG carriers ($n = 237$) No. (%)	Another mutation carriers ($n = 78$) No. (%)	P1	P2	P3	P4
Age at BC diagnosis, y									
Mean ± SD	51.4 ± 11.6	44.8 ± 9.3	47.8 ± 10.8	48.4 ± 11.9	51.0 ± 11.2	<0.001	<0.001	<0.001	0.750
≤40 years	1,274 (17.4)	51 (34.9)	76 (27.0)	57 (24.1)	10 (12.8)	<0.001	<0.001	0.008	0.293
>40 years	6,068 (82.6)	95 (65.1)	206 (73.0)	180 (75.9)	68 (87.2)				
Family history of any cancer						<0.001	<0.001	0.004	0.175
Yes	2,298 (31.3)	93 (63.7)	145 (51.4)	95 (40.1)	30 (38.5)				
No	5,044 (68.7)	53 (36.3)	137 (48.6)	142 (59.9)	48 (61.5)				
Family history of breast or ovarian cancer						<0.001	<0.001	<0.001	0.067
Yes	611 (8.3)	59 (40.4)	87 (30.9)	37 (15.6)	11 (14.1)				
No	6,731 (91.7)	87 (59.6)	195 (69.1)	200 (84.4)	67 (85.9)				
Tumor size						0.238	0.875	0.909	0.85
≤2 cm	3,167 (43.7)	55 (38.7)	121 (43.2)	100 (43.3)	33 (44.0)				
>2 cm	4,082 (56.3)	87 (61.3)	159 (56.8)	131 (56.7)	42 (56.0)				
Unknown	93	4	2	6	3				
ER status						<0.001	0.031	0.818	0.704
Negative	1,976 (28.1)	92 (64.3)	61 (22.2)	66 (28.8)	22 (30.1)				
Positive	5,050 (71.9)	51 (35.7)	214 (77.8)	163 (71.2)	51 (69.9)				
Unknown	316	3	7	8	5				
PR status						<0.001	0.017	0.961	0.838
Negative	2,453 (35.5)	96 (68.1)	77 (28.4)	78 (35.3)	26 (36.6)				
Positive	4,466 (64.5)	45 (31.9)	194 (71.6)	143 (64.7)	45 (63.4)				
Unknown	423	5	11	16	7				
HER2 status						<0.001	<0.001	0.065	0.758
Negative	4,980 (74.1)	130 (91.5)	234 (89.0)	179 (79.6)	50 (72.5)				
Positive	1,741 (25.9)	12 (8.5)	29 (11.0)	46 (20.4)	19 (27.5)				
Unknown	621	4	19	12	9				
TNBC						<0.001	0.317	0.034	0.590
Yes	927 (13.9)	82 (58.2)	42 (16.1)	42 (18.9)	11 (16.2)				
No	5,742 (86.1)	59 (41.8)	219 (83.9)	180 (81.1)	57 (83.8)				
Unknown	673	5	21	15	10				
Histology						0.002	0.338	0.136	0.795
Ductal	6,544 (89.1)	134 (91.8)	253 (89.7)	218 (92.0)	69 (88.5)				
Lobular	229 (3.1v	2 (1.4)	13 (4.6)	7 (3.0)	4 (5.1)				
Medullary	36 (0.5)	5 (3.4)	2 (0.7)	3 (1.3)	0 (0.0)				
Mucinous	158 (2.2)	1 (0.7)	4 (1.4)	2 (0.8)	1 (1.3)				
Other	375 (5.1)	4 (2.7)	10 (3.5)	7 (3.0)	4 (5.1)				
Grade						<0.001	0.979	0.685	0.730
I	596 (10.6)	1 (0.8)	9 (3.8)	13 (6.9)	9 (15.3)				
II	4,157 (74.1)	72 (59.5)	189 (80.8)	145 (76.7)	40 (67.8)				
III	860 (15.3)	48 (39.7)	36 (15.4)	31 (16.4)	10 (16.9)				
Unknown	1,729	25	48	48	19				
Lymph nodes status						0.273	0.065	0.603	0.185
Negative	5,030 (72.8)	107 (77.0)	180 (67.7)	161 (71.2)	46 (65.7)				
Positive	1,879 (27.2)	32 (23.0)	86 (32.3)	65 (28.8)	24 (34.3)				
Unknown	433	7	16	11	8				
DBC						0.001	<0.001	0.036	0.719
Yes	181 (2.5)	11 (7.5)	30 (10.6)	11 (4.6)	2 (2.6)				
No	7,161 (97.5)	135 (92.5)	252 (89.4)	226 (95.4)	76 (97.4)				

Abbreviations: Another mutation, mutations in other cancer susceptibility genes unrelated to breast cancer; BOCG, breast and/or ovarian cancer susceptibility genes; DBC, bilateral breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; PR, progesterone receptor; P1, *BRCA1* carriers vs. non-carriers; P2, *BRCA2* carriers vs. non-carriers; P3, other BOCG carriers vs. non-carriers; P4, other mutation carriers vs. non-carriers; TNBC, triple-negative breast cancer.

significantly associated with TNBC phenotype, representing most frequently mutation rate among the four molecular subgroups, particularly for *BRCA1* mutations. These data indicated that TNBCs exhibit genome instability and a more aggressive phenotype. In contrast, HER2-positive breast cancers exhibit the most less germline mutations among the four molecular subgroups not only in *BRCA1/ 2* genes, but also in other breast cancer susceptibility genes. Interestingly, HER2 is a stronger driver gene that could initially trigger tumorigenesis *per se*; this notion is well demonstrated in mouse models (37).

There is conflicting evidence (18, 19, 21, 23, 38) so far regarding survival of breast cancer patients who carry *BRCA1* or *BRCA2* mutations because previous studies are based on selected high-risk populations, small study populations, or patients with founder mutations. In our study, we are able to comprehensively analyze survival in patients who carried a mutation in *BRCA1* or

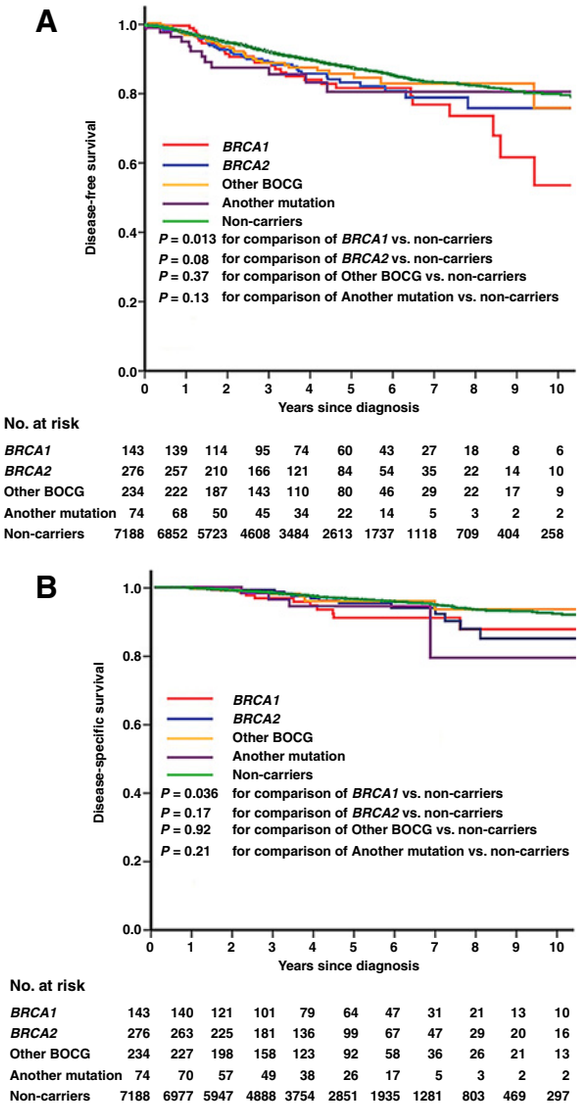

Figure 2.

Survival analyses by Kaplan–Meier according to mutation status in the 7,915 primary operable breast cancer patients. **A**, **B**, DFS and DSS by mutation status, respectively. Other BOCG, other breast, and/or ovarian cancer susceptibility genes; another mutation, mutations in another cancer susceptibility genes unrelated to breast cancer.

BRCA2 or other cancer susceptibility genes in a large consecutive unselected breast cancer series. We found that *BRCA1* mutation carriers had an unfavorable survival compared with non-carriers; this is not case for *BRCA2* mutation carriers and patients who carried mutations in other breast cancer susceptibility genes. Recent studies suggested that breast cancer patients with a mutation in *BRCA1/2* or other DNA repair genes are more likely to respond to poly-(ADP) ribose polymerase inhibitors (39, 40). Therefore, mutation carriers may benefit from the targeted therapy with optimal chemotherapy regimens.

There are two limitations in our study. First, although the sample size is large, it is a hospital-based population; second, the time of follow-up is relatively short (median 50 months); therefore, long-term follow-up is needed to investigate the survival in mutation carriers.

In conclusion, we found that 9.2% of breast cancer patients carry a pathogenic mutation in cancer susceptibility genes in this large consecutive unselected series. TNBCs have the highest prevalence of mutations in *BRCA1 /2* and other breast cancer susceptibility genes among the four molecular subgroups, whereas ER^-/PR^-HER2^+ breast cancers had the lowest mutations in these genes. Our findings have potential clinical implications, because strategies for early detection, prevention intervention, adjuvant chemotherapy regimens and targeted therapy are different between mutation carriers and non-carriers. As multiple-gene panel testing becomes more routine and cost-effective, our findings may be useful for selecting the right patients to receive multigene panel testing.

Disclosure of Potential Conflicts of Interest

J. Zhang is RD Director at Berry Genomics Ltd. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y. Xie
Development of methodology: J. Sun, H. Meng, M. Lv, J. Bai, J. Zhang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Sun, H. Meng, L. Yao, M. Lv, J. Bai, T. Ouyang, J. Li, T. Wang, Z. Fan, T. Fan, B. Lin, Y. Xie
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Sun, H. Meng, L. Yao, M. Lv, J. Bai, L. Wang, Y. Xie
Writing, review, and/or revision of the manuscript: J. Sun, H. Meng, Y. Xie
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Wang
Study supervision: Y. Xie

Grant Support

This study was supported by the National Natural Science Foundation of China (81372832), the National Science and Technology Support Program (2014BAI09B08), and the 973 project (2013CB911004).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 23, 2016; revised February 27, 2017; accepted July 13, 2017; published OnlineFirst July 19, 2017.

References

1. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol 2015;33:304–11.
2. Churpek JE, Walsh T, Zheng Y, Moton Z, Thornton AM, Lee MK, et al. Inherited predisposition to breast cancer among African American women. Breast Cancer Res Treat 2015;149:31–9.
3. Maxwell KN, Wubbenhorst B, D'Andrea K, Garman B, Long JM, Powers J, et al. Prevalence of mutations in a panel of breast cancer susceptibility genes in BRCA1/2-negative patients with early-onset breast cancer. Genet Med 2015;17:630–8.
4. Desmond A, Kurian AW, Gabree M, Mills MA, Anderson MJ, Kobayashi Y, et al. Clinical actionability of multigene panel testing for hereditary breast and ovarian cancer risk assessment. JAMA Oncol 2015;1:943–51.

5. Castera L, Krieger S, Rousselin A, Legros A, Baumann JJ, Bruet O, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. Eur J Hum Genet 2014;22:1305–13.
6. Li J, Meeks H, Feng BJ, Healey S, Thorne H, Makunin I, et al. Targeted massively parallel sequencing of a panel of putative breast cancer susceptibility genes in a large cohort of multiple-case breast and ovarian cancer families. J Med Genet 2016;53:34–42.
7. Thompson ER, Rowley SM, Li N, McInerny S, Devereux L, Wong-Brown MW, et al. Panel testing for familial breast cancer: calibrating the tension between research and clinical care. J Clin Oncol 2016;34:1455–9.
8. Kurian AW, Hare EE, Mills MA, Kingham KE, McPherson L, Whittemore AS, et al. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol 2014;32:2001–9.
9. Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer 2015;121:25–33.
10. Kapoor NS, Curcio LD, Blakemore CA, Bremner AK, McFarland RE, West JG, et al. Multigene panel testing detects equal rates of pathogenic BRCA1/2 mutations and has a higher diagnostic yield compared to limited BRCA1/2 analysis alone in patients at risk for hereditary breast cancer. Ann Surg Oncol 2015;22:3282–8.
11. Tung N, Lin NU, Kidd J, Allen BA, Singh N, Wenstrup RJ, et al. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. J Clin Oncol 2016;34:1460–8.
12. Foulkes WD, Wong N, Brunet JS, Begin LR, Zhang JC, Martinez JJ, et al. Germ-line BRCA1 mutation is an adverse prognostic factor in Ashkenazi Jewish women with breast cancer. Clin Cancer Res 1997;3:2465–9.
13. Ansquer Y, Gautier C, Fourquet A, Asselain B, Stoppa-Lyonnet D. Survival in early-onset BRCA1 breast-cancer patients. Institut Curie Breast Cancer Group. Lancet 1998;352:541.
14. Stoppa-Lyonnet D, Ansquer Y, Dreyfus H, Gautier C, Gauthier-Villars M, Bourstyn E, et al. Familial invasive breast cancers: worse outcome related to BRCA1 mutations. J Clin Oncol 2000;18:4053–9.
15. Foulkes WD, Chappuis PO, Wong N, Brunet JS, Vesprini D, Rozen F, et al. Primary node negative breast cancer in BRCA1 mutation carriers has a poor outcome. Ann Oncol 2000;11:307–13.
16. Eerola H, Vahteristo P, Sarantaus L, Kyyronen P, Pyrhonen S, Blomqvist C, et al. Survival of breast cancer patients in BRCA1, BRCA2, and non-BRCA1/2 breast cancer families: a relative survival analysis from Finland. Int J Cancer 2001;93:368–72.
17. Moller P, Borg A, Evans DG, Haites N, Reis MM, Vasen H, et al. Survival in prospectively ascertained familial breast cancer: analysis of a series stratified by tumour characteristics, BRCA mutations and oophorectomy. Int J Cancer 2002;101:555–9.
18. Robson ME, Chappuis PO, Satagopan J, Wong N, Boyd J, Goffin JR, et al. A combined analysis of outcome following breast cancer: differences in survival based on BRCA1/BRCA2 mutation status and administration of adjuvant treatment. Breast Cancer Res 2004;6:R8–R17.
19. El-Tamer M, Russo D, Troxel A, Bernardino LP, Mazziotta R, Estabrook A, et al. Survival and recurrence after breast cancer in BRCA1/2 mutation carriers. Ann Surg Oncol 2004;11:157–64.
20. Brekelmans CT, Seynaeve C, Menke-Pluymers M, Bruggenwirth HT, Tilanus-Linthorst MM, Bartels CC, et al. Survival and prognostic factors in BRCA1-associated breast cancer. Ann Oncol 2006;17:391–400.
21. Rennert G, Bisland-Naggan S, Barnett-Griness O, Bar-Joseph N, Zhang S, Rennert HS, et al. Clinical outcomes of breast cancer in carriers of BRCA1 and BRCA2 mutations. N Engl J Med 2007;357:115–23.
22. Bonadona V, Dussart-Moser S, Voirin N, Sinilnikova OM, Mignotte H, Mathevet P, et al. Prognosis of early-onset breast cancer based on BRCA1/2 mutation status in a French population-based cohort and review. Breast Cancer Res Treat 2007;101:233–45.
23. Huzarski T, Byrski T, Gronwald J, Gorski B, Domagala P, Cybulski C, et al. Ten-year survival in patients with BRCA1-negative and BRCA1-positive breast cancer. J Clin Oncol 2013;31:3191–6.
24. Brekelmans CT, Tilanus-Linthorst MM, Seynaeve C, vd Ouweland A, Menke-Pluymers MB, Bartels CC, et al. Tumour characteristics, survival and prognostic factors of hereditary breast cancer from BRCA2-, BRCA1- and non-BRCA1/2 families as compared to sporadic breast cancer cases. Eur J Cancer 2007;43:867–76.
25. Wang C, Zhang J, Wang Y, Ouyang T, Li J, Wang T, et al. Prevalence of BRCA1 mutations and responses to neoadjuvant chemotherapy among BRCA1 carriers and non-carriers with triple-negative breast cancer. Ann Oncol 2015;26:523–8.
26. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med 2015;17:405–24.
27. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.
28. De Leon Matsuda ML, Liede A, Kwan E, Mapua CA, Cutiongco EM, Tan A, et al. BRCA1 and BRCA2 mutations among breast cancer patients from the Philippines. Int J Cancer 2002;98:596–603.
29. Han SH, Lee KR, Lee DG, Kim BY, Lee KE, Chung WS. Mutation analysis of BRCA1 and BRCA2 from 793 Korean patients with sporadic breast cancer. Clin Genet 2006;70:496–501.
30. Ikeda N, Miyoshi Y, Yoneda K, Shiba E, Sekihara Y, Kinoshita M, et al. Frequency of BRCA1 and BRCA2 germline mutations in Japanese breast cancer families. Int J Cancer 2001;91:83–8.
31. Sugano K, Nakamura S, Ando J, Takayama S, Kamata H, Sekiguchi I, et al. Cross-sectional analysis of germline BRCA1 and BRCA2 mutations in Japanese patients suspected to have hereditary breast/ovarian cancer. Cancer Sci 2008;99:1967–76.
32. Aloraifi F, McDevitt T, Martiniano R, McGreevy J, McLaughlin R, Egan CM, et al. Detection of novel germline mutations for breast cancer in non-BRCA1/2 families. FEBS J 2015;282:3424–37.
33. Liu Y, Liao J, Xu Y, Chen W, Liu D, Ouyang T, et al. A recurrent CHEK2 p.H371Y mutation is associated with breast cancer risk in Chinese women. Hum Mutat 2011;32:1000–3.
34. Barrow E, Robinson L, Alduaij W, Shenton A, Clancy T, Lalloo F, et al. Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. Clin Genet 2009;75:141–9.
35. Engel C, Loeffler M, Steinke V, Rahner N, Holinski-Feder E, Dietmaier W, et al. Risks of less common cancers in proven mutation carriers with lynch syndrome. J Clin Oncol 2012;30:4409–15.
36. Win AK, Young JP, Lindor NM, Tucker KM, Ahnen DJ, Young GP, et al. Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. J Clin Oncol 2012;30:958–64.
37. Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell 1988;54:105–15.
38. Goodwin PJ, Phillips KA, West DW, Ennis M, Hopper JL, John EM, et al. Breast cancer prognosis in BRCA1 and BRCA2 mutation carriers: an International Prospective Breast Cancer Family Registry population-based cohort study. J Clin Oncol 2012;30:19–26.
39. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 2010;376:235–44.
40. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res 2006;66:8109–15.