Primary research

BRCA1 and *BRCA2* mutations in central and southern Italian patients

Laura Ottini*†, Cristina D'Amico*†, Cristiana Noviello*, Salvatore Lauro†, Maurizio Lalle†, Giuseppe Fornarini†, Orsola Anna Colantuoni‡, Claudia Pizzi‡, Enrico Cortesi†, Sandro Carlini§, Fiorella Guadagni§, Angelo Raffaele Bianco‡, Luigi Frati†, Alma Contegiacomo‡, Renato Mariani-Costantini*

*University 'Gabriele D'Annunzio', Chieti, †University 'La Sapienza', Rome, ‡University 'Federico II', Naples, and §Regina Elena Cancer Institute, Rome, Italy

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Statement of findings

Protein truncation test (PTT) and single-strand conformation polymorphism (SSCP) assay were used to scan the *BRCA1* and *BRCA2* genes in 136 unrelated Italian breast/ovarian cancer patients. In the sample tested, *BRCA1* and *BRCA2* equally contributed to site-specific breast cancer patients who reported one to two breast cancer-affected first-/second-degree relative(s) or who were diagnosed before age 40 years in the absence of a family history of breast/ovarian cancer. *BRCA1* and *BRCA2* mutations were mostly found in patients with disease diagnosis before and after age 50 years, respectively. Moreover, in cases with familial clustering of site-specific breast cancer, *BRCA1* mostly accounted for tumours diagnosed before age 40 years and *BRCA2* for tumours diagnosed after age 50 years. The *BRCA1* and *BRCA2* mutation spectrum was consistent with a lack of significant founder effects in the sample of patients studied.

Keywords: *BRCA1*, *BRCA2*, breast, carcinoma, germline mutations, Italy

Synopsis

Introduction: Germline *BRCA1* and *BRCA2* mutations account for most hereditary breast/ovarian cancers and are associated with male breast cancer. Furthermore, constitutional mutations in these genes may occur in breast/ovarian cancer patients that do not meet stringent criteria of autosomal-dominant predisposition. The relevance of *BRCA1* and *BRCA2* mutations in such patients is still debated.

Objectives: We sought to determine the impact of *BRCA1* and *BRCA2* mutations in a population of patients from central and southern Italy. We analyzed the *BRCA1* and *BRCA2* coding regions in 136 unrelated probands: 117 females with breast/ovarian cancer and 19 males with breast cancer. This population of patients was mostly representative of cases who are at risk for hereditary susceptibility, but who do not meet stringent criteria of autosomal-dominant predisposition.

Methods: Probands, subclassified as follows, were consecutively recruited depending on informed consent from patients attending breast cancer clinics in Rome and Naples. Selection criteria for females were as follows: breast cancer with breast cancer family history [one to two first-/second-degree relative(s), $n = 55$]; breast cancer diagnosed before age 40 years (no breast/ovarian cancer family history, $n = 28$); bilateral breast cancer (regardless of age and family history, $n = 10$); breast cancer associated with gastrointestinal,

CASH = Contraceptive and Steroid Hormones; PCR = polymerase chain reaction; PTT = protein truncation test; SSCP = single-strand conformation polymorphism.

Table 1

Oligonucleotide primers used for PTT and SSCP analyses

Exon screened	Primer
PTT	
BRCA1 exon 11	Br5′-S: 5′-GCTGCTTGTGAATTTTCTGAG-3′
	Br5′-A: 5′-GCCTGCAGTGATATTAACTGTCTG-3′
	Br3′-S: 5′-GAAGAAAGTGAACTTGATG-3′
	Br3′-A: 5′-TAAGTTTGAATCCATGCTTTG-3′
BRCA2 exon 10	B10-S: 5′-TTTGGAAAAACATCAGGGAATT-3′
	B10-A: 5′-AAACACAGAAGGAATCGTCATC-3′
BRCA2 exon 11	B11-1S: 5′-CATTCTTCTGTGAAAAGAAGCTG-3′
	B11-1A: 5′-TGGTTTGAATTAAAATCCTGC-3′
	B11-2S: 5′-TACATGAACAAATGGGCAGGAC-3′
	B11-2A: 5′-TCCAGTACCAACTGGGACAC-3′
	B11-3S: 5′-GATCAGAAACCAGAAGAATTGC-3′
	B11-3A: 5′-TTGGGATATTAAATGTTCTGGAGTA-3′
	B11-4S: 5′-TCACCTTGTGATGTTAGTTTG-3′
	B11-4A: 5′-GTTAGCATACCAAGTCTACTG-3′
SSCP	
BRCA1 exons 2, 3, 5–10, 12–24	Reported by Friedman *et al* [17]
BRCA2 exons 2–9, 12, 13, 15, 16, 19–27	Reported by Friedman *et al* [11]
BRCA2 exon 14	B14-S: 5′-GTGTACTAGTCAATAAAC-3′
	B14-A: 5′-CATCACACAAATTGTCAT-3′
BRCA2 exon 18	B18-S: 5′-GAATTCTAGAGTCACACTTCCT-3′
	B18-A: 5′-ACTGATTTTTACCAAGAGTGCA-3′

Sense primers used for PTT contain a T7 promoter and a eukaryotic translation initiation sequence: 5′-GGATCCTAATACGACTCACTATAGGGA-GACCACCATG-3′. A, antisense; S, sense.

pancreatic or uterine cancers [synchronous/metachronous or in first-degree relative(s), $n = 9$]; breast or ovarian cancer with family history of breast–ovarian/ovarian cancer (at least 1 first-/second-degree relative, $n = 10$); and ovarian cancer with no breast/ovarian cancer family history ($n = 5$). Males with breast cancer were recruited regardless of age and family history. *BRCA1* exon 11 and *BRCA2* exons 10 and 11 were screened by PTT. Coding *BRCA1* exons 2, 3, 5–10 and 12–24 and *BRCA2* exons 2–9 and 12–27 were screened by SSCP. Primers are listed in Table 1. In 27 cases, analyzed by PTT along the entire *BRCA1* coding sequence, *BRCA1* SSCP analysis was limited to exons 2, 5, 20 and 24. Mutations were verified by sequence analysis on two independent blood samples.

Results: Deleterious germline *BRCA1*/*BRCA2* mutations were detected in 11 out of 136 cases (8%). Only three *BRCA2* mutations were novel. One *BRCA2* mutation recurred in two unrelated probands. Table 2 shows the mutations and data concerning carriers and their families. Table 3 shows correlations between *BRCA1*/*BRCA2* mutations and sex, age at disease diagnosis and familial clustering of breast/ovarian cancer in the total patient population. Table 4 shows the proportions of *BRCA1* and *BRCA2* mutations in females with site-specific breast and breast–ovarian/ovarian cancer. Table 5 shows the frequency of *BRCA1*/*BRCA2* mutations in males.

BRCA1 and *BRCA2* mutations, respectively, accounted for four out of 68 (6%) and one out of 68 (1%) cases diagnosed before age 50 years, and for one out of 68 (1%) and five out of 68 (7%) cases diagnosed after age 50 years. *BRCA1* mutations were found in five out of 117 females (4%) and in none of 19 males (0%), and *BRCA2* mutations were found in four out of 117 females (3%) and in two out of 19 males (10%). The proportions of *BRCA1* and *BRCA2* mutations coincided in site-specific female breast cancers (four out of 102; ie 4% each). *BRCA1* and *BRCA2* equally contributed to female breast cancers, with no familial clustering in those diagnosed before age 40 years (one out of 28; 4% each), and to female breast cancers, all ages, with familial clustering in one to two relatives (three out of 55; ie 5% each). In the latter subset of cases, *BRCA1* mostly accounted for tumours diagnosed before age 40 years (two out of eight; 25%), and *BRCA2* for tumours diagnosed after age 50 years (three out of 34; 9%). Regardless of family history, the respective contributions of *BRCA1* and *BRCA2* to site-specific female breast cancers diagnosed before age 40 years were 8% (three out of 36) and 3% (one out of 36). One *BRCA1* mutation was detected among the 15 female probands from breast–ovarian/ovarian cancer families (7%). Among male breast cancers, *BRCA2* mutations were identified in one out of five (20%) cases with family history and in one out of 14 (7%) apparently sporadic cases. No *BRCA1* or *BRCA2* mutations were found in female probands with nonfamilial bilateral breast cancer (10 cases) or in those with breast cancer associated with gastrointestinal, pancreatic or uterine cancers, synchronous/metachronous or in first-degree relative(s) (nine cases). These cases were all diagnosed after age 40 years.

Discussion: Our results indicate a lack of relevant founder effects for *BRCA1*- and *BRCA2*-related disease in the sample of patients studied, which is consistent with other Italian studies and with ethnical and historical data. Overall, the

Table 2

Germline *BRCA1* and *BRCA2* mutations detected in selected samples from 136 unrelated probands and clinicopathologic correlations

Sex	Type of cancer	Family history	Gene	Exon	Codon	Nucleotide	Effect
Female	Br (26)	None	*BRCA1*	11	454	1479	delAG-ter454
Female	Ov (45)	Mo: Ov (57)	*BRCA1*	11	502	1623	5bpdel-ter505
Female	Br (64)	D: Br (24) D: Br (41)	*BRCA1*	11	1254	3880	delAG-ter1265
Female	Br (38)	Fa: Ga (64) Pa: Br (50)	*BRCA1*	5	61	300	Cys/Gly
Female	bil Br (40)	Mo: Br (49)	*BRCA1*	16	1655	5083	del19bp-ter1670
Male	Br (79)	S: Br (35) S: Br (55) S: Br (50) S: Col (47)	*BRCA2*	11	1293	4109	TTA-TAA (Lys-Stop)
Female	Br (55)	S: Br (49) S: Pan (52) B: Col (54)	*BRCA2*	11	1370	4339	CAG-TAG (Glu-Stop)
Female	Br (62)	Mo: Br (59)	*BRCA2*	11	1629	5117	TCA-TGA (Ser-Stop)
Male	Br (54)	None	*BRCA2*	11	1906	5950	delCT-ter1909
Female	Br (57)	S: Br (50)	*BRCA2*	11	2156	6696	delTC-ter2174
2Female	Br (34)	None	*BRCA2*	11	2156	6696	delTC-ter2174

Numbers in parentheses indicate age at onset. B, brother; bil Br, bilateral breast cancer; Br, breast cancer; Col, colorectal cancer; D, daughter; Fa, father; Ga, gastric cancer; Mo, mother; Ov, ovarian cancer; Pa, paternal aunt; Pan, pancreatic cancer; S, sister.

Table 3

***BRCA1* and *BRCA2* mutations by sex, age at disease diagnosis and presence of breast/ovarian cancer in first-/second-degree relative(s) in the total population of 136 breast/ovarian cancer probands analyzed**

	BRCA1		BRCA2		Total	
Characteristics of patients	Positive (%)	Negative	Positive (%)	Negative	Positive (%)	Negative
Age						
50 years or under	4 (6)	64	1 (1)	67	5 (7)	63
Older than 50 years	1 (1)	67	5 (7)	63	6 (9)	62
Sex						
Female	5 (4)	112	4 (3)	113	9 (8)	108
Male	0 (0)	19	2 (10)	17	2 (10)	17
Cancer in relative(s)						
Yes	4 (6)	66	4 (6)	66	8 (11)	62
No	1 (1)	65	2 (3)	64	3 (4)	63

Positive indicates the presence of deleterious mutations, whereas negative indicates the absence of such mutations.

contribution of *BRCA1* and *BRCA2* to breast/ovarian cancer in Italian patients appears to be less significant than in patients from communities with founder mutations. The present study is in agreement with direct estimates on other outbred populations, indicating that 7–10% of all female breast cancers that occur in patients aged under 40 years are due to *BRCA1*/*BRCA2*.

We found that *BRCA1* and *BRCA2* equally contributed to site-specific breast cancers who had one/two breast cancer-affected first-/second-degree relative(s) or who were diagnosed within age 40 years in the absence of family history. This is consistent with recent data that indicated that the respective frequencies of *BRCA1* and *BRCA2* mutations are comparable in early onset breast cancer. Considering the total population of patients analyzed here, however, *BRCA1* and *BRCA2* mutations were mostly found in cases with disease diagnosis before and after age 50 years, respectively. Moreover, in cases with familial clustering of site-specific

Table 4

Proportions of *BRCA1* and *BRCA2* mutations in 117 female breast/ovarian cancer probands, classified by number of cancer patients in the family, age at disease diagnosis, site-specific breast or breast–ovarian/ovarian cancer

	Age of proband at disease diagnosis							
	40 years or less		41–50 years		51 years or more		All ages	
Cancer patients in family†	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]
Breast								
One	4 (1/28)	4 (1/28)	0 (0/9)*	0 (0/9)*	0 (0/10)*	0 (0/10)*	2 (1/47)*	2 (1/47)*
Two or three	25 (2/8)	0 (0/8)	0 (0/13)	0 (0/13)	3 (1/34)	9 (3/34)	5 (3/55)	5 (3/55)
Total	8 (3/36)	3 (1/36)	0 (0/22)	0 (0/22)	2 (1/44)	7 (3/44)	4 (4/102)	4 (4/102)
Breast/ovary								
None/one	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/3)	0 (0/3)	0 (0/5)	0 (0/5)
None to two/ one or two	–	–	25 (1/4)	0 (0/4)	0 (0/6)	0 (0/6)	10 (1/10)	0 (0/10)
Total	0 (0/1)	0 (0/1)	20 (1/5)	0 (0/5)	0 (0/9)	0 (0/9)	7 (1/15)	0 (0/15)

†Including proband; numbers of *BRCA1*- and *BRCA2*-positive cases over number of cases in each age subset are given in parentheses. *The 19 breast cancer patients with no familial clustering of breast/ovarian cancer diagnosed above age 40 years included 10 cases with bilateral breast cancer and nine patients with breast cancer associated with gastrointestinal, pancreatic or endometrial cancer (synchronous/metachronous, four cases; in a first-degree relative, five cases).

Table 5

Proportions of *BRCA1* and *BRCA2* mutations in 19 male breast cancer probands classified by number of cancer patients in the family and age at disease diagnosis

	Age of proband at disease diagnosis					
	41–50 years		51 years or more		All ages	
Cancer patients in family*	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]	*BRCA1* [% (*n*)]	*BRCA2* [% (*n*)]
One	0 (0/1)	0 (0/1)	0 (0/13)	8 (1/13)	0 (0/14)	7 (1/14)
Two or three	0 (0/3)	0 (0/3)	0 (0/2)	50 (1/2)	0 (0/5)	20 (1/5)
Total	0 (0/4)	0 (0/4)	0 (0/15)	13 (2/15)	0 (0/19)	10 (2/19)

*Including proband; numbers of *BRCA1*- and *BRCA2*-positive cases over number of cases in each age subset are given in brackets.

breast cancer, *BRCA1* mostly accounted for tumours diagnosed before age 40 years, and *BRCA2* for tumours diagnosed after age 50 years. This is in agreement with a trend, which has been observed in other populations, for the proportion of cases with *BRCA2* mutations to increase, and the proportion with mutations in *BRCA1* to decrease, as the age at cancer onset increases.

As in other studies, the frequency of *BRCA1*/*BRCA2* mutations taken together was lower than the estimated frequencies at comparable ages for all susceptibility alleles derived from the Contraceptive and Steroid Hormones (CASH) study. The discrepancy between direct data deriving from *BRCA1*/*BRCA2* mutational analysis and CASH estimates could be due to several factors, including contribution of gene(s) other than *BRCA1*/*BRCA2*, differences between populations and relative insensitivity of mutational screening.

Only *BRCA1* mutations were found in breast/ovarian and site-specific ovarian cancer families. *BRCA2*, but not *BRCA1* mutations were found in the male breast cancers. The overall proportion of males with *BRCA2* mutations was high when compared with data from other studies on outbred populations, but was low compared with data from populations with founder effects.

The present results should be regarded as an approximation, because the following types of mutation are predicted to escape detection by the screening strategy used: mutations in noncoding regions; missense mutations within *BRCA1* exon 11 and *BRCA2* exons 10 and 11; large gene deletions; and mutations within the first and last 180 nucleotides of the amplicons analyzed by PTT.

Full article

Introduction

The proportion of breast cancers that are attributable to autosomal-dominant susceptibility genes is estimated to be approximately 7% in the general population [1–4]. Germline mutations of the *BRCA1* and *BRCA2* genes are estimated to contribute to the majority of the breast cancers that have very early disease onset, strong family history and/or association with ovarian cancer [4–6]. *BRCA1* and *BRCA2* also account for a proportion of common breast/ovarian cancer patients that typically do not meet stringent criteria for highly penetrant autosomal-dominant cancer predisposition, but rather report one or two disease-affected relatives and/or manifest an early disease onset [4,6,7]. Knowledge of the contribution of *BRCA1* and *BRCA2* to breast cancer in these patients is still incomplete [4–8]. A better understanding of the frequencies of *BRCA1* and *BRCA2* mutations in such moderate risk patients is fundamental to our appreciation of the importance of these genes as a cause of disease in the general population. Furthermore, *BRCA2* and, to a lesser extent, *BRCA1* also appear to be responsible for an important, but still debated proportion of male breast cancers [4,9–14].

We analyzed the entire *BRCA1* and *BRCA2* coding regions in 136 unrelated probands: 117 female breast/ovarian cancer patients and 19 male breast cancer patients. This sample, selected from patients attending breast cancer clinics in Rome and Naples, was primarily drawn from moderate-risk families originating from central and southern Italy, a geographic region that is known to be ethnically heterogeneous [15]. We used a screening strategy based on a combination of PTT and SSCP analyses, techniques that are mutually complementary in sensitivity and that may identify more than 80% of mutations [16].

Patients and methods

Patients

A total of 136 probands, subclassified as listed below according to selection criteria, were consecutively recruited depending on informed consent from among breast/ovarian cancer patients attending the breast cancer clinics participating in the study in Rome and Naples. The patients originated from the regions of Latium and Abruzzo (central Italy) and Campania and Molise (southern Italy). They included 55 patients with female breast cancer, any age, with breast cancer in one or two first-/second-degree relative(s); 28 patients with female breast cancer diagnosed before age 40 years, who reported no family history of breast/ovarian cancer; 10 patients with female breast/ovarian cancer, any age, with a family history of ovarian/breast–ovarian cancer in at least one first-/second-degree relative; 19 patients with male breast cancer, selected regardless of age and family history; five patients with ovarian cancer, any age, who reported no familial history of breast/ovarian cancer; 10 patients with bilateral breast cancer, selected regardless of age, who reported no family history of breast/ovarian cancer; and nine patients with breast cancer associated with gastrointestinal, pancreatic or uterine cancers, synchronous/metachronous, or in first-degree relative(s), selected regardless of age. Analysis of genomic DNA, RNA and cDNA preparations from peripheral blood lymphocytes were performed following standard procedures. The research protocol was approved by the ethical committee of the University 'Gabriele D'Annunzio'.

BRCA1 and *BRCA2* mutational analysis

All patients were analyzed for constitutional mutations throughout the entire *BRCA1* and *BRCA2* coding regions. *BRCA1* exon 11 and *BRCA2* exons 10 and 11 were screened by PTT from genomic DNA using the primers listed in Table 1. Coding *BRCA1* exons 2, 3, 5–10 and 12–24, and *BRCA2* exons 2–9 and 12–27 were screened by SSCP analysis using previously reported primers [11,17]. Primers for *BRCA2* exons 14 and 18 and for a 281 bp *BRCA2* fragment that encompassed the intron 10/exon 11 boundary are reported in Table 1. In 27 cases, who were analyzed by PTT along the entire *BRCA1* coding sequence as described by Friedman *et al* [17], *BRCA1* SSCP analysis was limited to exons 2, 5, 20 and 24, to identify missense mutations that are reportedly frequent in these exons and to allow a better investigation of the 5′ and 3′ ends of the coding sequence. PTT and polymerase chain reaction (PCR)-SSCP were performed as described previously [11,17,18]. When truncated peptides or variant SSCP conformers were identified, genomic DNA was reamplified and directly sequenced with the PCR product sequencing kit (Sequenase Version 2.0, USB-Amersham, Cleveland, OH, USA). Mutations were verified on two independent blood samples.

Results

Deleterious germline *BRCA1*/*BRCA2* mutations were detected in 11 out of 136 cases (8%). Table 2 shows the mutations and data concerning carriers and their families. Table 3 shows correlations between *BRCA1*/*BRCA2* mutations and sex, age at disease diagnosis and familial clustering of breast/ovarian cancer in the total patient population. Table 4 shows the proportions of *BRCA1* and *BRCA2* mutations in females with site-specific breast and breast–ovarian/ovarian cancer. Table 5 shows the frequency of *BRCA1*/*BRCA2* mutations in males.

The five deleterious *BRCA1* mutations (Table 2) included four frameshift mutations (*BRCA1* 1479delAG, *BRCA1* 1623del5bp, *BRCA1* 3880delAG, *BRCA1* 5083del19bp)

and one missense mutation (*BRCA1* 300TtoG). These mutations were already reported in the literature [18–22] or in the Breast Cancer Information Core electronic database (http://nchgr.nih.gov/Intramural_research/Lab_transfer/Bic/). In addition to the above described deleterious mutations, the neutral *BRCA1* coding variants Glu1038Gly and, in homozygosity, Ser1613Gly were found in two patients [20]. A novel C to G transversion, affecting the conserved C/T residues of the consensus sequence for the 3′-splice site of *BRCA1* intron 22, was also found in a 60-year-old woman with synchronous breast and gastric cancers, but no family history of cancer. Sequence analysis from cDNA and genomic DNA revealed normal exon 23 and exon 24 transcripts. Allele expression analysis was not feasible, because the patient was homozygous at multiple *BRCA1* polymorphisms.

Five deleterious *BRCA2* mutations, all localized in exon 11 and including three nonsense and two frameshift mutations, were identified in six patients (Table 2). *BRCA2* 4109TtoA, *BRCA2* 4339CtoT and *BRCA2* 5117CtoG are novel, whereas *BRCA2* 5950delTC and *BRCA2* 6696delTC were previously reported [23,24]. One mutation (*BRCA2* 6696delTC) recurred in two unrelated probands. The haplotype of the mutation-bearing chromosomes could not be reconstructed, because DNA samples from relatives of the two patients were not available.

BRCA1 and *BRCA2*, respectively, accounted for four out of 68 (6%) and one out of 68 (1%) patients diagnosed before age 50 years, and for one out of 68 (1%) and five out of 68 (7%) cases diagnosed after age 50 years. *BRCA1* mutations were found in five out of 117 females (4%) and in none of 19 males (0%), and *BRCA2* mutations were found in four out of 117 females (3%) and in two out of 19 males (10%). *BRCA1* and *BRCA2* mutations accounted for the same number of cases among patients with family history of breast/ovarian cancer (four out of 66; ie 4% each), whereas *BRCA1* and *BRCA2* mutations, respectively, accounted for one out of 66 (1%) and for two out of 64 (3%) of the cases without a family history (Table 3).

In site-specific female breast cancers, the proportions of *BRCA1* and *BRCA2* mutations coincided (four out of 102, ie 4% each). *BRCA1* and *BRCA2* equally contributed to female breast cancers with no familial clustering diagnosed before age 40 years (one out 28; 4% each) and to female breast cancers, all ages, with familial clustering in one or two relatives (three out of 55; 5% each). In the latter subset of cases, *BRCA1* mostly accounted for tumours diagnosed before age 40 years (two out of eight; 25%) and *BRCA2* for tumours diagnosed after age 50 years (three out of 34; 9%). Regardless of family history, the respective contributions of *BRCA1* and *BRCA2* to site-specific female breast cancers diagnosed before age 40 years were 8% (three out of 36) and 3% (one out of 36). One *BRCA1* mutation was detected among the 15 female probands from breast–ovarian/ovarian cancer families (7%). This patient was among the 10 who had a family history of breast/ovarian cancer (one out of 10, 10%; Table 4).

Among male breast cancers, *BRCA2* mutations were identified in one out of five (20%) patients with a family history and in one out of 14 (7%) apparently sporadic cases. No *BRCA1* or *BRCA2* mutations were found in female probands with nonfamilial bilateral breast cancer (10 cases) and with breast cancer associated with gastrointestinal, pancreatic or uterine cancers, synchronous/metachronous or in first-degree relative(s) (nine cases). These cases were all diagnosed after age 40 years (Table 5).

Discussion

We screened the coding sequences of the *BRCA1* and *BRCA2* genes in 136 breast/ovarian cancer patients, including 102 females with breast cancer who were mostly at moderate risk for mutation-carrier status, 15 females with breast–ovarian/ovarian cancer and 19 males with breast cancer. The sensitivity of the combined PTT/SSCP screening assays is reportedly high [16]. The present results should be regarded as an approximation, because the following types of mutation are predicted to escape detection: mutations in noncoding regions, which are estimated to account for a minimum of 10% of pathogenetic *BRCA1* and *BRCA2* mutations [5]; missense mutations in functionally important regions within *BRCA1* and *BRCA2* exons 11; large deletions undetectable by PCR-based assays; and mutations within the first and last 180 nucleotides of the amplicons analyzed by PTT. In spite of these limitations, the present study contributes evidence that is useful for assessing the importance of *BRCA1* and *BRCA2* mutations in patients who are not in high-risk families from outbred populations.

In contrast to studies on North and East European populations [10,14,25–27], the present results indicate a lack of relevant founder effects for *BRCA1*- and *BRCA2*-related disease in the sample of patients analyzed, which is in agreement with other Italian studies [24,28–30] and with ethnical and historical data [15]. The *BRCA1* mutations detected in the present study were previously reported in families with high cancer incidence of different ethnic or geographic origin, but not in other Italian surveys [24,28–30]. Interestingly, three of the six *BRCA2* mutations were nonsense and three were novel. *BRCA2* 6696delTC, reported in another series of Italian breast cancer patients [24] but only twice in the Breast Cancer Information Core database, was the only mutation detected more than once. This mutation may represent a candidate frequent mutation in the Italian population. A possible common origin of the mutation detected in two

unrelated patients could not be verified, however, because the haplotypes of the mutation-bearing chromosomes could not be reconstructed.

The contributions of *BRCA1* and *BRCA2* to breast/ovarian cancer in Italian patients appear to be less significant than in patients from communities with founder mutations [4,10,31,32]. The present results are in agreement with recent direct estimates on other outbred populations, which indicate that 7–10% of all female breast cancers that occur before age 40 years are due to *BRCA1*/*BRCA2* [4,8,32,33]. Moreover, the 8% *BRCA1* mutation rate found in women with site-specific breast cancer diagnosed before age 40 years, regardless of family history, is consistent with an indirect estimate that 5.3% of all female breast cancers among those who are under 40 years old may be due to mutations in *BRCA1* [34].

Overall, *BRCA1* and *BRCA2* equally contributed to site-specific breast cancer in patients who reported one or two breast cancer-affected first-/second-degree relative(s) or who were diagnosed before age 40 years in the absence of a family history. This is consistent with recent data [4,7] that indicated that the respective frequencies of *BRCA1* and *BRCA2* mutations are comparable in early onset breast cancer. Considering the total population of patients analyzed here, however, *BRCA1* and *BRCA2* mutations were mostly found in cases with disease diagnosis before and after age 50 years, respectively. Moreover, in cases with familial clustering of site-specific breast cancer, *BRCA1* mostly accounted for tumours diagnosed before age 40 years, and *BRCA2* for tumours diagnosed after age 50 years. This is in agreement with a trend, observed in other populations, for the proportion of cases with *BRCA2* mutations to increase, and the proportion with mutations in *BRCA1* to decrease as the age at cancer onset increases [4,31,35,36]. As in other studies [4], the frequency of *BRCA1*/*BRCA2* mutations taken together was lower than the estimated frequencies at comparable ages for all susceptibility alleles derived from the CASH study [3]. The discrepancy between direct data derived from *BRCA1*/*BRCA2* mutational analysis and CASH estimates could be due to several factors, including contribution(s) of gene(s) other than *BRCA1*/*BRCA2*, differences between populations and relative insensitivity of mutational screening.

In the present study, *BRCA1* mutations were detected in only one out of 10 cases from breast/ovarian and site-specific ovarian cancer families. This is a low proportion compared with studies that suggested that *BRCA1* and *BRCA2* are responsible for the large majority of breast/ovarian cancer families, with the greater proportion due to *BRCA1* [2–5,7,19,31]. In this respect, the limits of mutation detection techniques and the small number of breast/ovarian and site-specific ovarian cancer cases tested should be taken into account, together with the fact that most of the probands examined here were from families with only one case of ovarian cancer.

As expected, *BRCA2* mutations were detected in male breast cancer patients. *BRCA2* mutations were found in 20% of the males reporting familial clustering of breast cancer. In the males with no family history of breast/ovarian cancer, the proportion of carriers of *BRCA2* mutations (7%) was comparable to that obtained for *BRCA1* and *BRCA2* combined in site-specific female breast cancer patients (8%). The overall proportion of cancer-affected males with *BRCA2* mutations (10%) was high compared with data from other outbred populations, but was lower than that reported for populations with founder effects [4,11–14,37].

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Authors' affiliations: Laura Ottini, Cristina D'Amico, Cristiana Noviello and Renato Mariani-Costantini (Department of Oncology and Neurosciences, University 'Gabriele D'Annunzio', Chieti, Italy), Laura Ottini, Cristina D'Amico, Salvatore Lauro, Maurizio Lalle, Giuseppe Fornarini, Enrico Cortesi and Luigi Frati (Department of Experimental Medicine and Pathology, University 'La Sapienza', Rome, Italy), Orsola Anna Colantuoni, Claudia Pizzi, Angelo Raffaele Bianco and Alma Contegiacomo (Department of Endocrinology and Oncology, University 'Federico II', Naples,Italy), and Sandro Carlini and Fiorella Guadagni (Regina Elena Cancer Institute, Rome, Italy)

Correspondence: Professor Renato Mariani-Costantini, Section of Molecular Pathology, Department of Oncology and Neurosciences, University 'Gabriele D'Annunzio', Via dei Vestini, 66013 Chieti, Italy. Tel. +39 0871 355 4109/4116; Fax: +39 0871 355 4110; e-mail: mariani_cost@axrma.uniroma1.it/r.mariani@istpat.unich.it