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MOLECULAR PROFILE AND CLINICAL VARIABLES IN BRCA1-POSITIVE BREAST CANCERS. A POPULATION-BASED STUDY

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Purpose: **To evaluate the clinical features of breast cancer patients with genetic susceptibility to this disease and to investigate the contribution of BRCA1 germline mutations to the phenotype of these tumors.**
Patients and methods: **We reviewed the clinical and pathological records of 102 women with suspected inherited susceptibility to breast cancer consecutively seen at the Genetic Oncology Service of Parma, Italy. Sixty-two patients with a high probability of harboring a germline, cancer-predisposing mutation were tested for BRCA1 mutations. Exon 11 was screened using the protein truncation test and detected mutations were confirmed by direct sequencing (DS). All other exons were analyzed by DS.**
Results: **Among the 62 patients with a completed mutation analysis, 48 (77.4%) had wild-type BRCA1, six (9.6%) had variants of unclear significance, eight (13%) had deleterious mutations. BRCA1-associated breast cancers (BABC) were significantly less likely to be diagnosed at stage I than breast cancers in women without mutations (12.5% *vs* 51%; *P* = 0.045), more likely to have a high proliferation rate (100% *vs* 24%, *P*<0.001), and more likely to be histological grade 3 (100% *vs* 14%, *P*<0.001), estrogen and progesterone receptor negative (87.5% *vs* 13%, *P*<0.001; 75% *vs* 23%, *P* = 0.004), and p53 positive (87.5% *vs* 30%, *P* = 0.023). All tumors with BRCA1 mutations were HER-2/neu negative compared with 57% of the non-BRCA1 tumors (*P* = 0.04). There were no significant differences between BABC and non-BABC in 20-year relapse-free survival, 20-year event-free survival, and 20-year overall survival.**
Conclusion: **In this population-based study, BABC seems to present with adverse molecular features when compared with non-BABC, although the prognosis appears to be similar.**

Key words: BRCA1, breast cancer phenotype, genetic testing, hereditary breast cancer, molecular markers.

Introduction

In 2000, there were about 796,000 new breast cancers diagnosed and about 314,000 deaths due to breast cancer around the world[1]. Excluding cancers of the skin, breast cancer is the most common cancer among women, accounting for one out of every three cancer diagnoses. In Western Europe one in 15 women (7%) will develop breast cancer during their lifetime and the National Cancer Institute (NCI) estimates that about one in 50 women will develop breast cancer by age 50 years and about one in ten women in the United States will develop breast cancer by age 80 years[2].

Breast cancer is considered a multifactorial disorder caused by both non-genetic and genetic factors. The clustering of breast cancer in families has been recognized for many years, suggesting that there may be an inherited component, and it has been estimated that 5-10% of all breast cancers arise in individuals carrying a germline mutation[3]. Clinical features suggesting a genetic predisposition include bilateral breast cancer and bilateral premalignant lesions, such as lobular carcinoma *in situ*[4]. Early age at onset is also generally considered an indicator of genetic susceptibility to breast cancer and has been demonstrated to be associated with a higher risk in relatives[5].

A substantial proportion of hereditary breast cancers can be attributed to mutations in one of two genes, BRCA1 and BRCA2. Early studies of families with multiple cases of breast and ovarian cancer suggested that BRCA1 mutation carriers may have a lifetime breast cancer risk of up to 84% and an ovarian cancer risk of up to 44%[6]. However, other studies of less selected families have suggested that the risks may be somewhat lower than these initial estimates[7]. BRCA1 and BRCA2 mutations are thought to confer a similar susceptibility to breast cancer, but BRCA2 mutations may pose a lower risk of ovarian cancer. Moreover, BRCA2 has been found to contribute to fewer cases of early-onset breast cancer than BRCA1[8].

Although several studies have suggested that the presence of a family history of breast cancer may have an impact on the prognosis of women with breast cancer[9,10], the biological basis of such an influence remains unclear. Several studies have shown that BRCA1-associated breast cancers display a high prevalence of adverse histopathological features suggestive of an aggressive cancer phenotype[3,11,12] but other studies failed

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to demonstrate a worse prognosis in breast cancer patients carrying BRCA1 mutations compared to patients with sporadic breast cancers[3,11,13].

In an attempt to better define the clinical features and outcome of breast cancer patients with genetic susceptibility to this disease and to help address the questions of the contribution of a BRCA1 germline mutation to the phenotype of these tumors and of its role as an independent prognostic factor, we report our experience with 102 breast cancer patients, all of whom had a suspected inherited susceptibility to breast cancer development.

Materials and methods

Patients

The study cohort was composed of 102 women with breast cancer who were part of the breast cancer population referred to the Medical Oncology Unit of the University Hospital of Parma, Italy, from December 1976 to July 2003. Some of these patients perceived themselves to be at an elevated risk of inherited susceptibility to breast cancer. Others were suspected to have such a risk by relatives or their treating physician. The entire group was consecutively seen at the Genetic Oncology Service of the University Hospital of Parma between June 1999 and November 2003.

Pre-genetic testing education and counseling

For every patient, extensive family information was collected and their pedigrees were traced in order to assess the possible family history of breast cancer and other malignancies. For such patients, the probability of having a BRCA1 cancer-predisposing mutation was calculated by the Shattuck-Eidens model[14]; a cutoff >10% was used to identify women at high risk.

According to the criteria adopted by our institution, patients were referred for BRCA1 mutation analysis if they had at least one first and one second-degree relative with breast cancer or one first-degree relative affected by ovarian cancer. Patients with no affected first-degree relatives were referred for genetic testing if they had a Shattuck-Eidens score ≥10% and at least one of the following features: 1) breast cancer diagnosis before age 40 years, 2) bilateral breast cancer, 3) occurrence of both breast and ovarian cancer (BOC).

All patients were offered full genetic counseling. Before the selected subjects donated a DNA sample for germline BRCA1 testing, they were asked to consent to such testing. They were given the opportunity to decline to learn the result.

Clinical and pathological data

Date of birth, date of diagnosis, pathological stage, nodal involvement, histological type and grade, hormone receptor status, proliferation rate, p53 and HER-2/neu status were determined from the pathology findings and the clinical records. Tumor samples were stained de novo for HER-2/neu when this marker was not available from the pathology records. Immunohistochemistry (IHC) staining was carried out on formalin-fixed, paraffin-embedded material, using the HercepTest® developed by DAKO.

According to the criteria adopted by our institution, hormone receptor status was labeled "positive" when the fraction of cells stained by the corresponding immunoreaction was higher than 5% and the proliferation rate was considered "high" when 15% or more cells were stained by Ki 67 antibody. HER-2/neu and p53 were scored from 0 to 3 with a score of 2 or 3 recorded as "positive" and 0 or 1 as "negative".

Treatment details were abstracted from the medical records. When necessary, clarification was obtained from the patient, next of kin, or treating physician. The date of last follow-up evaluation and vital status were determined through review of clinical records and through telephonic contact with the patient, next of kin, or primary physician.

Genetic testing

Genomic DNA was isolated from peripheral-blood lymphocytes. We analyzed BRCA1 exon 11, which contains 60% of the coding sequence, by the protein truncation test (PTT)[15,16]. Mutations detected by PTT were confirmed by direct sequencing (DS). The remaining 21 BRCA1 coding exons were analyzed by DS.

For PTT analysis, exon 11 was amplified by polymerase chain reaction in three overlapping fragments of 1000-2000 base pairs using previously described primers[16], which contained a T7 promoter, an eukaryotic translation initiation sequence and gene-specific sequences. The 5' and 3' ends of exon 11 in both genes were screened separately to identify any potential decrease in the sensitivity of PTT resulting from the use of such large fragments. All samples showing altered PTT patterns were directly sequenced to confirm the presence of a mutation. However, to permit the identification of missense variants on exon 11, complete exon 11 sequencing was performed in 38 of the 54 samples (70%) that did not show altered PTT patterns. The remaining coding region and intron-exon boundaries were amplified using standard PCR procedures with primers and conditions as described[14].

Amplified products were directly sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers in a Beckman Coulter CEQ™ 2000XL sequencer. Chromatographic tracings of each amplicon were analyzed by a proprietary computer-based review followed by visual inspection and confirmation. Genetic variants were compared with the wild-type sequence and were checked on the Breast Cancer Information Care (BIC) database[17], and classified on this basis. In any case, positive results were confirmed by repeating every step of the analysis from DNA extraction. Mutations causing a premature stop codon in BRCA1 or missense mutations that are known to cause phenotypic cancer were termed BRCA1 mutations. Missense mutations that have been shown not to cause

phenotypic cancer were considered polymorphisms, while missense mutations with insufficient data to be considered either BRCA1 mutations or polymorphisms were termed variations of unclear significance.

Statistical analysis

Overall, relapse-free, contralateral breast cancer-free, and event-free survivals were calculated using the method of Kaplan and Meier[18]. Overall survival was defined as the interval between the initial breast cancer diagnosis and death from any cause. Relapse-free survival was calculated from the date of initial diagnosis to the date of last follow-up evaluation, any relapse (including ipsilateral breast tumor recurrence for women who underwent breast-conserving therapy), or death, whichever occurred first. Contralateral breast cancer-free survival was defined as the time from initial breast cancer diagnosis to contralateral breast cancer diagnosis or last follow-up evaluation, whichever was earliest. Patients who underwent prophylactic contralateral mastectomy were censored for contralateral breast cancer-free survival at the time of surgery. Event-free survival was calculated as the interval between the date of initial breast cancer diagnosis and the date of last follow-up evaluation, development of metachronous contralateral breast cancer or ovarian cancer, any relapse, or death, whichever occurred first. The log-rank test was used to compare survival distributions of individuals with and without germline BRCA1 mutations[19]. Other comparisons between BRCA1-associated breast cancers (BABC) and non-BRCA1-associated tumors were made with Fisher's exact test[20]. P values of <0.05 were considered significant. The Cox regression analysis was used to determine the independent effect of BRCA1 mutations on event-free survival[21]. The SPSS software (version 8.0) was used in all analyses.

Results

For the entire cohort of 102 women, the median age at the time of initial diagnosis was 43 years (range, 23 to 79). Thirty patients (29%) had a breast cancer diagnosis before age 40 and 20 patients (19%) had contralateral breast cancer. Ovarian cancer was found in 12 patients (11.7%). A history of breast or ovarian cancer in a first-degree relative was elicited from 42 women (41%). An additional 41 women (40%) described breast or ovarian cancer in a second-degree relative, with no affected first-degree relative. The median follow-up duration from the time of initial breast cancer diagnosis was 76 months for surviving patients.

Sixty-five (63.7%) women were considered at high risk for BRCA1 mutation as explained in the Materials and Methods section. Sixty-two of these hereditary breast cancer or hereditary breast-ovarian cancer patients underwent genetic testing for BRCA1. The three women refusing the test attributed their decision to major concerns about themselves and/or their children. Table 1 shows the clinical characteristics of the study cohort.

Table 1 - Clinical characteristics of the study cohort (first breast cancer)

Parameter	High risk* patients		Low risk* patients	
	No.	%	No.	%
No. of patients	65	100	37	100
Age at initial diagnosis (years)				
Median	42		48.5	
Range	23-79		29-71	
Family history of breast or ovarian cancer	52	80	31	83
Laterality of initial breast cancer (left/right)	34/31	15/22		
Histology of initial breast cancer				
Infiltrating ductal	49	75.4	20	54
Medullary or medullary features	2	3	1	3
Infiltrating lobular	5	7.7	12	32
DCIS	4	6.2	3	8
LCIS	1	1.5	0	
Other/unknown	4	6.2	1	3
Tumor stage				
IS	5	7.7	3	8
1	34	52.4	20	54
2	20	30.7	10	27
3	2	3	0	
4	1	1.5	3	8
Unknown	3	4.7	1	3
No. of nodes involved (invasive only)				
0	29	48.3	30	88.2
1-3	17	28.3	2	5.8
4-9	7	11.7	1	3
10+	4	6.7	1	3
Unknown	3	5		
Final TNM stage				
IS	5	7.7	3	8
I	28	43	17	46
II	23	35.4	13	35.2
III	5	7.7	3	8.1
IV	1	1.5		
Unknown	3	4.7	1	2.7
Early-onset BC (<40 years)	21	32	9	24
Bilateral BC	18	27	2	5
BOC	12	18	0	
Shattuck-Eidens score				
≤10%	30	46	37	100
>10%	35	54	0	

*for BRCA1 mutation; DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; IS, *in situ*; BC, breast cancers; BOC, breast-ovarian cancers.

Among the 62 patients with a complete mutation analysis, germline BRCA1 mutations were detected in eight (13%) and 48 (77.4%) had wild-type BRCA1. Frameshift mutations were detected in five women, one of whom carried the mutation 185delAG, a common alteration that is frequently found in Ashkenazi Jewish individuals. In another two cases, small mutations located in exon 11 (3600del11 and 1499insA) that were previously reported in individuals from Italy were detected. All the frameshift mutations and the nonsense mutation Y1429X, found in one patient, result in premature truncation of the protein product and are presumed to be clinically significant.

The splice mutation IVS5-11T>G was found in one patient. This mutation has been reported in families with hereditary breast and ovarian cancer from Western Europe. An additional patient was noted to have a nucleotide deletion that resulted in the splice mutation IVS13+1delG. This mutation is similar to previously described splice mutations such as IVS13+1G>A and IVS13+1G>T.

Six women (9.6%) had variants of unclear significance. In four cases the variant detected resulted in amino acid substitution. None of these women were carriers of the same mutation. Polymorphisms were detected in 42 additional cases (data not shown).

With regard to the main entry criteria for BRCA1 genetic testing, mutations were detected in 18.7% (3/16), 11.7% (2/17), 25% (3/12) and 0% (0/17) of women with a family history, early-onset breast cancer (<40 years), breast-ovarian cancer and bilateral breast cancer, respectively. Interestingly, two of eight (25%) patients with detected BRCA1 mutations had a Shattuck-Eidens score <10%.

The clinical and pathological features of the tested patients are reported in Tables 2 and 3. A history of breast or ovarian cancer in a first- or second-degree relative was reported by five of eight (62.5%) women with BRCA1 mutations and by 41 of 48 (85.4%) women without mutations. There was no family history of breast or ovarian cancer in two women with 5154del5 and IVS5-11T>G mutations, respectively.

Of the six women with variants of unknown significance, one had no family history of breast or ovarian cancer and five had a history of breast or ovarian cancer in a first- or second-degree relative.

There were no statistically significant differences in tumor stage and axillary node involvement between women with or without germline BRCA1 mutations. However, women with mutations were significantly less likely to present with stage I (T1N0M0) disease (12.5% *vs* 51%; $P = 0.045$). Estrogen receptor (ER) status was reported for all the BRCA1-related and for 46 (96%) BRCA1-unrelated tumors: receptors were negative in seven (87.5%) of eight tumors of the former group, compared with six (13%) of the latter ($P<0.001$). Progesterone receptor (PR) negativity was noted in six of eight (75%) mutation carriers, compared with 10 of 43 (23%) non-carriers for whom the data were available ($P = 0.004$). Histological grade was described for 43 (89.5%) tumors from women without mutations and seven (87.5%) tumors from women with mutations. Histological grade III disease was noted in seven of

Table 2 - Clinical characteristics of the breast cancer patients tested for BRCA1 mutation analysis (first breast cancer)

Parameter	No mutation		Mutation		US variants	
	No.	%	No.	%	No.	%
No. of patients	48	100	8	100	6	100
Age at initial diagnosis (years)						
<40	13	27.1	4	50	3	50
≥40	35	72.9	4	50	3	50
Family history of breast or ovarian cancer	41	85.4	5	62.5	5	83
Histology of initial breast cancer						
Infiltrating ductal	32	66.7	8	100	6	100
Medullary or medullary features	2	4.2				
Infiltrating lobular	5	10.4				
DCIS	4	8.3				
LCIS	1	2.1				
Other/unknown	4	8.3				
Tumor stage						
IS	5	10.4				
1	23	48	4	50	6	100
2	15	31.2	4	50		
3	2	4.2				
4	0					
Unknown	3	6.2				
Number of nodes involved (invasive only)						
0	18	41.9	6	75	4	66.8
1-3	14	32.6	1	12.5	1	16.6
4-9	5	11.6	1	12.5	1	16.6
10	4	9.3				
Unknown	2	4.6				
Bilateral BC	15	31.2	1	12.5	2	33.3
BOC	9	18.7	3	37.5	0	

US, unclear significance; DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; IS, *in situ*; BC, breast cancer; BOC, breast-ovarian cancer.

Table 3 - Pathological characteristics of the breast cancer patients tested for BRCA1 mutation analysis (first breast cancer)

Parameter	No mutation	Mutation	*P* value	US variants
	No. (%)	No. (%)		No. (%)
No. of patients	48 (100)	8 (100)		6 (100)
TNM stage (invasive only)				
Stage I	21 (51)	1 (12.5)	0.045	5 (83)
Stage II-IV	20 (41)	7 (87.5)		1 (17)
Histological grade				
Low (1-2)	37 (77.5)	0	<0.001	4 (66.6)
High (3)	6 (12.5)	7 (87.5)		1 (16.7)
Unknown	5 (10)	1 (12.5)		1 (16.7)
Estrogen receptor status				
Positive	40 (83.5)	1 (12.5)	<0.001	5 (83.3)
Negative	6 (12.5)	7 (87.5)		1 (16.7)
Unknown	2 (4)			
Progesterone receptor status				
Positive	33 (68.5)	2 (25)	0.004	5 (83.3)
Negative	10 (21)	6 (75)		
Unknown	5 (10.5)			1 (16.7)
Proliferation rate				
Low	32 (66.5)	0	<0.001	2 (33.3)
High	10 (21)	6 (12.5)		2 (33.3)
Unknown	6 (12.5)	2 (25)		2 (33.3)
p53 status				
Positive	14 (30%)	7 (87.5)	0.023	3 (50)
Negative	34 (70%)	1 (12.5)		2 (33.3)
Unknown				1 (16.7)
HER-2/neu status				
Positive	13 (27)	0	0.04	0
Negative	17 (35.5)	8 (100)		4 (66.6)
Unknown	18 (37.5)			2 (33.3)

US, unclear significance.

seven cases from BRCA1 heterozygotes, compared with six of 43 cases (14%) from women without germline mutations ($P<0.001$). Tumor proliferation rate was known for 48 patients: it was classified as high in six of six mutation carriers and in 10 of 42 (24%) non-carriers ($P<0.001$).

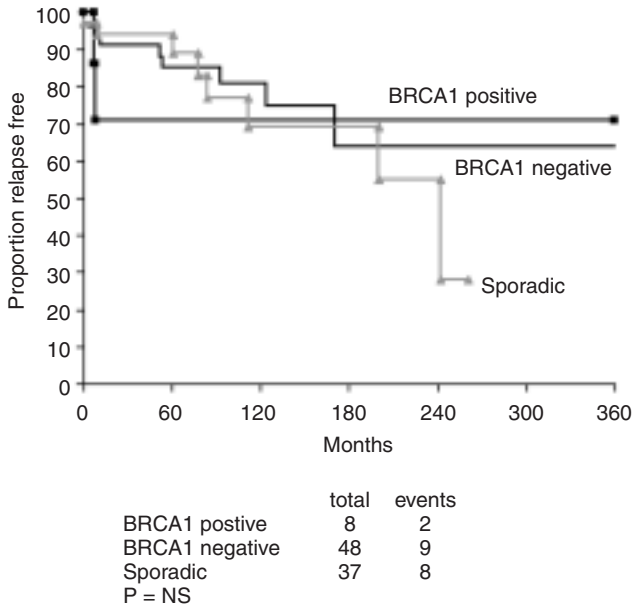
HER-2/neu status was reported for all the BRCA1-related and for 30 (62.5%) BRCA1-unrelated tumors. Interestingly enough, HER-2/neu was negative in eight of eight (100%) mutation carriers, compared with 17 (57%) non-carriers ($P = 0.04$). BRCA1 also correlated with p53 positivity: seven of eight (87.5%) BRCA1 tumors were p53 positive compared with 14 of 48 (30%) non-BRCA1 tumors ($P = 0.023$).

Two control groups were compared with BRCA1-related tumors regarding clinical outcome: the high-risk group for cancer-predisposing genetic mutations with wild-type BRCA1 (48 patients) and the low-risk group for such mutations that did not undergo BRCA1 genetic testing (37 patients). Breast cancers in the latter group were considered sporadic.

Relapses occurred in two of eight (25%) patients with breast cancer and germline mutations. The site of relapse was locoregional in both cases. Among women without mutations, nine of 48 (18.7%) relapsed. In this group the site of first relapse was locoregional in four and distant in five patients. Eight (21%) patients in the low-risk group had a relapse. The site was locoregional in five cases. The 20-year relapse-free survival rate was 71.4% in the group with BRCA1 mutations, 64.4% in the group without mutations, and 55% in the low-risk group (Figure 1). The difference in relapse-free survival between the three groups was not significant (log-rank $P = 0.47$).

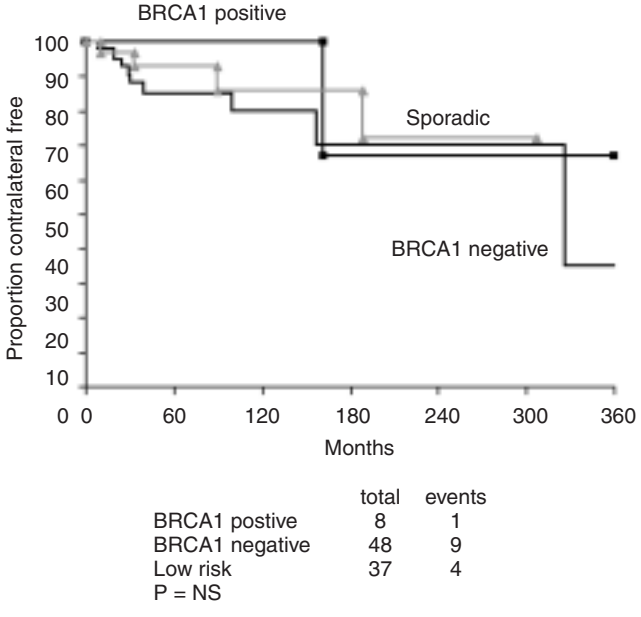
Contralateral breast cancer developed in one of eight (12.5%) women with germline BRCA1 mutations, compared with nine of 48 (18.7%) women without BRCA1 mutations and four of 37 (11%) women with sporadic breast cancers. Among those without mutations, four cases were diagnosed synchronously (within three months of diagnosis of the index cancer). No synchronous contralateral breast cancers were noted among women with BRCA1 mutations and women with sporadic tumors. The 20-year contralateral breast cancer-free survival rate was 66.6% among women with BRCA1 mutations, 70.4% among those without mutations, and 71% in the group of sporadic breast cancers. This difference was not significant (log-rank $P = 0.57$) (Figure 2).

Ovarian cancer was diagnosed in three of eight (37.5%) patients with BRCA1 mutations (IVS5-11T>G, 3600del11 and 5154del5). In one of these women the ovarian cancer was diagnosed before the index breast cancer. No malignancies other than those of the breast or ovary were observed with the exception of a bone osteosarcoma that occurred before the breast cancer diagnosis in mutation carrier 1499insA. Among women without BRCA1 mutations and those with sporadic breast cancers, 19 of 48 (39.5%) and 10 of 37 (27%) developed ovarian cancer, respectively. In five women with BRCA1-unrelated tumors, the ovarian neoplasia was diagnosed before the breast cancer. The median event-free survival duration for women with BRCA1 mutations was 144 months, compared with 170 months and 190 months in patients without mutations and with sporadic breast cancers, respectively (log-rank $P = 0.95$) (Figure 3). Cox regression analysis failed to demonstrate a significant influence of BRCA1 mutation status on event-free survival. No significant difference



	total	events
BRCA1 postive	8	2
BRCA1 negative	48	9
Sporadic	37	8
P = NS		

Figure 1 - Relapse-free survival.



	total	events
BRCA1 postive	8	1
BRCA1 negative	48	9
Low risk	37	4
P = NS		

Figure 2 - Contralateral breast cancer-free survival.

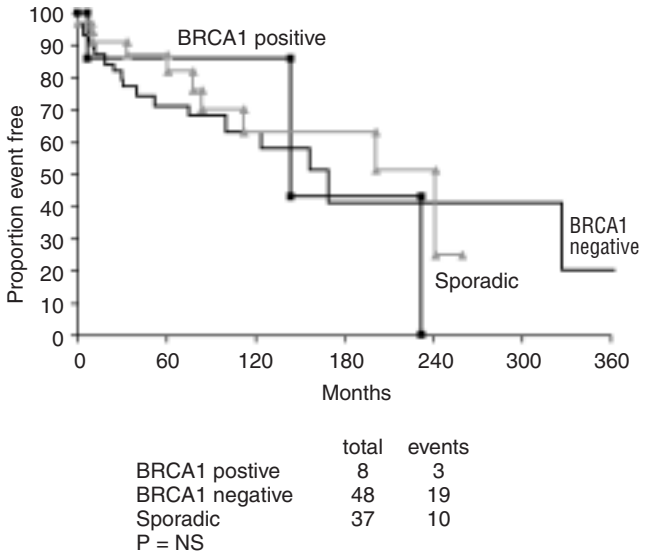


Figure 3 - Event-free survival.

in overall survival between BRCA1-associated breast cancers and non-BABC was observed (Figure 4).

Discussion

In this series of Italian women with breast cancer and suspected inherited susceptibility to cancer development, we investigated the contribution of BRCA1 germline mutations to the phenotype of these tumors.

The study cohort was part of a group of women with breast cancer evaluated at a single cancer center. All the women with a high likelihood of having a breast cancer-predisposing mutation underwent genetic testing; in this way the potential bias of including mutation carriers in a historical control group was avoided. The combination of PTT on exon 11 (followed by direct sequencing of samples with an altered pattern) and DS on the remaining 21 exons, a very sensitive and efficient tool for mutation detection[22,23], ensured that practically all mutation carriers were likely to have been identified. However, to permit the identification of missense variants on exon 11, complete exon 11 sequencing was performed in 38 of the 54 samples (70%) that did not show altered PTT patterns.

There were no statistically significant differences in tumor stage and axillary node involvement between women with or without germline BRCA1 mutations. However, individuals with mutations were significantly less likely to present with stage I disease.

The detection of a BRCA1 mutation did show a statistically significant association with high histological grade, high proliferation rate, p53 positivity, ER/PR and HER-2/neu negativity. These results are similar to those reported previously in the literature[3,11,12,24]. Interestingly, none of the seven BRCA1-positive tumors were HER-2/neu positive, compared with 17 (57%) of the 30 non-BRCA1 tumors for which the data were available ($P = 0.04$).

Armes *et al.* proposed an intriguing mechanism of the interaction of BRCA1 with molecular markers. They suggested that once a BRCA1 mutation is followed by p53 dysfunction, the cell needs no more help to become cancerous. So there is little selective pressure for cancer cells to become ER-, PR-, or HER-2/neu-positive, hence the unique molecular profile of BRCA1 mutant tumors[25].

Despite these histological markers of poor prognosis, several studies failed to demonstrate a worse clinical outcome in breast cancer patients carrying BRCA1 mutations compared to their counterparts with sporadic disease[3,11,13]. Two groups have described a more favorable clinical outcome in women with BABC. Porter et al. described 35 women with breast cancer who were members of BRCA1-linked kindreds. Survival among these women was superior to that of age-matched controls from a historical population of breast cancer patients[26]. Marcus *et al*. contrasted the outcome of 72 female breast cancer patients from 26 BRCA1-linked families to that of non-age-matched historical controls. In this series, women with presumed BABC appeared to have an improved disease-free survival, although methodological limitations precluded statistical analysis. There was no improvement in overall survival among hereditary cases[27].

In a study cohort of 91 Ashkenazi women with early-onset breast cancer, there were no significant differences between BABC and non-BABC in five-year relapse-free survival, five-year event-free survival, or five-year overall survival. However, women with germline BRCA1 mutations were significantly more

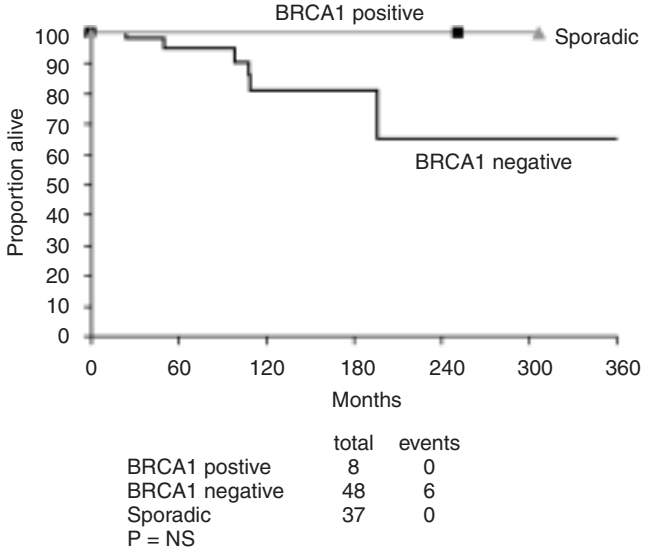


Figure 4 - Overall survival.

likely to develop contralateral breast cancer at five years (31% *vs* 4%, $P = 0.0007$)[3].

The present study did not demonstrate any differences in relapse-free, contralateral breast cancer-free, event-free or overall survival between women with germline BRCA1 mutations and those without, although the power to detect these variables is limited by the small size of the study. Two control groups were compared with BRCA1-related tumors regarding clinical outcome: a high-risk group for cancer-predisposing genetic mutations with wild-type BRCA1 and a low-risk group for such mutations that did not undergo BRCA1 genetic testing. No differences in clinical outcome were observed between the two groups and the women with BRCA1-related breast cancers. The relatively high frequency of second malignancies among women with BRCA1 mutations (particularly ovarian cancers) was reflected in a trend towards an lower event-free survival in this group, although this did not reach statistical significance.

The rate of BRCA1-positive patients in our study was small (13%). This finding may reflect the criteria used to define high-risk patients and the lack of Ashkenazi Jewish ancestry. However, a mutation frequency of at least 10% is the threshold generally adopted to select a population for BRCA1 mutation screening[28,29]. Interestingly, two of the eight (25%) patients with detected BRCA1 mutations would have been considered at low risk of having such a cancer-predisposing mutation if only the Shattuck-Eidens model has been used (risk score <10%).

Despite the small number of BRCA1 mutation carriers, several correlations with molecular markers and clinical variables were statistically significant. Other correlations showed trends but may have lacked the power to be significant. In addition, the inclusion of a substantial number of prevalent breast cancer cases may have obscured an influence of BRCA1 status on early outcome (survival bias). Since the risk of breast cancer recurrence is greatest in the three to five years after diagnosis, the survival experience of individuals who undergo genetic testing is likely to vary with the interval from diagnosis. Women who have survived several years before testing have passed through the period of greatest risk and are less likely to relapse subsequent to testing. A prognostic factor that positively influences short-term outcome would be expected to be enhanced among such women. Alternatively, a negative prognostic factor would be underrepresented among prevalent cases, as women who manifest that factor would relapse and thus not be available for later testing. However, even in a cohort of women tested for BRCA1 mutation analysis within two years of their breast cancer diagnosis, BRCA1 mutation status had no influence on outcome[3].

Our findings show that even in a population-based study, BRCA1-positive tumors have a unique molecular profile, reinforcing the notion that, at some level, the various BRCA1 mutations share a common mechanism of tumorigenesis, which is in part different from the molecular pathways of non-BRCA1 tumors. Whether markers such as ER, PR, HER-2/neu, Ki67, histological grade, and p53 maintain the same prognostic significance in BRCA1 carriers as in sporadic cancers and whether the more aggressive biological phenotype of BRCA1 tumors may justify more aggressive treatment is still a matter of debate and needs to be further elucidated. To avoid the biases inherent in a small retrospective study and definitively address the independent prognostic significance of germline BRCA1 mutations, a large prospective trial of incident hereditary breast cancers is necessary.

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