Acta Biomed 2016; Vol. 87, N. 1: 54-63 © Mattioli 1885

Original article

Triple negative status and BRCA mutations in contralateral breast cancer: a population-based study

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Summary. *Background and aim of the work: BRCA1/2* mutation carriers diagnosed with breast cancer have a strong life-time risk of developing contralateral breast cancer (CBC). We performed a population-based study with the aim of estimating the proportion of CBC associated with *BRCA1/BRCA2* mutations, and the contribution of germline mutations to both molecular and clinical features of these tumors. *Methods:* Fifty-five women with invasive CBC consecutively seen at the at the Genetic Oncology Service of the University Hospital of Parma from 2000 to 2011 were subjected to *BRCA1/2* testing. Fifty-five case-matched, unilateral breast cancer (UBC) patients (pts), which tested negative for *BRCA1/2* mutations, were selected as control group. *Results:* BRCA mutations were detected in 13 (24%) of 55 CBC pts. Women with *BRCA1* mutations, and to a lesser extent *BRCA2* mutations, were significantly more likely to present with high histologic grade, negative hormone receptor status and high proliferation rate in both first and second primary breast cancers than BRCA-negative, CBC tumors. A diagnosis of triple-negative breast cancer (TNBC) was significantly more frequent in women with BRCA mutations in comparison with BRCA-negative, UBC controls. There were no survival differences between BRCA-positive and non-BRCA tumors. *Conclusions:* Results of the present study indicate that both first primary and second primary breast cancers in BRCA carriers are qualitatively distinct from BRCA negative CBC, and from sporadic UBC controls. These findings highlight relevant clinical considerations about the potential value of BRCA testing in women with CBC as well as therapeutic, preventive, and surveillance implications for patients carrying a mutation. (www.actabiomedica.it)

Key words: BRCA, breast cancer, contralateral, genetic testing, triple-negative

Introduction

Breast cancer is the most frequently diagnosed malignant disease and the second leading cause of cancer deaths among women (1). Incidence increases with age, and the probability of a women developing breast cancer is 1 in 69 in her 40s, 1 in 38 in her 50s, and 1 in 27 in her 60s (1).

Germline mutations in either the *BRCA1* or the *BRCA2* gene account for the majority of breast cancers in high-risk families and confer a 36% to 84% lifetime risk of first primary breast cancer (2-5). About 10-15% of all breast cancer patients will develop contralateral breast cancer (CBC) during the first 20 years after initial diagnosis (2,6), and the 15-year CBC risk for women with a BRCA mutation has been estimated at between 30 and 40% (7-9).

Breast cancer patients display diverse pathologic and clinical features, some of which have prognostic and/or predictive significance. Based on molecular

profiling of tumors, breast cancers have been divided into those with high expression of the estrogen receptor (ER) gene (luminal A and luminal B subtypes), and those that do not express ER (10,11). Within the ER-negative group, tumors that overexpress the *HER2* oncogene are named HER2-positive subtype (11). ER-negative tumors that express genes found in basal epithelial cells and can be stained with antibodies to keratin 5/6 have been identified as basal-like tumors (11). The majority of these are believed to consist of tumors that do not express ER, progesterone receptor (PR), or HER2 (ie, triple-negative breast cancer [TNBC]) (12). Several studies have shown that TNBC is more aggressive and displays a higher rate of CBC than other molecular subtypes (10,13).

BRCA1-mutation carriers are more likely to be diagnosed with TNBC than non-carriers (10,14). In contrast, carriers of *BRCA2* mutations seem to share similar pathological characteristics with non-carriers (14-16). To our knowledge, there are no reported data on the correlation between molecular markers and clinical variables in a population of women with CBC and known BRCA status. To address this, we performed a population-based observational study with the aim of estimating the proportion of CBC associated with *BRCA1/BRCA2* mutations, and the contribution of germline mutations to both molecular and clinical features of these tumors.

Methods

Subject Selection

Fifty-five women with invasive CBC consecutively seen at the at the Genetic Oncology Service of the University Hospital of Parma between 1st June 2000 and 31st December 2011 were subjected to *BRCA1/2* testing. In addition, 55 controls with sporadic, BRCA-negative, unilateral breast cancer (UBC) were individually matched to each CBC patient by age (± 3 years) and year of diagnosis (± 2 years). Both CBC and UBC patients were selected independently of their family history of breast or ovarian cancer and, before donating a DNA sample for germ-line *BRCA1/BRCA2* testing, they were asked to provide written informed consent for such testing.

The investigators obtained regional and institutional review board approval for study conduction.

Clinical and Pathological Data

Birth, diagnosis date, tumor stage, nodal involvement, histological type and grade, hormone receptor status, proliferation rate, and HER2 status of all study patients were reviewed to confirm accuracy of variables recorded within the PCR database. Tumor stage was defined according to the American Joint Committee on Cancer (AJCC) system (17). Histologic diagnosis and grade were defined using the World Health Organization classification of malignant breast tumors and the Elston and Ellis grading scheme, respectively (18). ER, PR and Ki67 status were determined by immunohistochemistry (IHC) as previously described (19,20). Tumors with greater than 10% stained cells were considered to have positive hormone receptor status. The percentage of Ki67-positive cells, which were defined by the presence of brown staining nuclei, was averaged for each tumor sample over four high-power fields. Ki67 status was considered as "high" when 15% or more cells were stained by Ki-67 antibody. According to the American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) guidelines (21), patients were considered to have HER2-positive disease if the primary or metastatic lesion showed IHC staining of 3+ (uniform, intense membrane staining of > 30% of invasive tumor cells) or had gene amplification (ratio of *HER2* to chromosome 17 copy number greater than 2.2) by fluorescence *in situ* hybridization (FISH). Patients were considered to have HER2-negative disease if they either had negative expression by IHC (0, 1+) or did not have gene amplification by FISH. Available tumor samples were centrally evaluated "de novo" for HER2 amplification, when this marker was not reported from pathology records, or previous tests gave inconclusive results (e.g. IHC 2+). *BRCA Genetic Testing* Genomic DNA was isolated from peripheral blood lymphocytes using the Qiage (Chatsworth, CA) DNA extraction kit. The entire BRCA1 and BRCA2 coding regions and the splice junctions were amplified from genomic DNA using 41 and 50 primers sets, respectively (primer sequences and polymerase chain reaction [PCR] protocols are avail-

able upon request). Amplified products were screened with Denaturing High Performance Liquid Chromatography (DHPLC) analysis. DHPLC was carried out on an automated instrument (WAVE DNA fragment analysis system Transgenomic Inc., Omaha, NE). Five μl of the PCR product was denatured at 95°C for 10 min and subsequently reannealed by gradually lowering the temperature to 25°C. PCR products were then eluted with a linear acetonitrile gradient adjusted to the size of the PCR fragment, at a flow rate of 0.9 ml/min. Appropriate temperature(s) of analysis were determined for each amplicon basing on DHPLC melting algorithm and on previously published data (22). Each DNA sample was run by itself and mixed at an equimolar ratio with the homozygous control PCR. Under conditions of partial heat denaturation and acetonitrile gradient, heteroduplexes formed in PCR samples carrying sequence variations displayed reduced retention times compared to their homoduplex counterparts. Fragments showing heterozygous profile, alone or mixed, were directly sequenced in forward and reverse directions in a Beckman Coulter CEQ™ 2000XL sequencer. Genetic variants were detected by comparison with a consensus wild-type sequence constructed for each gene and were confirmed by repeated analysis, including PCR amplification of the indicated gene regions and sequence determination.

Classification of Breast Cancers by BRCA Status

All sequence variants were named according to the nomenclature used by the Breast Cancer Information Core (BIC) nomenclature (23), with nucleotide numbering starting at the first transcribed base of BRCA1 and BRCA2. BRCA missense variants were analyzed using web-based algorithms (24). According to the genetic test results, breast cancer patients were classified into the following categories:

a. BRCA-positive: Women with mutations causing a premature stop codon in *BRCA1/BRCA2* genes or missense mutations that are known to cause phenotypic cancer.
b. BRCA-negative: Women with no *BRCA1/BRCA2* mutations, or bearing genetic variants that have been shown not to cause phenotypic cancer (polymorphisms).
c. Unclassified variants (UV): Women bearing genetic variants of uncertain significance with insufficient data to be considered either *BRCA1/BRCA2* mutations or polymorphisms.

Statistical Analysis

The study was designed to estimate the proportion of CBC associated with *BRCA1/BRCA2* mutations, and to evaluate the contribution of germline mutations to the clinico-pathological features of these tumors. Molecular profile and clinical variables of BRCA-positive, CBC cases were compared with two different controls: i) women with BRCA-negative CBC (internal control group), and ii) women with BRCA-negative UBC (external control group). The Fisher's exact test was used for analysis of categorical data (25). A P value of < 0.05 was considered significant. The prognostic significance of BRCA-positive status was evaluated on overall survival (OS), defined as the time between the date of first breast cancer diagnosis and the date of death from any cause or the last date the patient was known to be alive. Survival distributions will be estimated by the Kaplan–Meier method and compared using the log-rank test (26,27). Differences were considered to be significant if the log-rank P-value was <0.05. Statistical analyses were carried out using the SPSS software (version 8.0).

Results

Study Population

For the cohort of 55 women with CBC, the median age at the time of initial diagnosis was 45 years (range, 24 to 75). Family history of breast and/or ovarian cancer, which was defined by the presence of at least one first and one second-degree relative with breast cancer or one first-degree relative affected by ovarian cancer, was elicited from 13 (25%) out 52 women. None of the 55 women were known to be genetically related. No significant differences in clinical and tumor characteristics between the UBC control group and the CBC cohort were observed (Table 1).

Table 1. Clinical and tumor characteristics of the study population

Characteristics	CBC cohort* (total, n = 55) No. (%)	UBC controls* (total, n = 55) No. (%)	P
Age at initial diagnosis (years)			
Median (range)	45 (24-75)	43 (23-75)	
Family history†			
Positive	13 (25)	11 (20)	0.64
Negative	39 (75)	44 (80)	
Breast-Ovarian Cancer	4 (7)	5 (9)	1.0

	1st tumor	2nd tumor	Reference tumor	P^1	P^2
Breast cancer histology					
Infiltrating ductal	44 (81)	45 (82)	42 (76)	0.81	0.63
Medullary or medullary features	3 (5)	1 (2)	2 (4)		
Infiltrating lobular	5 (9)	5 (9)	5 (9)		
Other/unknown	3 (5)	4 (7)	6 (11)		
Tumor stage					
I-II	39 (71)	46 (85)	38 (83)	0.24	0.78
III-IV	16 (29)	8 (15)	8 (17)		
Histologic grade					
G1-G2	29 (66)	36 (67)	26 (58)	0.51	0.41
G3	15 (34)	18 (33)	19 (42)		
Estrogen receptor					
Positive	37 (71)	40 (74)	33 (73)	0.82	1.0
Negative	15 (29)	14 (26)	12 (27)		
Progesterone receptor					
Positive	36 (69)	34 (63)	24 (55)	0.14	0.41
Negative	16 (31)	20 (37)	20 (45)		
Proliferation rate					
Low	26 (60)	28 (52)	25 (56)	0.67	0.83
High	17 (40)	26 (48)	20 (44)		
HER2 status					
Negative	39 (87)	43 (80)	32 (71)	0.11	0.35
Positive	6 (13)	11 (20)	13 (29)		

Abbreviations: CBC, contralateral breast cancer; UBC, unilateral breast cancer; No, number; yrs, years.
*Numbers in these categories may not sum to the total because of missing data.
†At least one first and one second-degree relative with breast cancer or one first-degree relative affected by ovarian cancer.
P^1 Comparison of 1st tumor characteristics of CBC cohort with tumor characteristics of UBC controls.
P^2 Comparison of 2nd tumor characteristics of CBC cohort with tumor characteristics of UBC controls.

BRCA Mutation Analysis

Germline *BRCA* mutations (9 *BRCA1* and 5 *BRCA2*) were detected in 13 (24%) out of 55 tested patients (Table 2). Frameshift mutations were detected in 8 women: five in *BRCA1* and three in *BRCA2*. Three splice and 3 nonsense mutations were also reported. All these mutations result in premature truncation of the protein product and are presumed to be clinically significant. The most common mutation was *BRCA1* 14499insA, detected in two women. The *BRCA1* 1499insA, 4035delTT, 5154del5, 4603G>T

Table 2. BRCA mutations detected in women with contralateral breast cancer

Patient (n)	Gene	Exon	Type	Nucleotide change	Amino Acid change	Designation
Definite mutations						
1	BRCA1	11	Frameshift	ins†A	Stop479	1499insA
2	BRCA1	11	Frameshift	insA	Stop479	1499insA
3	BRCA1	11	Frameshift	delAAAG	Stop735	2274del4
4	BRCA1	11	Frameshift	delTT	Stop1328	4035delTT
5	BRCA1	13	Nonsense	C to A	Y1429X	4406C to A
6	BRCA1	13	Splice	delG	/	IVS‡13+1delG
7	BRCA1	14	Splice	G to T	R1495M	4603G to T
8	BRCA1	17	Frameshift	del5	Stop1680	5154del5
9	BRCA1	21	Splice	G to A	/	IVS21+G to A
10	BRCA2	11	Frameshift	delGT	Stop 1284	4075delGT
11	BRCA2	11	Frameshift	delAAAC	Stop 959	3034delAAAC
12	BRCA2	11	Nonsense	C to G	S1630X	5117C to G
5	BRCA2	16	Nonsense	7966C to T	Q2580X	7966C to T
13	BRCA2	18	Frameshift	delGA	Stop2762	8475delGA
Unclassified variants						
14	BRCA1	8	Missense	C to T	T176M	646C to T
15	BRCA1	16	Missense	C to A	S1577T	4849C to A
16	BRCA2	3	Missense	T to A	/	IVS2-7T>A
17	BRCA2	22	Missense	A to G	/	IVS21-17A to G

*Del, deletion; †Ins, insertion; ‡IVS, intervening sequence

and the *BRCA2* S1630X mutations were already described in families with hereditary breast and ovarian cancer from Italy (14,28-30). One patient was found to have mutations in both the *BRCA1* (4406CtoA) and *BRCA2* (7966CtoT) genes (31). Furthermore, missense variants of uncertain significance were detected in 4 women (Table 3). Polymorphisms were detected in 16 additional cases (data not shown).

Correlation between BRCA Mutation Status and Clinico-Pathological Features

Correlations of BRCA status with both molecular and clinical variables of the CBC study cohort are summarized in Table 3. Mutations were detected in 9 (50%) out of 18 women diagnosed with first primary breast cancer at the age of 40 years or younger (five [28%] in *BRCA1*, three [17%] in *BRCA2*, and one [5%] in both *BRCA1* and *BRCA2*). A direct comparison of age distribution between *BRCA1* and *BRCA2* mutation carriers was not statistically significant (P = 1.0). BRCA-positive cases tended to be diagnosed at younger ages than BRCA-negative controls (P = 0.005). Predictably, a known family history of breast and/or ovarian cancer was more frequently observed in *BRCA1* and *BRCA2* mutation carriers (P = 0.03) and they also showed higher ovarian cancer incidence rate (P = 0.04). There were no statistically significant differences in tumor stage of first and second primary breast cancers between women with or without germline BRCA mutations but, women with *BRCA1* mutations, and to a lesser extent *BRCA2* mutations, were significantly more likely to present with high histologic grade, negative hormone receptor status, and high proliferation rate in both first and second primary breast cancers than BRCA-negative tumors. Though the relationship between BRCA mutations and HER2 negative status was only marginally significant, it was notable that almost all evaluable BRCA-positive tumors were HER2 negative (Table 3). To confirm that histopathological characteristics of both first and second primary breast cancers diagnosed in BRCA carriers were qualitatively distinct from those of BRCA-negative tumors, we compared the molecular profile of BRCA-positive, CBC cases with an external control group of women with BRCA-negative UBC. As

Table 3. Molecular and clinical variables by BRCA status

Characteristic	BRCA-1 mutation*† (total, n = 9) No. (%)		BRCA-2 mutation*† (total, n = 5) No. (%)		No BRCA mutation* (total, n = 38) No. (%)		P		UV*‡ (total, n = 4) No. (%)	
Family history§										
Positive	5 (56)		2 (50)		7 (19)		0.03		0	
Negative	4 (44)		2 (50)		29 (81)				4 (100)	
Breast-Ovarian Cancer	2 (22)		1 (20)		1 (3)		0.04		0	
Contralateral breast cancer										
Synchronous	0		1 (20)		11 (29)		0.14		1 (25)	
Metachronous	9 (100)		4 (80)		27 (71)				3 (75)	
Age at diagnosis of 1st tumor ≤ 40	6 (67)		4 (80)		8 (21)		0.005		1 (25)	
Median time between 1st and 2nd tumor-yrs	4.9		3.4		6.1		0.38		2.9	
	1st tumor	2nd tumor	1st tumor	2nd tumor	1st tumor	2nd tumor	P^1	$P2^2$	1st tumor	2nd tumor
Tumor stage										
I-II	7 (78)	8 (100)	5 (100)	5 (100)	24 (63)	32 (84)	0.17	0.31	4 (100)	2 (50)
III-IV	2 (22)	0	0	0	14 (37)	6 (16)			0	2 (50)
Histologic grade										
G1-G2	2 (24)	1 (11)	1 (20)	3 (60)	25 (83)	31 (84)	0.001	<0.001	1 (50)	1 (25)
G3	6 (76)	8 (89)	4 (80)	2 (40)	5 (17)	6 (16)			1 (50)	3 (75)
Estrogen receptor										
Positive	1 (11)	0	2 (40)	3 (60)	33 (92)	35 (95)	<0.001	<0.001	1 (33)	2 (50)
Negative	8 (89)	9 (100)	3 (60)	2 (40)	3 (8)	2 (5)			2 (67)	2 (50)
Progesterone receptor										
Positive	1 (11)	0	2 (40)	2 (40)	32 (89)	31 (84)	<0.001	<0.001	1 (33)	1 (25)
Negative	8 (89)	9 (100)	3 (60)	3 (60)	4 (11)	6 (16)			2 (67)	3 (75)
Proliferation rate										
Low	0	0	1 (20)	2 (40)	23 (74)	26 (70)	<0.001	<0.001	2 (100)	0
High	6 (100)	9 (100)	4 (80)	3 (60)	8 (26)	11 (30)			0	4 (100)
HER2 status										
Negative	7 (88)	9 (100)	5 (100)	5 (100)	26 (84)	28 (74)	0.65	0.04	2 (100)	2 (67)
Positive	1 (12)	0	0	0	5 (16)	10 (26)			0	1 (33)

Abbreviations: No, number; %, percentage; yrs, years.
*Numbers in these categories may not sum to the total because of missing data.
†The double heterozygote for BRCA-1 and BRCA-2 mutations was included in both the BRCA-1 and BRCA-2 groups.
‡U.V., unclassified variants. Only cases with UV not combined with mutations were considered.
§At least one first and one second-degree relative with breast cancer or one first-degree relative affected by ovarian cancer.
P^1 Comparison of first tumor characteristics between BRCA-positive and BRCA-negative patients.
P^2 Comparison of second tumor characteristics between BRCA-positive and BRCA-negative patients.

shown in Table 4, a diagnosis of TNBC was significantly more frequent in women with BRCA mutations compared with non-BRCA controls. Notably, women with BRCA mutations were commonly diagnosed with TNBC in both breasts with no difference in TNBC occurrence rate between first and second primary breast cancer ($P = 1.0$). The two control groups of women with BRCA-negative CBC, and with BRCA-

Table 4. Molecular profile of BRCA-positive, CBC cases compared with BRCA-negative, UBC

Characteristic	BRCA-1 mutation*† (total, n = 9) No. (%)		BRCA-2 mutation*† (total, n = 5) No. (%)		UBC controls* (total, n = 55) No. (%)	P	
	1st tumor	2nd tumor	1st tumor	2nd tumor	Reference tumor	P1	P2
Tumor stage							
I-II	7 (78)	8 (100)	5 (100)	5 (100)	38 (83)	0.58	0.39
III-IV	2 (22)	0	0	0	8 (17)		
Histologic grade							
G1-G2	2 (24)	1 (11)	1 (20)	3 (60)	26 (58)	0.12	0.03
G3	6 (76)	8 (89)	4 (80)	2 (40)	19 (42)		
Estrogen receptor							
Positive	1 (11)	0	2 (40)	3 (60)	33 (73)	<0.001	<0.001
Negative	8 (89)	9 (100)	3 (60)	2 (40)	12 (27)		
Progesterone receptor							
Positive	1 (11)	0	2 (40)	2 (40)	24 (55)	0.05	0.004
Negative	8 (89)	9 (100)	3 (60)	3 (60)	20 (45)		
Proliferation rate							
Low	0	0	1 (20)	2 (40)	25 (56)	0.007	0.01
High	6 (100)	9 (100)	4 (80)	3 (60)	20 (44)		
HER2 status							
Negative	7 (88)	9 (100)	5 (100)	5 (100)	32 (71)	0.4	0.07
Positive	1 (12)	0	0	0	13 (29)		
Triple-negative							
Yes	7 (88)	9 (100)	3 (60)	2 (40)	12 (27)	0.001	<0.001
No	1 (12)	0	2 (40)	3 (60)	32 (73)		

Abbreviations: CBC, contralateral breast cancer; UBC, unilateral breast cancers; No, number; yrs, years.
*Numbers in these categories may not sum to the total because of missing data.
†The double heterozygote for BRCA-1 and BRCA-2 mutations was included in both the BRCA-1 and BRCA-2 groups.
P1 Comparison of 1st tumor characteristics of BRCA-positive cases with tumor characteristics of BRCA-negative controls.
P2 Comparison of 2nd tumor characteristics of BRCA-positive cases and reference tumor characteristics of BRCA-negative controls.

negative UBC were compared with BRCA-positive cases regarding clinical outcome. The median follow-up periods for CBC and UBC cohorts were 13.5 years (minimum 5.3 years) and 14.6 years (minimum 5.5 years), respectively. Despite the increased frequency of TNBC subtype observed in the BRCA-positive group, there were no survival differences between BRCA-positive and non-BRCA tumors (Figure 1).

Discussion

In this series of 55 Italian women with contralateral breast cancer (CBC), we investigated the contribution of *BRCA1* and *BRCA2* germline mutations to the phenotype of these tumors. Fifty-five women with sporadic unilateral breast cancer (UBC) and negative BRCA status were selected from the same breast cancer population as control group, and were compared with BRCA-positive CBC patients regarding molecular status and clinical variables. Our results show that in a population-based study CBCs with *BRCA1* mutations (and to a lesser, but still significant extent - *BRCA2* mutations) have distinct clinico-pathological features from non-BRCA cancers (both CBC and UBC controls). According to previously reported studies (32-34), we confirm that BRCA mutations result in breast cancers that are highly likely to be triple-negative in character. Our data also demonstrate, for the first time to our knowledge, that CBC cases with BRCA mu-

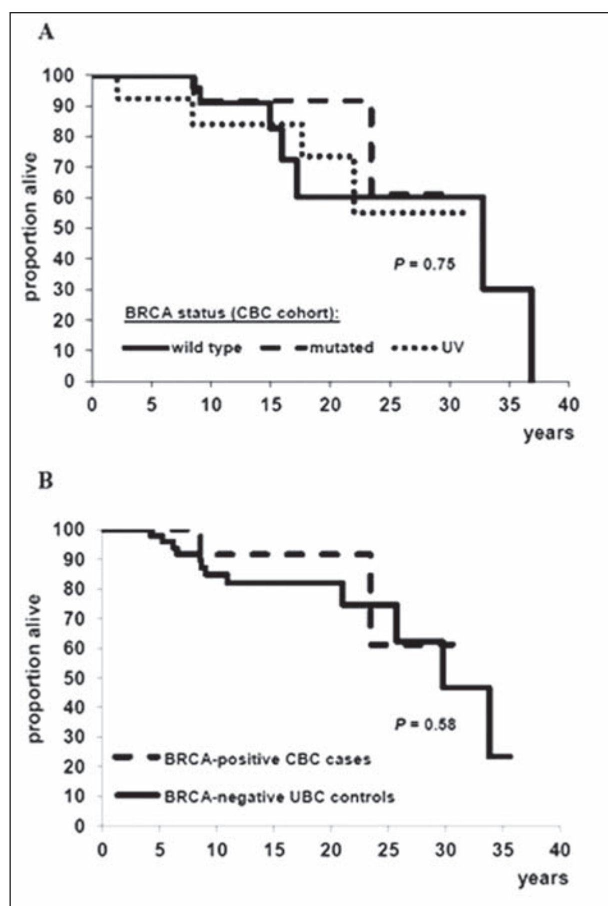

Figure 1. Overall survival (OS). **A**, OS of contralateral breast cancer (CBC) patients by BRCA status (BRCA wild type, mutated, unclassified variants [UV]). **B**, OS of BRCA-positive, CBC cases and BRCA-negative, unilateral breast cancer (UBC) controls

tations are commonly diagnosed with TNBC in both breasts with no difference in TNBC occurrence rate between first and second primary breast cancer. The notion that, at some level, the various BRCA mutations share a common mechanism of tumorigenesis is therefore reinforced (14,35).

Although there have been several studies that estimate the risk of CBC in women with a *BRCA1* or *BRCA2* mutation, there has been little research on the predictors of CBC risk. In a recent population-based study, CBC risk was substantially greater for BRCA mutation carriers diagnosed with their first cancer at younger ages (2). In another study, BRCA mutation carriers diagnosed with breast cancer younger than 40 years of age had a 15-year contralateral breast cancer risk of 42% (annual risk 2.8%) compared with 19% risk for women over the age of 50 years at time of diagnosis (annual risk 1.3%) (9). In our study, the rate of BRCA mutations in CBC cases was relatively high (24%). Furthermore, BRCA mutations were detected in 50% of women diagnosed with first primary breast cancer at the age of 40 years or younger, and nearly all of these tumors were TNBC. Assuming that a mutation frequency of at least 10% is the threshold generally adopted to select a population for BRCA mutation screening (36,37), our findings suggest that the combination of early-onset breast cancer and primary TNBC phenotype can provide an efficient model for identifying individuals with high risk for both BRCA mutations and CBC. Several studies reported no difference or poorer survival outcomes in BRCA mutation carriers compared with non-carriers (38-40). An equal or improved prognosis for BRCA mutation carriers compared with wild-type subjects was also described (41,42). In the present study, two control groups were compared with BRCA-positive, CBC patients regarding clinical outcome: i) women with BRCA-negative CBC, and ii) women with BRCA-negative UBC. No significant differences in OS were observed between the two control groups and the women with BRCA mutations. Although the relatively small sample size of our study limits the power to detect small but significant differences in survival, such results may be considered somewhat in contrast to the increased frequency of poor prognostic TNBC phenotype observed in our BRCA-positive cases. However, a recent study demonstrated that a positive *BRCA1* mutation status was an independent prognostic factor for lower distant breast cancer recurrence risk in TNBC patients (43). Furthermore, in a previous study, Gonzalez-Angulo et al. showed improved recurrence-free survival for *BRCA1*-positive TNBC patients treated with surgery and anthracycline-taxane chemotherapy when compared with women with *BRCA1*-negative TNBC (44). All these findings indicate that BRCA-associated TNBC should be considered as a biologically and prognostically distinct subtype of TNBC, and may be useful for counseling patients with regard to life expectancy, affecting the choice of chemotherapy regimens and providing a unique molecular profile for molecular-targeted therapies (43). Limitations of our study include both

the small sample size and the retrospective design. Because our analysis was limited to BRCA-tested women who survived breast cancer, we cannot exclude the possibility that findings might differ if otherwise eligible deceased women were included (2). Similarly, the criteria used by our Genetic Oncology Service to screen patients for BRCA genetic testing may have been determined a potential selection bias in favor of high-risk breast cancer kindreds. Even so, almost 50% of our BRCA-positive, CBC cases did not have a significant family history of breast and/or ovarian cancer, and none of BRCA-carriers were of Ashkenazi Jewish ancestry. Another study limitation is the lack of data regarding mutations in other breast cancer predisposing genes. Recent reports indicate that loss-of-function mutations in *PALB2* gene determine a ninefold increase in the risk for breast cancer in carriers, which translates into a 35% risk by age 70 in women carriers (45). These data suggest the breast-cancer risk for *PALB2* mutation carriers may overlap with that for *BRCA2* mutation carriers (45). Interestingly, most of the PALB2-related breast cancers were reported to be estrogen receptor–positive, but there also appeared to be an increase in women with TNBC compared with non-mutation carriers (34,45,46). However, due to the lack of prospective information in unselected patients, the role of *PALPB2* mutations in individuals with TNBC who test negative for *BRCA1/BRCA2* mutations should be confirmed in future studies. In conclusion, the present study shows that almost one-fourth of an unselected population of women with CBC have deleterious BRCA mutations. Both first primary and second primary breast cancers in BRCA carriers are qualitatively distinct from BRCA-negative CBC, and from sporadic UBC controls. These data highlight relevant clinical considerations about the potential value of BRCA testing in women with CBC as well as therapeutic, preventive, and surveillance implications for patients carrying a mutation.

Acknowledgements

Authors would like to thank Dr. Paolo Sgargi (Cancer Registry of Parma Province, University Hospital of Parma, Italy) for his contribution regarding this work. This study was supported in part by a grant from the Regione Emilia-Romagna Breast Cancer Screening Program.

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Received: 23 September 2015
Accepetd: 16 October 2015
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