MUTATION IN BRIEF

BRCA1 and BRCA2 Mutations in Breast/Ovarian Cancer Patients from Central Italy

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We report on the screening of the entire BRCA1/BRCA2 coding sequence by SSCP, PTT, and direct sequencing in 68 Italian families with recurrent breast or ovarian cancer. For each investigated proband, the probability of being carrier of a BRCA1/BRCA2 mutation was evaluated using the BRCAPRO software. We detected BRCA1/BRCA2 mutations in 8 patients (11.7%). However, if considering only patients with a carrier probability >10%, the detection rate was 36.8%, confirming the usefulness of the BRCAPRO software. One change (BRCA1 4172insT) was a novel mutation not reported in BIC database. © 2003 Wiley-Liss, Inc.

KEY WORDS: cancer, breast cancer, ovarian cancer, BRCA1, BRCA2, BRCAPRO software

INTRODUCTION

Breast Cancer (BC) is the most common malignancy in women in the western world (Parkin, 1998). Hereditary BC accounts for 5-10% of all cases and is characterised by dominant inheritance, early onset, severe course of the disease, bilaterality and frequent association with ovarian cancer (OC) (Claus et al., 1996). Linkage analysis in large kindreds with hereditary BC lead to the identification of two genes, BRCA1 (17q21, MIM* 113705) and BRCA2 (13q14, MIM* 600185), whose mutations induce a 5-fold elevated lifetime risk of BC and OC (Miki et al., 1994; Wooster et al., 1995; Struewing et a., 1997). The prevalence of BRCA1/BRCA2 mutations in families with BC and OC varies widely among populations, with some ethnic groups presenting a small number of common mutations, likely due to a "founder effect", and others showing several unique mutations (Szabo and King, 1997). However, the majority of studies carried out in many world countries indicates that the prevalence of BRCA1/BRCA2 mutations is lower than that originally suggested by early studies on large families with several affected members (Ford et al., 1998; Malone et al., 1998; Osorio et al., 2000; Konstantopoulou et al., 2000; Llort et al., 2002; Tereschenko et al., 2002). Studies performed in Italy reported different prevalences of BRCA1/BRCA2 mutations, likely due to the different selection criteria and to the variability of the employed techniques (Caligo et al., 1996; De Benedetti et al., 1996; Montagna et al., 1996; De Benedetti et al., 1998; Santarosa et al., 1998; Santarosa et al., 1999; Ottini et al., 2000; Baudi et al., 2001).

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We performed a screening of BRCA1/BRCA2 exons and their flanking sequences in 68 families from Central Italy with familial recurrence of BC or OC in which the individual risk of patients to be carriers of BRCA1/BRCA2 mutations was evaluated using the BRCAPRO (CAGene) software.

MATERIALS AND METHODS

Cases and families

Sixty-eight families with history of BC/OC were selected after interview among 128 individuals who underwent genetic counselling at the Pescara Hospital from April 2000 to April 2002 in the context of a public program of screening for the prevention of Breast Cancer in the Abruzzo Region (Central Italy).

Familial cases were selected on the bases of the presence of: a) three affected first or second degree relatives with BC or OC at any age; b) two affected first or second degree relatives with BC before 50 years, with bilateral BC at any age or OC at any age, or with male BC at any age.

Sporadic cases were also selected in presence of: a) BC before 40 years; b) OC before 47 years; c) Bilateral BC before 43 years; d) BC and OC before 52 years; e) Bilateral BC and OC before 56 years; f) Male BC at any age.

For each family a proband was chosen for molecular analysis. When affected member was not available, a first-degree unaffected relative was analyzed. Selected probands were 64 females and 4 males (Pats. 1-68). Forty-two female probands were affected by BC with a mean age at diagnosis of 43.5 years (range 22-73 years) (pats. 1-43), while the only male with BC was 77 years old at diagnosis (pat. 7). Eight patients, with a mean age at diagnosis of 42,7 years (range 34-60 years) had OC (pats. 44-51). Two other patients were affected by thyroid and prostate cancers, respectively (pats. 52-53). The remaining 15 subjects were not affected by any cancer (pats 54-68). All participant gave written informed consent. Data on selected probands are summarised in Table 1.

Assessment of genetic risk using BRCAPRO

For each proband we calculated the risk of being carrier of a BRCA1/BRCA2 mutation using the BRCAPRO (CAGene) program, a statistical model and software using Mendelian genetics and Bayesian updating. This program calculates the risk of being carrier of BRCA1/BRCA2 mutations ("Carrier probability," CP) on the basis of the individual's cancer status and the history of BC or OC among first- and second-degree relatives (Berry et al., 2002). Using this program, we classified our patients as "BRCAPRO positive" when their carrier probability was >10%, and "BRCAPRO negative" when the carrier probability was <10%.

Mutation screening

The entire coding region and splicing boundaries of BRCA1 and BRCA2 were examined in all 68 probands on genomic DNA, isolated from peripheral blood samples according to standard procedures (Maniatis, 1989).

All BRCA1 exons were amplified by PCR using primer pairs and cycling condition described by Friedmann (1994), except for exons 6 (Simard et al. 1994) and 7. For this latter exon we derived a new primers pair: 7F CTTAACACAACAAAGAGCAT (nt 24361-24386); 7R CAAGACTCCATCTCAAAAA (nt 24690-24672).

For BRCA2 we used primers reported by Hakansson (1997) except for exons 2, 3 and 5-6 (Friedmann et al. 1997), 12 and 20. For these latter two exons we derived the following new primer pairs: 12F ATATTATTTGCCTTAAAAAC (nt 83840-83859); 12R CTATAGAGGGAGAGAACAGATA (nt 84019-83999); 20F CCTGGCCTGATACAAT (nt 110301-110316); 20R TTTACCTTCATGTTCTTCAA (nt 110420-110401).

BRCA1 and BRCA2 mutation screening was performed using Single Strand Conformational Polymorphism analysis (SSCP) for exons 1, 3-10 and 12-24 of BRCA1 and for exons 2-9 and 12-27 of BRCA2 (Orita et al. 1989). Due to the poor resolution of SSCP on exon 2 of BRCA1 in our experience, this exon was analysed by direct sequencing. Exon 11 of BRCA1 and exons 10-11 of BRCA2 were screened using the Protein Truncation Test (PTT) (Friedmann et al., 1994) with primers reported by Ottini et al. (2000).

Samples showing a variant band after SSCP or PTT analysis, exon 2 of BRCA1 and long fragments of exons 10 and 11 of BRCA2 were sequenced using an ABI Prism 310 Genetic Analyzer according to the manufacturer's instructions. Sequencing was performed on both DNA strands. Detected mutations were confirmed by repeating both PCR analysis and sequencing.

Table 1. Clinical Data of the 20 Probands Showing Carrier Probability > 0.1 (BRCAPro positive) and/or BRCA1/BRCA2 Mutations

Pat.	Sex/Age	Cancer	Age at diagnosis	Family history	Carrier probability BRCA1	Carrier probability BRCA2	Carrier probability Total	Mutation	BIC Entries
1	F/55	bil BrCa	46-54	1 BC	**0.118**	0.042	**0.160**	BRCA1 2715C>T (R866C), 2731C>T (P871L)	116
2	F/54	bil BrCa	54	2 BC	**0.119**	**0.173**	**0.292**	no	
3	F/22	BrCa	22	1 BC	**0.256**	0.039	**0.295**	no	
10	F/41	BrCa	40	2 MBC	0.028	**0.896**	**0.924**	no	
15	F/35	BrCa	35	1 BC	**0.300**	0.030	**0.330**	no	
17	F/40	BrCa	39	3 BC	**0.639**	0.099	**0.738**	BRCA1 4172insT (1351fsX1355)	/
25	F/45	bil BrCa	44	/	**0.237**	0.028	**0.265**	no	
28	F/33	BrCa	33	4 BC 1 OC	**0.868**	0.074	**0.952**	BRCA2 6696delTC (2156fsX2174)	9
33	F/38	BrCa	38		**0.175**	0.092	**0.267**	no	
38	F/41	BrCa	38	1 BC 1 LuC 1 GC	**0.181**	0.013	**0.194**	no	
42	F/30	BrCa	29	1 OC	**0.150**	0.024	**0.174**	no	
43	F/54	BrCa	46	1 BC 1 bilBC	0.171	**0.269**	**0.440**	BRCA2 9189del4 (2987fsX2999)	1
46	F/60	OvCa	60		**0.110**	0.016	**0.126**	no	
49	F/39	OvCa	39	2 BC 4 OC	**0.613**	0.125	**0.738**	BRCA1 1499insA (461fsX479)	1
51	F/34	OvCa	34	3 OC	**0.309**	0.016	**0.325**	BRCA1 3596del4 (1159fsX1208)	3
52	M/51	Prostate	51	1 BC	0.001	0.001	0.002	BRCA2 5445del4 (1739fsX1739)	1
55	F/35	/	/	1 bilBC 1 OC	**0.381**	0.040	**0.421**	no	
60	F/30	/	/	4 BC 1 CC 1 LC	**0.222**	0.098	**0.320**	no	
62	M/41	/	/	4 BC	**0.329**	0.098	**0.427**	BRCA1 IVS20+60dup12	23
66	F/26	/	/	6 BC	**0.158**	0.014	**0.172**	no	

BrCa= Breast Cancer; bilBrCa= bilateral Breast Cancer; OvCa= Ovarian Cancer; MBC= Male Breast Cancer; CC= Colon Cancer; LuC= Lung Cancer; GC= Gastric Cancer; LC= Larinx Cancer

Table 2: Frequency BRCA1/BRCA2 Polymorphisms Among Probands and 50 Healthy Controls

Polymorphism	Frequency among probands	Frequency among controls
BRCA1 Lys1183Arg	20%	19%
BRCA1 Gln356Arg	4%	6%
BRCA1 Glu1038Gly	15.4%	19%
BRCA1 Ser1040Asn	4%	3%
BRCA2 Asp1420Tyr	1.5%	3%
BRCA2 nt 203 G/A	35%	26%
BRCA2 nt 909 (+56) C/T	1.5%	2,4%

RESULTS

Carrier probability

Among probands selected for molecular analysis, 19 were BRCAPRO positive (28%) with a mean CP of 40% (range 12,7%-93,5%), while 49 were BRCAPRO negative (72%), with a mean CP of 2.4% (range: 0,1 – 8,5%).

BRCA1/BRCA2 mutations

Eight of the 68 index cases showed a mutation (11.7%) involving in five cases BRCA1 (7.3%) (Pats. 1, 17, 49, 51, 62) and in 3 cases BRCA2 (4.4%) (Pats. 28, 43, 52). Four of the BRCA1 changes were frameshift mutations, involving in three cases exon 11. The first was a 4172insT (1351fsX1355) detected in a 39 year-old female affected by BC with CP of 73% (pat. 17); the second mutation was a 1499insA (461fsX479), evidenced in a 39 year-old OC female with CP of 73% (pat 49); the third was a 3596del4 (1159fsX1208) in a 34 year-old OC female with CP of 32% (pat. 51). This latter mutation was also found in the 38 year-old healthy female cousin of the patient. The last BRCA1 frameshift mutation was a IVS20+60dup12, detected in an unaffected 41 year-old male with familiarity for BC and CP of 42% (pat.62), and in his healthy male twin. The remaining BRCA1 change consisted of two missense mutations within exon 11, namely 2715C>T (R866C) and 2731C>T (P871L), detected in a 46 years old female with bilateral BC and CP of 16% (pat. 1). Segregation analysis carried out on healthy parents and the sister of this patient demonstrated the cis-position of the two mutations. These two missense mutations were not present in 50 healthy females with no history of familiar cancer investigated as controls.

All the three BRCA2 rearrangements were frameshift mutations. The first one was a 6696delTC in exon 11 (2156fsX2174) in a 33 year-old BC female with CP of 93% (pat. 28). The second was a 5445del4 in exon 11 (1739fsX1739) in a 51 year-old male, affected by prostate cancer, with familiarity for BC and CP of 0.2% (pat. 52). The last mutation was a 9189del4 in exon 23 (2987fsX2999) detected in a 46 year-old BC female with CP of 44% (pat. 43). Clinical and molecular data of the 20 patients with BRCAPro positive calculation and/or BRCA1/BRCA2 mutations are reported in Table 1.

Several nucleotide changes known as polymorphisms have been detected in this study. Their prevalence compared to the frequency among 50 healthy controls is reported in Table 2.

Seven out of the 19 BRCAPRO-positive patients had mutations of BRCA1/BRCA2 (36.8%) (pats. 1, 17, 28, 43, 49, 51) while only one of the 49 BRCAPRO-negative patients showed a mutation of BRCA2 (2%) (pat. 52). A diagram showing the relationship between the presence of BRCA1/BRCA2 mutations, the age at diagnosis, and the carrier probability is shown in Fig. 1.

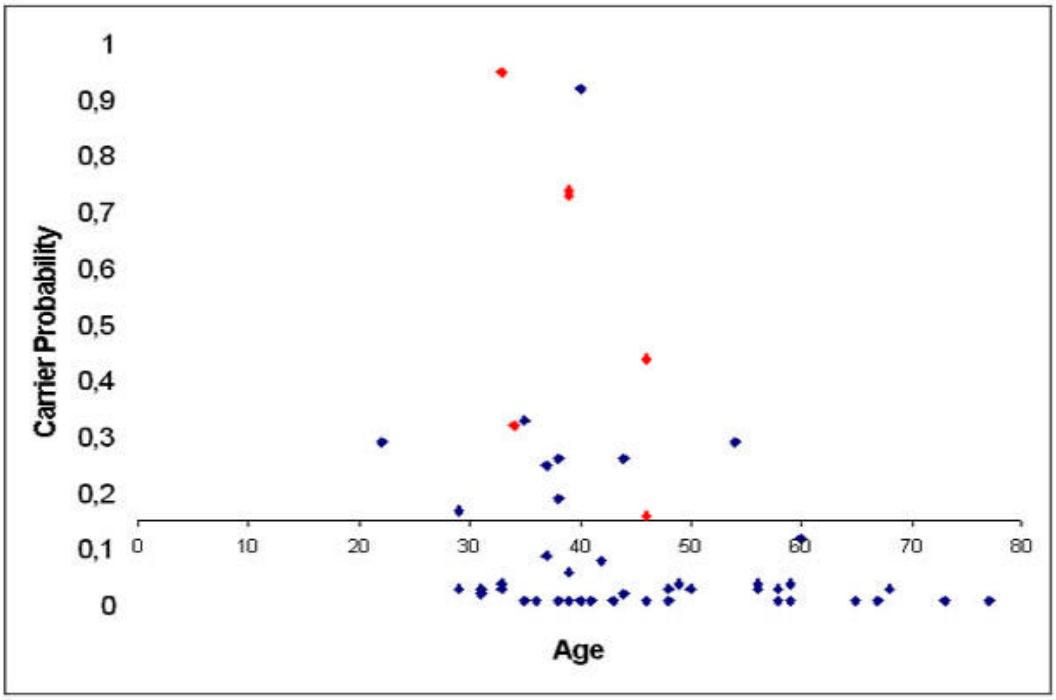

Figure 1. Diagram showing the distribution of BRCA1/BRCA2 mutations in relationship with the age of diagnosis and the carrier probability as calculated by the BRCAPRO software. Red spots= patients with mutation; blue spots= patients without mutation.

DISCUSSION

Molecular analysis of BRCA1 and BRCA2 in different populations have demonstrated a very large mutational spectrum and a variable mutation prevalence related to the different employed techniques, to the criteria of selection and to the ethnic origin of patients. We analyzed BRCA1 and BRCA2 genes in 68 probands from selected unrelated Italian families with history of BC or OC. This analysis allowed to detect eight different mutations, with a detection rate of 11.7%. In the two previous studies carried out in Italy by screening the entire BRCA1/BRCA2 sequences, Ottini et al. (2000) detected mutations in 8% of 136 Italian patients, while Santarosa et al. (1999) disclosed mutations in 37% of cases studying 57 selected Italian families. These different detection rates are clearly due to the different criteria of selection employed, since Santarosa et al. (1999) used more stringent criteria of autosomal-dominant predisposition. In fact, in the present study, considering only the 19 patients with a carrier probability >10% as detected by the BRCAPRO program, the detection rate of BRCA1/BRCA2 mutations would have been 36.8%, like Santarosa et al. (1999). Interestingly, this detection rate is very close to the mean carrier probability of the 19 BRCAPRO positive probands (40%). This confirms that BRCAPRO is an useful and very sensitive tool for the selection of patients to enrol in a screening analysis for BRCA1/BRCA2 mutations (Berry et al., 2002). Moreover, in six out of seven cases, the BRCAPRO program correctly identified which of the two genes was mutated. Thus, the selection of patients using the BRCAPRO is also useful to indicate which gene should be analysed at first.

One out of the 8 BRCA1/BRCA2 changes detected in this study was a novel mutation not reported in the BIC DataBase. This change (BRCA1 4172insT) consisted of a frameshift mutation leading to a stop codon within exon 11 of BRCA1. All the remaining mutations have been already reported in the BIC DataBase. Five of these (BRCA1 1499insA and 3596del4; BRCA2 9189del4, 6696delTC and 5445del4) were again frameshift mutations leading to truncated proteins. Interestingly, one BRCA2 mutation (5445del4) was detected in a patient with prostate cancer. Interestingly, an increased risk of prostate cancer in male carriers of BRCA2 mutations belonging to families with multiple cases of BC, have been previously suggested (Bratt et al., 2000; Osorio et al., 2000).

Among the detected mutations, the BRCA1 1499insA and 3596del4bp and the BRCA2 6696delTC have been previously detected in Italian patients (De Benedetti et al., 1996; De Benedetti et al., 1998; Montagna et al., 1996; Ottini et al., 2000; Nedelcu et al., 2002). On the other hand, we did not find in any case the BRCA1 5083del19bp change, mutation recurring in individuals of Southern Italian descent because of founder effect (Baudi et al., 2001; Nedelcu et al., 2002).

Another alteration was a duplication of 12 bp within intron 20 of BRCA1 (IVS20+60 dup12), detected in an healthy male proband of a BC family. This mutation has been previously associated to an increased risk of familial BC (Sobczak 1997, Robledo 1997). However, the mechanism by which this intronic mutation would disrupt the function of BRCA1 and its pathogenetic role are still debated (Scholl 1999, Dork 1999). Unfortunately, in the present case it was not possible to investigate the segregation of this mutation with the disease in the family. The last change consisted in the presence of two missense mutations within exon 11 of BRCA1 in a patient affected by bilateral BC. Both mutations (Arg866Cys, Pro871Leu) are reported in the BIC DataBase as Unclassified Variants. Genotype analysis of the patient compared to her healthy relatives showed that the two mutations were present in a cis- configuration, and that only the patient was carrier of this genotype. Although the role played by missense mutations in the pathogenesis of BC is still unclear, we hypothesise that the presence of two changes in the same allele could affect the function of the BRCA1 protein. This is confirmed also by the absence of both changes in 50 subjects with no history of familiar cancer investigated as controls. Moreover, recent reports have suggested that R866C change is likely to affect the BRCA1 function because it's a nonconservative substitution affecting a conservative site inside a region of BRCA1 which has a documented role in DNA double-stranded break repair (Fleming et al., 2003).

In conclusion, our data indicate that the prevalence of BRCA1/BRCA2 mutations in families with BC or OC from the Abruzzo Region in Central Italy is 11.7%, confirming that, in the clinical practice, the majority of families attending genetic counselling for hereditary BC show a low risk of BRCA1/BRCA2 mutations (Ford et al., 1998; Osorio et al., 2000). The selection of patients to be analysed using the BRCAPRO program allows to increase the detection rate of the molecular analysis up to 36.8% and is also useful to identify which of the two genes is more likely mutated in a specific proband, allowing to improve the cost/effectiveness ratio of the molecular analysis.

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