Characteristics of breast cancer patients tested for germline BRCA1/2 mutations by next-generation sequencing in Ramathibodi Hospital, Mahidol University

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Research Article

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Abstract

Germline mutations in BRCA1/2 are the most common cause of hereditary breast and ovarian cancer (HBOC) syndrome. A few studies have reported the prevalence of germline BRCA mutations in Asian patients with breast cancer. Here, we aimed to explore the prevalence and characteristics of breast cancers in Thai patients with germline BRCA1/2 mutations. We retrospectively reviewed breast cancer patients tested for germline BRCA1/2 mutations in our institute during 2014–2018. BRCA mutations were detected using next-generation sequencing and confirmed using Sanger sequencing. We analyzed the characteristics of patients with or without BRCA mutations, disease-free survival (DFS), and associated factors. Among the 67 included patients, 12 (18%) were BRCA1/2 carriers (6 each), 4 (6%) harbored variants of uncertain significance, and 51 (76%) were non-carriers. We discovered two novel frameshift mutations in BRCA2 (c.2380delA and c.8855dupT). Mean ages at breast cancer diagnosis in BRCA1 carriers, BRCA2 carriers, and non-carriers were 39.8, 46.2, and 42.0 years, respectively. The 12 tumors of BRCA carriers were mostly the luminal-B subtypes. Two of these tumors were HER2-positive luminal-B; however, the triple-negative subtype was not observed. After adjusting for stages and luminal subtypes, BRCA carriers experienced worse 3-year DFS than non-carriers (81.5% vs. 90.3%, HR 2.04 (0.64–6.49), P = 0.229). The stage at diagnosis was the sole factor significantly associated with 3-year DFS (100%, 84.8%, and 72.7%; stages I, II, and III, respectively). In summary, breast cancers in Thai patients with germline BRCA1/2 mutations were mostly the luminal-B subtypes and experienced a worse prognosis than those without mutations.

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females in most countries [1]. In Thailand, breast cancer is the third most frequent cancer and the third most common cause of cancer death of both sexes [2]. Breast cancer, which primarily occurs in women and 1% of men, is typically acquired through multistep accumulations of somatic mutations, whereas 5–10% of breast cancers are inherited through germline mutations. Germline mutations in the breast cancer susceptibility genes, BRCA1 and BRCA2, are the most common causes of hereditary breast and ovarian cancer (HBOC) syndrome [3]. A large prospective study found that women who inherit deleterious germline mutations of BRCA1 or BRCA2 have very high cumulative risks for developing breast cancer (e.g., risks to 80-year-olds are 72% and 69% for BRCA1 and BRCA2 carriers, respectively) [4].

The main characteristics of patients with breast cancer with BRCA mutations include a family history of breast cancer, younger age at diagnosis, male breast cancer, or multiple tumors (bilateral breast cancer or breast and ovarian cancer) in the same patient [5]. Furthermore, patients with BRCA1 mutations are most commonly younger at diagnosis and associated with the triple-negative breast cancer (TNBC) subtype. In contrast, patients with BRCA2 mutations are mainly associated with estrogen receptor (ER)-positive breast cancer [6–8]. Furthermore, a more advanced stage at diagnosis and the presence of multiple foci of breast cancers are more common in patients with BRCA1 and BRCA2 mutations compared with patients with sporadic tumors. Abundant data show that patients with breast cancer with BRCA1/2 mutations (BRCA carriers) experience higher mortality rates than non-carriers [5, 8].

BRCA1 and BRCA2 are tumor suppressor genes located at chromosome 17q21 and 13q12.3, respectively [9–12]. BRCA1 and BRCA2 proteins are essential for repairing DNA-double strand breaks (DSBs) by homologous recombination, cell growth regulation, and control of cell division [3, 13, 14]. Genetic alterations of these genes occur in 5% of all breast cancers and 15–25% of familial breast cancers worldwide [7, 15]. The prevalence and phenotype of BRCA mutations vary according to country and race. The prevalence of BRCA mutations depends on the risk of breast cancer development; namely, a low prevalence in patients with sporadic breast cancer, but higher in selected high-risk cases such as breast cancer with a strong family history, bilateral breast cancer, and multiple organ cancer. Only a few studies have investigated the prevalence of BRCA mutations in Asian patients with breast cancer. Data from Korea shows a BRCA1/2 prevalence of 8.9% in high-risk patients without family history and 22.3% in patients with family history [16]. In China, the prevalence of BRCA1/2 in high-risk patients is 9.1%, but only 3.5% in sporadic breast cancer patients [17]. Similarly, there is limited data on the prevalence and characteristics of BRCA-associated breast cancer in Thailand. Only two studies have investigated BRCA1/2 mutations in selected Thai patients with breast cancer with and without familial history of HBOC [18, 19]. One group of investigators in Thailand used the Multiplex Ligation dependent Probe Amplification (MLPA) method to screen for BRCA1/2 large genomic rearrangement in Thai patients with familial breast cancer; they only found BRCA1 alteration in 1% of high-risk patients with breast cancer [19].

Sanger sequencing is the gold standard for germline BRCA mutation testing. Unfortunately, Using Sanger sequencing in a routine setting is considered too time-consuming and costly due to the nature of germline BRCA1/2 mutation, which is not clustered in any specific exon region. Next-generation sequencing (NGS) offers a promising alternative for BRCA testing because it takes a shorter turnaround time and relatively inexpensive compared with conventional Sanger sequencing. However, NGS requires a dedicated system operated by skilled technicians and requires complex data analysis.

Before the approval of a poly(adenosine diphosphate [ADP]–ribose) polymerase (PARP) inhibitor for patients with breast cancer with BRCA mutations [20], few testing sites were available in Thailand. This is likely explained by the limited number of testing laboratories, the lack of geneticists offering pre-and post-test counseling, and costly out-of-pocket payments.

The Center for Medical Genomics (CMG) Ramathibodi Hospital started using NGS for routine BRCA mutation testing in 2014. The number of BRCA mutation testing services in Thailand recently increased since the approval of PARP inhibitors as the treatment for cancers with BRCA mutations. In the present study, we explored the prevalence and characteristics of breast cancers in Thai patients tested for germline BRCA1/2 mutations.

Methods

Patients

We first screened all patients tested for germline mutations of BRCA1 and BRCA2 at the CMG Ramathibodi Hospital from January 2014 to December 2018. We excluded patients with cancers other than breast cancer or patients with breast cancer who enrolled in other clinical studies; we also excluded those whose

data in the electronic medical records (EMRs) of Ramathibodi Hospital were unavailable. The EMRs were retrospectively reviewed. We collected demographic data at diagnosis, including sex, age, and Eastern Cooperative Oncology Group (ECOG) performance status as well as TNM staging according to AJCC 8th edition [21]. Besides, we also collected tumor characteristics, including pathological subtype, histological grading, ER, progesterone receptor (PR), human epidermal growth factor receptor (HER2), and Ki-67 status as described in the pathological reports. There are some variations from time to time in the reporting systems for ER and PR immunohistochemistry (IHC) staining. In the present study, ER and PR were considered negative if the nuclei of tumor cells were stained < 1%. HER2 expression, which was routinely assessed through IHC staining intensity, was classified as negative, 1+, 2+, or 3+. Furthermore, fluorescence in situ hybridization (FISH) was performed to confirm and document registration for trastuzumab reimbursement. Luminal subtypes were classified according to the Saint Gallen Guidelines 2015 [22]. Treatments and outcomes such as surgical management, adjuvant therapies, recurrent/metastasis of disease, secondary primary cancer, and death were also collected.

The Ethics Committee on Human Rights related to research involving human subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University approved this study (MURA2019/988).

Analysis of germline mutations in BRCA1 and BRCA2 detected by NGS

Genomic DNA was extracted from the peripheral blood of patients. Analyses of germline mutations of BRCA1 and BRCA2 were performed using NGS at the CMG. All coding regions in BRCA1 and BRCA2 were sequenced, aligned with the Homo sapiens genome assembly GRCh37 (hg19) published by the Genome Reference Consortium [23, 24]. Sequence variants were classified in decreasing order of clinical importance as “Pathogenic,” “Likely pathogenic,” “Variants of Uncertain Significance (VUS),” “Likely benign,” or “Benign,” according to the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines [25]. Selected variants identified as “Pathogenic” or “Likely pathogenic” were confirmed using Sanger sequencing.

Statistical analysis

Descriptive statistical analysis was used to describe patients’ characteristics. Clinical characteristics were expressed as numbers and percentages for categorical variables, and continuous data were expressed as the mean ± standard deviation (SD). Comparisons of characteristics between carriers of BRCA1/2 and non-carriers were performed using Fisher’s exact test for categorical variables and one-way analysis of variance for continuous variables; P-value < 0.05 indicates a significant difference. Disease-free survival (DFS) was defined as the time from the date of diagnosis to the date of the first event (local recurrence or distant metastasis, or second primary cancer, or death from any cause). Patients who were alive without disease recurrence were censored at the cut-off date (December 31, 2019). Median DFS was estimated using the Kaplan–Meier method. To identify prognostic factors of DFS, we applied Cox regression analysis. BRCA status, stage at diagnosis, and luminal subtypes, along with variables with P-value less than 0.1 from the univariate Cox regression model, were adjusted by multivariate analysis. Stata software, version 16, was applied to perform all analyses [26].

Results

Pathogenic germline mutations of BRCA1/2

One hundred forty-two patients had been tested for germline mutations of BRCA1/2 by NGS at CMG from January 2014 to December 2018. Seventy-five patients were excluded for reasons, as described in **Fig. 1**. Finally, 67 patients with breast cancer were included in this study. The majority of the included patients (51, 76.1%) were regarded as high risk for HBOC (diagnosed with breast cancer at ≤ 40 years or TNBC at ≤ 60 years, male breast cancer, bilateral breast cancer, or multiple organ cancers).

Pathogenic BRCA mutations confirmed using Sanger sequencing were detected in 12 (17.9%) patients (6 BRCA1 carriers and 6 BRCA2 carriers). VUS were detected in 4 (6.0%) patients, and the others were BRCA non-carriers. Among the 12 mutations identified, 5 were frameshifts, 4 were nonsense, 2 were at splice acceptor positions, and 1 was missense. The mutations in BRCA1 were clustered in specific exonic regions, and the mutations of BRCA2 were more widely distributed across exonic regions. The positions of these BRCA1/2 mutations were shown in **Fig. 2**. Among the 6 pathogenic mutations in BRCA1, 3 were located in exon 10 and 3 in exon 16. Among the 6 pathogenic mutations of BRCA2, 3 were located in exon 11, 2 in exon 22, and 1 in exon 25.

We discovered two novel frameshift mutations in BRCA2 [c.2380delA (p.Met794Cysfs) and c.8855dupT (p.Met2952Ilefs)]. We detected the another BRCA2 mutation [c.8888_8889insA (p.Arg2964Lysfs)] reported in previous studies from China and Singapore [27, 28]. The other nine mutations detected here were reported in previous studies of patients residing in Asia, the United States, and Europe (ClinVar [29] or HGMD databases [30]). The characteristics of the BRCA carriers and associated mutations were presented in Table 1. We detected 5 VUS of BRCA2 in 4 of 67 (6.0%) patients. All were missense mutations, and one of the patients carried two VUS. VUS were not detectable in BRCA1 (**Fig. 2**).

Patients' Clinical Characteristics And Dfs

Among 67 patients, 98.5% were female, except one male with breast cancer who was a BRCA non-carrier. The mean age at diagnosis of breast cancer was 42.0 ± 10.3 years (BRAC1, 39.8 ± 7.4 years; BRCA2, 46.2 ± 16.0 years). Among 10 (14.9%) patients with bilateral breast cancer, 4 had synchronous lesions, and 6 had metachronous lesions. Most patients presented with early-stage breast cancer, and 50% had stage II disease. BRCA carriers and VUS patients tended to be luminal B subtypes, whereas the most common tumor subtype of non-carriers was TNBC (27.4%), followed by the HER2-negative luminal-B subtype (21.6%). Patients’ demographic data were presented in Table 2.

Surgery was performed on 98.5% of patients, including mastectomy (n = 51) and breast-conserving surgery (BCS) (n = 15). Almost all BRCA carriers underwent mastectomy, except one who underwent BCS and was subsequently found to harbor a BRCA mutation. Adjuvant radiotherapy was administered to 56.7% of patients for locally advanced breast cancer (T3–T4) and lymph node metastasis. Adjuvant chemotherapy was provided to 66% of patients; 19 (28.4%)

patients received only anthracycline-based chemotherapy, 16 (23.9%) patients received sequential treatment with anthracycline-based followed by a taxane, 2 patients received taxane-based chemotherapy, and 7 patients received other regimens. Platinum-based adjuvant chemotherapy was not used. Patients with hormone receptor-positive breast cancer underwent adjuvant endocrine therapy. Trastuzumab was used as a 1-year adjuvant treatment for 16 confirmed HER2-positive patients.

Among 12 tumors of BRCA carriers, 8 were the HER2-negative luminal-B, and 2 were the HER2-positive luminal-B subtype. Unexpectedly, the TNBC subtype was not observed among the BRCA carriers. The clinicopathological features of BRCA1 and BRCA2 carriers were summarized in Table 3.

The results of subgroup analysis of BRCA non-carriers were shown in **Supplementary Table S1**. Overall, there were no significant differences in clinical and pathological characteristics between early-onset (≤40 years) and late-onset (> 40 years) patients; however, early-onset patients received adjuvant chemotherapy at a significantly higher frequency compared with late-onset patients (P = 0.017). Early-onset patients tended to have higher grades and stages compared with late-onset patients.

At the cut-off date (December 31, 2019), the median follow-up was 2.7 years (range 0.2–17.2 years), 51 patients were disease-free, 16 experienced a second primary tumor (n = 8), distant metastases (n = 7), or local recurrence (n = 1). Among the seven patients who developed metastasis, 2 were BRCA carriers, 1 had VUS, and 4 were BRCA non-carriers; 6 patients received palliative chemotherapy, and 1 patient received palliative endocrine therapy. One BRCA carrier received platinum-based chemotherapy followed by a PARP inhibitor, and another BRCA carrier received anthracycline and cyclophosphamide.

The 3-year DFS rate was 87.7% (Table 4). BRCA1/2 carriers had lower 3-year DFS rates vs non-carriers, although the difference was not statistically significant (81.5% vs 90.3%, hazard ratio (HR) 95% confidence interval (CI), 2.04 (0.64–6.49); P = 0.229). Similarly, patients with VUS had inferior 3-year DFS compared with non-carriers (79.0% vs 90.3%; HR (95% CI), 1.60 (0.20–12.99); P = 0.658). TNM staging was a significant prognostic factor for 3-year DFS, with higher stage associated with lower 3-year DFS. Three-year DFS rates were 100%, 84.8%, and 72.7% for stages I, II, and III, respectively, with HR (95% CI), 2.88 (0.73–11.33); P = 0.131 and 6.82 (1.12–41.33); P = 0.037 for stages II and III compared with stage I, respectively.

After adjusting for the TNM stage and luminal subtypes using multivariate Cox regression analysis, BRCA1/2 carriers had inferior DFS compared with non-carriers, although the difference was not statistically significant, HR (95% CI), 2.50 (0.48, 12.97); P = 0.275. Similarly, patients with VUS had inferior DFS compared with non-carriers, HR (95% CI), 1.25 (0.12–12.81); P = 0.852. After adjustments in multivariate analysis, the TNM stage at diagnosis remained significantly associated with 3-year DFS. Stages II and III had inferior DFS compared with stage I, HR (95% CI), 6.32 (0.90–44.48); P = 0.064 and 16.30 (1.38–192.81); P = 0.027, respectively (Table 4).

Discussion

Among 67 patients included in the present study, we detected 6 (8.95%) BRCA1 carriers, 6 (8.95%) BRCA2 carriers, 4 (6.0%) patients with VUS, as well as 51 (76.1%) non-carriers. The 12 BRCA carriers had tumors mostly with luminal-B subtypes (8 HER2-negative luminal-B, 2 HER2-positive luminal-B, and 2 unclassified because of missing data for hormonal receptor and HER2). BRCA carriers had inferior 3-year DFS compared to non-carriers, although the difference was not statistically significant after adjusting for TNM stage and luminal subtype. The TNM stage at diagnosis was only the significant factor associated with 3-year DFS (higher stage lower 3-year DFS).

The majority of the previous reports from Thailand focused on BRCA in ovarian cancer [31,32]. In contrast, limited data are available regarding BRCA mutations in Thai patients with breast cancer. A few studies conducted in Thailand have included breast cancer in their report; one study investigated in the highly selective patients with breast and/or ovarian cancer with strong familial history of HBOC [18], and the other study screened for BRCA1/2 large genomic rearrangement with MLPA method in high-risk patients with familial breast cancer [19]. Our present retrospective study documents the prevalence and clinical characteristics of Thai patients with breast cancer, including BRCA carriers, non-carriers, and those with VUS as detected by NGS. Although most studies on BRCA1/2 mutations involved non-Asian populations, retrospective studies of high-risk Asian populations showed the frequencies of BRCA1 and BRCA2 mutations between 2.3%–18.6% and 2.3%–11.4%, respectively [7]. Furthermore, data from Korea demonstrated the prevalence of BRCA1/2 carriers in high-risk patients without or with familial history of breast cancer between 8.9-22.3% [16]. The prevalence of BRCA1/2 carriers in the Chinese population is 9.1% [17]. Here we showed the frequencies of BRCA1 and BRCA2 mutations in high-risk Thai patients at 8.95% and 8.95%, respectively, consistently with the previous studies [7,16,17].

It is well accepted that family history, age at diagnosis, and race are predictive factors for the probability of an individual carrying a germline BRCA1/2 mutation [33,34]. Whereas the mean age at diagnosis of breast cancer in BRCA1/2 carriers and non-carriers was similar in the present study, the age at diagnosis of breast cancer in BRCA1 carriers was lower than that of BRCA2 carriers (39.8 years vs. 46.2 years), consistently with previous studies showing the age at diagnosis of breast cancer in BRCA1 and BRCA2 carriers ranging between 30–45 years [7,35]. BRCA1/2 carriers have a higher risk of developing contralateral or second primary breast cancer, with long term-risks ranging from 60%–70% [35]. In the present study, we found 3 out of 12 patients (25%) with BRCA1/2 mutations that had bilateral breast cancer (one with metachronous bilateral breast cancer and two with synchronous bilateral breast cancer). This was siwmilar to a previous study in Korea finding 22.1% of BRCA1/2 carriers with bilateral breast cancer [36]. Breast cancer in BRCA1 carriers is typically associated with the TNBC subtype, and incidence of TNBC is higher in younger patients and lowered in the elderly [7,35,37]. Nevertheless, hormone receptor-positive breast cancers in BRCA1 carriers are detectable in the elderly and postmenopausal patients [38,39]. HER2 overexpression has been detected only in 0-8% of BRCA-associated breast cancer, lower than in sporadic breast cancer [35]. However, in the present study, all breast cancers of BRCA1 carriers were mostly the HER2-negative luminal-B subtype, the patients were younger (27–46 years), and the TNBC subtype was not observed. Furthermore, BRCA2 carriers in the present study had both HER2-negative luminal-B tumors and HER2-positive luminal-B tumors. Therefore, we should consider testing for BRCA germline mutation in patients suspected of HBOC presenting with luminal-B subtype breast cancer; this is relevant because BRCA carriers are candidates for specific

treatments (platinum-based chemotherapy or PARP inhibitors) that increase response rates and prolong survival. Such patients with BRCA mutations are candidates for screening for BRCA-associated cancer other than breast cancer to detect the early-stage disease.

The outcomes of breast cancer in BRCA carriers were conflicting. Previous systematic reviews and a meta-analysis found significantly worse overall survival (OS) of BRCA carriers than non-carriers, although the difference in recurrence-free survival is not statistically significant [40,41]. Other studies conducted in France and Switzerland found significantly superior 5-year DFS of BRCA1 carriers, although 5-years DFS of BRCA2 carriers is not significantly better than non-carriers [42]. In contrast, a study conducted in Korea found significantly inferior 10-year DFS and more contralateral breast cancer in BRCA1/2 carriers than non-carriers, but no significant difference in 10-year OS [43]. Furthermore, a study of Korean patients found that clinical nodal status is the only significant factor associated with DFS, even after adjusting for clinical nodal stage, BRCA status, hormonal receptor status, and Ki-67. Consistent with these findings, in the present study, adjusted multivariate analysis revealed that the only significant factor associated with DFS was the stage at diagnosis, whereas BRCA1/2 carriers had inferior DFS compared with BRCA non-carriers; however, the difference was not statistically significant.

The strength of this study is the use of reliable and comprehensive NGS techniques to analyze BRCA sequences. Using NGS, novel genetic alterations could be found and graded. However, our study has some limitations, such as its retrospective analysis of patients tested at a single institution which primarily relied on affordable patients despite the indications for germline BRCA testing according to the NCCN guideline. Thus, it probably not represents the true prevalence of BRCA mutation in Thai patients. Moreover, we could not identify a significant association between BRCA status and clinical outcomes because of the study's relatively small sample size. To better identify the actual prevalence of germline BRCA mutations in Thai breast cancer patients, a multicenter prospective study with a larger number of subjects is required.

In summary, this study describes characteristics, treatment outcomes, and prognostic factors of patients with breast cancer treated at a single university hospital in Thailand. We found that breast cancer in BRCA carriers was significantly associated with the luminal-B subtype, and BRCA1 and BRCA2 carriers showed a trend of inferior 3-year DFS than non-carriers. A longer follow-up of this study is required to determine the long-term survival outcomes.

Declarations

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Conflict of Interests

All authors declare no conflict of interests.

Competing interests

The authors declare that they have no competing interests.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Songporn Oranratnachai (SO): Methodology, Formal analysis, Visualization, Writing – Original draft, and contributed to every draft thereafter. Watchalawalee Yamkaew (WY): Data curation, Visualization, Writing – Original draft, and contributed to every draft thereafter. Atchara Tunteeratum (AT): Supervision and contributed to later drafts of the manuscript. Thongchai Sukarayothin (TS): Supervision and contributed to later drafts of the manuscript. Nareenart Iemwimangsa (NI): Resource, Formal analysis, and contributed to later drafts of the manuscript. Ravat Panvichien (RP): Conceptualization, Writing – Review and editing, and contributed to later drafts of the manuscript. All authors analyzed the results, read, and approved the manuscript for submission.

Ethics approval and consent to participate

The Ethics Committee on Research involving Human Subjects of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University approved this study. As this was a retrospective study of anonymized patients' data, informed consent was not required.

Consent for publication

Not applicable

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Tables

Table 1 Details of breast cancer in BRCA carriers

Age (years)	Sex	Histology	Luminal subtype	Stage	Uni/Bilateral	2nd primary cancer	Recurrence/ Metastasis	BRCA1/2 mutations			
								BRCA1/2 gene	Nucleotide change	Protein change	T
40	F	IDC	Unknown	IA	Unilateral	Ovarian cancer		BRCA1	c.5072C>A	p.Thr1691Lys	M
45	F	ILC	HER2 - Luminal B	IA	Unilateral			BRCA1	c.2643dupA	p.Cys882Metfs	F
45	F	IDC	HER2 - Luminal B	IIA	Unilateral	Ovarian cancer		BRCA1	c.3748G>T	p.Glu1250Ter	N
27	F	IDC	HER2 - Luminal B	IIB	Unilateral		Liver metastasis	BRCA1	c.4987-1G>C	?	S a
36	F	IDC	HER2 - Luminal B	IIB	Unilateral	Ovarian cancer		BRCA1	c.2500G>T	p.Gly834Ter	N
46	F	IDC	Unclassified	IIB	Bilateral			BRCA1	c.5074+3A>G	?	S a
76	F	IDC	HER2 - Luminal B	IIB	Unilateral	PPC		BRCA2	c.9382C>T	p.Arg3128Ter	N
37	F	IDC	HER2 - Luminal B	IIB	Unilateral			BRCA2	c.5116_5119delAATA	p.Asn1706Leufs	F
35	F	IDC	HER2 - Luminal B	IIB	Bilateral			BRCA2	c.2380delA	p.Met794Cysfs	F
36	F	IDC	HER2 + Luminal B	IIIA	Bilateral			BRCA2	c.8855dupT	p.Met2952Ilefs	F
53	F	IDC	HER2 - Luminal B	IIIC	Unilateral			BRCA2	c.6229A>T	p.Lys2077Ter	N
40	F	IDC	HER2 + Luminal B	IIIC	Unilateral		Liver metastasis	BRCA2	c.8888_8889insA	p.Arg2964Lysfs	F

F, Female; HER2, human epidermal growth factor receptor; IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; PPC, Primary peritoneal cancer

Table 2 Demographic data of breast cancer patients according to BRCA status

Characteristics N, (%)	Total N = 67	BRCA1/2 carriers N = 12	Patients with VUS N = 4	BRCA1/2 Non-carriers N = 51	P-value
Sex					1.000
Female	66 (98.5)	12 (100)	4 (100)	50 (98.0)	
Male	1 (1.5)	0	0	1 (2.0)	
Age (mean ± SD, years)	42.0 ± 10.3	43.0 ± 12.4	42.0 ± 8.1	41.7 ± 10.1	0.590
Age at diagnosis					1.000
£ 40 years	36 (53.7)	7 (58.3)	2 (50.0)	27 (52.9)	
> 40 years	31 (46.3)	5 (41.7)	2 (50.0)	24 (47.1)	
Cancer affected: breast cancer only					0.455
Unilateral	57 (85.0)	9 (75.0)	4 (100)	44 (86.3)	
Metachronous bilateral	6 (9.0)	1 (8.3)	0	5 (9.8)	
Synchronous bilateral	4 (6.0)	2 (16.7)	0	2 (3.9)	
ECOG performance status					0.239
0	66 (98.5)	11 (91.7)	4 (100)	51 (100)	
1	1 (1.5)	1 (8.3)	0	0	
TNM staging					0.367
0	3 (4.6)	0	0	3 (6.1)	
I	19 (29.2)	2 (16.7)	0	17 (34.7)	
II	32 (49.2)	7 (58.3)	3 (75.0)	22 (44.9)	
III	11 (16.9)	3 (25.0)	1 (25.0)	7 (14.3)	
Missing data (N = 2)					
ER					0.072
Negative	22 (35.5)	1 (9.1)	1 (25.0)	20 (42.5)	
Positive	40 (64.5)	10 (90.9)	3 (75.0)	27 (57.5)	
Missing data (N = 5)					
PR					0.120
Negative	21 (35.0)	1 (10.0)	1 (25.0)	19 (41.3)	
Positive	39 (65.0)	9 (90.0)	3 (75.0)	27 (58.7)	
Missing data (N = 7)					
HER2					0.159
Negative	40 (69.0)	8 (80.0)	1 (25.0)	31 (70.4)	
Equivocal	1 (3.4)	0	1 (25.0)	1 (2.3)	
Overexpression (Positive)	16 (27.6)	2 (20.0)	2 (50.0)	12 (27.3)	
Missing data (N= 9)					
Ki-67					1.000
<20%	7 (13.2)	1 (11.1)	0	6 (15.0)	
³20%	46 (86.8)	8 (88.9)	4 (100)	34 (85.0)	
Missing data (N = 14)					
Luminal subtypes					0.058
Luminal-A	6 (9.0)	0	0	6 (11.8)	
HER2-negative Luminal-B	19 (28.3)	8 (66.6)	0	11 (21.6)	
HER2-positive Luminal-B	13 (19.4)	2 (16.7)	2 (50.0)	9 (17.6)	

Characteristics N, (%)	Total N = 67	BRCA1/2 carriers N = 12	Patients with VUS N = 4	BRCA1/2 Non-carriers N = 51	P-value
TNBC	15 (22.4)	0	1 (25.0)	14 (27.4)	
HER2-positive Non-luminal	3 (4.5)	0	0	3 (5.9)	
Unclassified	11 (16.4)	2 (16.7)	1 (25.0)	8 (15.7)	
Surgery					0.257
Mastectomy	51 (77.3)	11 (91.7)	4 (100)	36 (72.0)	
BCS	15 (22.7)	1 (8.3)	0	14 (28.0)	
Missing data (N = 1)					
Adjuvant chemotherapy					0.639
No	20 (29.8)	2 (16.7)	1 (25.0)	17 (33.3)	
Yes	47 (70.2)	10 (83.3)	3 (75.0)	34 (67.7)	
Hormone therapy					0.653
No	24 (35.8)	3 (25.0)	1 (25.0)	20 (39.2)	
Yes	43 (64.2)	9 (75.0)	3 (75.0)	31 (60.8)	
Adjuvant Radiation					0.261
No	29 (43.3)	5 (41.7)	0	24 (47.1)	
Yes	38 (56.7)	7 (58.3)	4 (100)	27 (52.9)	

BCS, breast conservation surgery; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; HER2, human epidermal growth factor receptor; PR, progesterone receptor; SD, standard deviation; TNBC, triple-negative breast cancer

Table 3 Comparison of breast cancer in BRCA1 and BRCA2 carriers

Characteristics	BRCA1 carriers	BRCA2 carriers	P-value
	N = 6	N = 6	
Age (mean ± SD, years)	39.8 ± 7.4	46.2 ± 16.0	0.400
Age at diagnosis			1.00
≤ 40 years	3 (50.0)	4 (66.7)	
> 40 years	3 (50.0)	2 (33.3)	
ER			0.455
Negative	1 (20.0)	0	
Positive	4 (80.0)	6 (100)	
Missing data (N = 1)			
PR			0.400
Negative	1 (25.0)	0	
Positive	3 (75.0)	6 (100)	
Missing data (N = 2)			
HER2			0.467
Negative	4 (100)	4 (66.7)	
Equivocal	0	0	
Overexpression	0	2 (33.3)	
Missing data (N = 2)			
Luminal subtypes			0.212
Luminal-A	0	0	
HER2-negative Luminal-B	4 (66.7)	4 (66.7)	
HER2-positive Luminal-B	0	2 (33.3)	
TNBC	0	0	
HER2-positive Non-luminal	0	0	
Unclassified/Unknown	2 (33.33)	0	
Stage			0.091
I	2 (33.3)	0	
II	4 (66.7)	3 (50.0)	
III	0	3 (50.0)	

ER, estrogen receptor; HER2, human epidermal growth factor receptor; PR, progesterone receptor; SD, standard deviation; TNBC, triple-negative breast cancer

Table 4 Cox regression analysis of prognostic factors associated with 3-year disease-free survival

Factors	N	3-year DFS (%)	Univariate analysis		Multivariate analysis	
			HR (95%CI)	P-value	HR (95%CI)	P-value
BRCA						
BRCA 1/2 Non-carriers	50	90.3	1		1	
BRCA1/2 Carriers	12	81.5	2.04 (0.64, 6.49)	0.229	2.50 (0.48, 12.97)	0.275
Patients with VUS	4	79.0	1.60 (0.20, 12.99)	0.658	1.25 (0.12, 12.81)	0.852
Age						
£ 40 years	36	90.8	1			
> 40 years	30	84.4	1.19 (0.42, 3.42)	0.741		
T stage						
Tis	3	100	-			
T1	22	100	1			
T2	30	80.1	4.32 (1.01, 18.42)	0.048		
T3	10	78.8	4.12 (0.90, 18.91)	0.069		
Lymph node metastasis						
Negative	36	90.4	1			
Positive	29	84.3	1.84 (0.58, 5.79)	0.299		
TNM Staging						
0	3	100	-		-	
I	19	100	1		1	
II	32	84.8	2.88 (0.73, 11.33)	0.131	6.32 (0.90, 44.48)	0.064
III	11	72.7	6.82 (1.12, 41.33)	0.037	16.30 (1.38, 192.81)	0.027
ER status						
Negative	22	76.8	1			
Positive	40	92.1	0.49 (0.16, 1.46)	0.198		
PR status						
Negative	21	76.7	1			
Positive	39	91.7	0.67 (0.20, 2.22)	0.515		
HER2 status						
Negative	40	82.4	1			
Equivocal	2	100	-			
Overexpression	16	93.8	0.48 (0.10, 2.31)	0.359		
Ki-67						
< 20 %	7	85.7	1			
³ 20 %	46	84.3	0.97 (0.12, 8.10)	0.981		
Luminal subtype						
Luminal-A	6	83.3	1		1	
HER2-negative Luminal-B	15	93.8	0.46 (0.04, 4.78)	0.513	0.14 (0.01, 2.30)	0.171
HER2-positive Luminal-B	13	92.3	0.33 (0.02, 5.50)	0.441	0.14 (0.01, 3.01)	0.212
TNBC	15	65.3	1.21 (0.13, 11.16)	0.868	1.23 (0.13, 11.78)	0.866
HER2-positive Non-luminal	3	100	0.57 (0.03, 10.52)	0.706	0.72 (0.03, 16.67)	0.836
Unclassified	10	100	0.42 (0.04, 4.74)	0.487	0.65 (0.04, 11.87)	0.775
Cancer affected						
Unilateral	56	89.3	1			

Factors	N	3-year DFS (%)	Univariate analysis		Multivariate analysis	
			HR (95%CI)	P-value	HR (95%CI)	P-value
Metachronous bilateral	6	66.7	7.44 (2.26, 24.50)	0.001		
Synchronous bilateral	4	100	1.77 (0.21, 14.79)	0.599		
Adjuvant treatment						
No	19	94.1	1			
Yes	47	85.1	2.44 (0.66, 9.03)	0.181		
Surgery treatment						
BCS	15	92.3	1			
Mastectomy	51	86.5	2.07 (0.46, 9.29)	0.342		
Radiation therapy						
No	28	88.9	1			
Yes	38	86.5	1.03 (0.35, 2.97)	0.961		

BCS, breast conservation surgery; CI, confidence interval; DFS, disease free survival; ER, estrogen receptor; HER2, human epidermal growth factor receptor; HR, hazard ratio; PR, progesterone receptor; T, tumor size; Tis, carcinoma in situ; TNBC, triple-negative breast cancer; VUS, Variants of Uncertain Significance

Figures

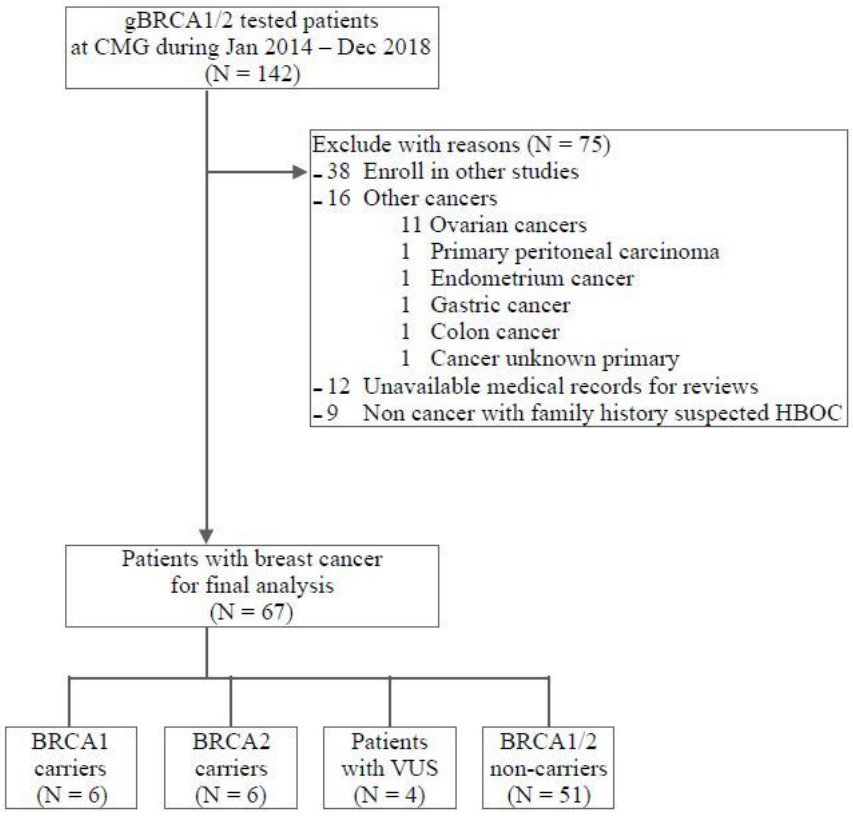

Figure 1

Patient selection

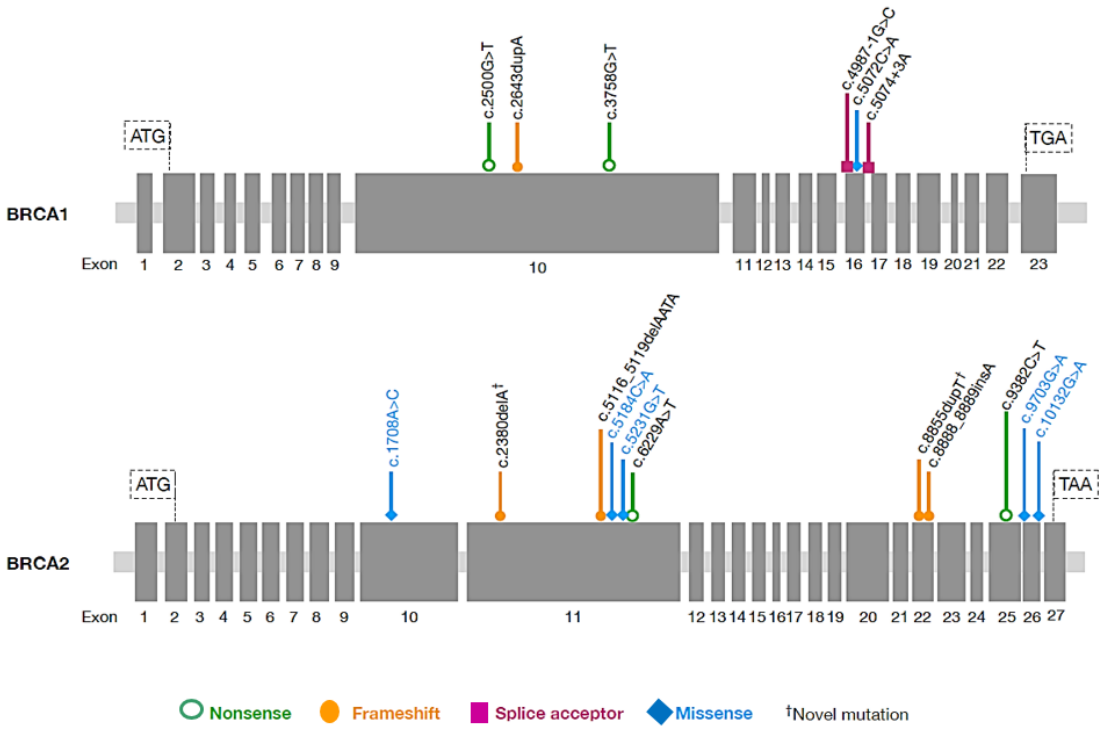

Figure 2

Diagram of BRCA1 and BRCA2 genes, indicating the position of pathogenic variants identified here. Exons are indicated by boxes and numbered according to the Locus Reference Genomic (LRG) description. The ATG translation initiation sites and termination codons are indicated by longer lines. Black letters = pathogenic mutation in BRCA1 and BRCA2, Blue letters = VUS in BRCA2