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BRCA1 mutations and clinicopathological features in a sample of Italian women with early-onset breast cancer

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Abstract

Breast cancer in young women is uncommon and often presents with unfavourable biopathological features. Although early age at onset could suggest a genetic susceptibility to cancer, the appropriateness of *BRCA1* testing for women with early-onset breast cancer and modest family history (FH) is controversial. 40 Women diagnosed with breast cancer at the age of 35 years or less, unselected for FH, were screened for germ line *BRCA1* mutations by automated sequencing of exons 2, 5, 6, 11, 13 and 20. Overall, deleterious mutations were evidenced in 6 (15%) patients. With regard to FH, mutations were detected in 14%, 11% and 29% of women with none, weak and strong FH, respectively. Large tumour size, grade 3, lack of oestrogen receptors and high proliferation rate were significantly more common in mutation carriers (MC). Our data support both the appropriateness of testing young breast cancer patients and the frequency of unfavourable features in *BRCA1*-related breast cancer. It is hypothesised that *BRCA1* mutations partially justify the high rate of aggressive breast cancer in young patients and that combining age and breast cancer phenotype could help to identify probable MC.

Keywords: *BRCA1*; Breast cancer phenotype; Early-onset breast cancer; Hereditary breast cancer; Genetic testing

1. Introduction

Breast cancer is the most common malignancy in females, occurring in approximately 1 in 8 women in Western countries. Although breast cancer is a typical disease of middle-aged and older women, approximately 4–8% of total cases occur in patients aged less than 40 years. In our geographical area (Province of Modena, Italy), out of 1835 incident cases in the period 1988–1992, 83 (4.5%) were diagnosed before the age of 40 years, and 28 (1.5%) before 35 years of age [1].

Early age at onset is generally considered an indicator of genetic susceptibility to cancer, as evidenced for retinoblastoma, Wilms' tumour and familial colonic polyposis [2–4]. Since early-onset breast cancer has also been demonstrated to be associated with higher risk in relatives [5–7], age at diagnosis has been suggested to be a useful variable for identifying subgroups of breast cancer patients in whom genetic factors could play a significant role [8]. The identification and cloning of two major breast cancer susceptibility genes, *BRCA1* and *BRCA2* [9,10], offer the opportunity to confirm the presence of germ line mutations in patients with high prior probability of carrying an inherited predisposition. Nevertheless, previous studies on *BRCA1* mutations in early-onset breast cancer patients yielded inconsistent results, raising controversy about the opportunity of testing patients on the basis of age alone [11–16]. Moreover, *BRCA2* has been determined to contribute to fewer cases of early-onset breast cancer than *BRCA1* [17].

Several studies have shown that early-onset breast cancer displays histological features suggestive of an aggressive cancer phenotype [18–20]. In addition, germ line mutations in the *BRCA1* gene have been related to a similarly aggressive cancer phenotype [21–25]: it could be hypothesised that the association of a significant proportion of early-onset breast cancer with *BRCA1* mutations might be responsible for the high frequency of histologically aggressive tumours in young women.

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Nevertheless, in spite of a higher prevalence of adverse pathobiological features, most studies failed to demonstrate a worse prognosis in breast cancer patients carrying *BRCA1* mutations [26–28].

To help address both the questions of the proportion of early-onset breast cancer associated with *BRCA1* and of the contribution of germ line mutations to the phenotype of these tumours, we analysed a sample of young breast cancer patients, who were not selected on the basis of family history (FH), for *BRCA1* mutations and clinicopathological features.

2. Patients and methods

2.1. Patients

Among breast cancer patients registered at the Division of Medical Oncology of the University of Modena and Reggio Emilia in the years 1988–1998, 56 were aged 35 years or less at disease onset. Among these, 46 patients remained alive and were proposed for genetic analysis. After a pre-test counselling session in which rationale, methods and implications of the molecular analysis were explained, 40 patients gave their informed consent and were enrolled in the study. The 6 women refusing the test attributed their decision to major concerns about themselves and/or their children. The study was carried out with ethical committee approval.

2.2. Family history ascertainment

For every enrolled patient, extensive family information was collected, and their pedigrees traced, in order to assess the possible family history of breast cancer and other malignancies. According to the criteria adopted by our institution, and previously described in [29], the case was defined as Hereditary Breast Cancer (HBC) or Hereditary Breast-Ovarian Cancer (HBOC) if, in addition to the early-onset, the following family features were satisfied: (i) at least 3 relatives affected by breast cancer (or 2 by breast cancer and 1 by OC in HBOC) in two different generations; (ii) first-degree relationship between at least 1 of the 3 breast cancer cases and the other 2 (in cases of male interposition a relationship of different degree was allowed).

A patient who did not meet such criteria was classified on the basis of the occurrence of breast cancer in first-, second- or third-degree relatives or on the absence of breast cancer in relatives.

2.3. Clinical and pathological data

The following data were obtained from medical and, when available, from pathological records: birth and diagnosis date, pathological stage, nodal involvement, histological type and grade, hormone receptor status and proliferation rate. According to the criteria adopted by our institution, hormone receptor status was labelled as 'positive' when the fraction of cells stained by the corresponding immunoreaction was higher than 10%, proliferation rate was considered as 'high' when 15% or more cells were stained by Ki67 antibody.

2.4. Molecular analysis

Genomic DNA was isolated from peripheral blood lymphocytes by standard procedures. Exons 2, 5, 6, 11, 13 and 20 of the *BRCA1* gene were amplified by the polymerase chain reaction (PCR), using primer pairs previously described by Friedman and colleagues [30]. After purification with MicroSpin columns (Boehringer Mannheim, Germany), PCR products (0.2 pmol per sample) were directly sequenced in forward and reverse directions using the AmpliTaq dye Deoxy Terminator Cycle sequencing Kit (Applied Biosystems and Perkin Elmer, USA) according to the manufacturer's instructions. The sequence reactions were run and analysed using a 377 ABI PRISM sequencer (Applied Biosystems, USA). Consensus sequences obtained with the Autoassembler program (Perkin Elmer, USA) were compared with the wild-type sequence using the DNAsis program (Pharmacia, USA).

When heterozygosity was suggested, but not definitely identified by DAS, the fragment containing the putative mutation was subcloned into the pCR 3.1 vector (Invitrogen, USA). The fragment containing the putative mutated region was amplified as described above. The ligation reaction was performed at 16°C overnight, using 40 ng of PCR product, 5U of T4 Ligasi and Ligation Buffer (Gibco, USA). Plasmid transformation was carried out using XL1 BLUE MRF competent bacteria and plating was performed as described by Hanahan [31]. Single colonies were recovered and incubated in 3 ml of SOB medium (2% tryptone, 0.5% yeast extracts, 10 mM NaCl, 2.5 mM KCl, H_2O) containing 50 μg/ml of ampicillin at 37°C. Plasmid DNA was extracted using the High pure™ isolation kit (Boehringer Mannheim, Germany), *Eco*RI digests were used (Boehringer Mannheim, Germany) to check subcloning, and recombinant plasmids were sequenced using the T7 promoter primer.

Variants detected by the molecular analysis were checked on the BIC database (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/Member/index.html), and classified on this basis. In any case, positive results were confirmed by repeating every step of the analysis from DNA extraction.

2.5. Statistical analysis

Comparison between *BRCA1*-related and *BRCA1*-unrelated tumours was performed with the Chi-square test. A P value of < 0.05 was considered significant.

Table 1
BRCA1 mutations detected in women with early-onset breast cancer

Patient (*n*)	Exon	Nucleotide change	Type	Effect
Definite mutations				
84	5	300T→G	Missense	Cys61Gly
37	5	300T→G	Missense	Cys61Gly
58	11	3596delAAAG	Frameshift	1208ter
38	11	4034delTT	Frameshift	1328ter
124	20	5382insC	Frameshift	1829ter
86	20	5382insC	Frameshift	1829ter
Unclassified variants				
104	11	2641 G→A	Missense	Arg840Gly
106	11	3238G→A	Missense	Ser1040Asn
95	11	3238G→A	Missense	Ser1040Asn

ter, termination.

3. Results

All patients had breast cancer diagnosed between 22 and 35 years (mean age: 31.5). Definite mutations of *BRCA1* were detected in 6 (15%) out of 40 tested patients (Table 1). Frameshift mutations, causing premature termination of protein translation, were detected in 4 women, 2 of whom carried the mutation 5382insC, which is a very common alteration, being frequently found in Ashkenazi Jewish individuals. In the other 2 cases, small deletions, located in exon 11 and previously reported in individuals from Italy, were detected. The missense mutation 300T→G, causing the amino acid change Cys61Gly, was found in 2 patients. This missense mutation has already been reported in families with hereditary breast and ovarian cancer, and is considered a deleterious mutation: its pathological role is probably related to its position in the zinc-finger domain of the BRCA1 protein. Furthermore, variants of unknown significance were detected in 3 women: 2 of them, unrelated, were carriers of the same missense mutation 3238G→A (Table 1). Polymorphisms were detected in 13 additional cases (data not shown).

As shown in Table 2, 2 out of 6 mutation carriers did not report any family history of breast cancer, and another 1 reported only a second-degree relative diagnosed with breast cancer; in the remaining 3 cases, pedigree was suggestive of increased genetic risk. Mutations were detected in 14, 11 and 29% of women with none, weak and strong FH, respectively. 2 carriers were in the

Table 2
Distribution of *BRCA1* mutation carriers by family history and age

	Patients (*n*)	Mutation-carriers (*n*)	%
Family history			
HBOC	2	1	50
HBC	5	1	20
First-degree	7	1	14
Second-degree	11	1	9
Third-degree	1	0	0
None	14	2	14
Total	40	6	15
Age (years)			
<30	11	2	18
30–35	29	4	14
Total	40	6	15

HBOC, hereditary breast cancer ovarian cancer; HBC, hereditary breast cancer.

Table 3
Clinical features of the breast cancer patients enrolled in the study

Parameter	Patients (*n*)		
	BRCA1− (34) *n* (%)	*BRCA1*+ (6) *n* (%)	All (40) *n* (%)
Tumour stage			
Tis	1 (3)	0	1 (3)
1	13 (38)	0	13 (33)
2	11 (32)	5 (83)	16 (40)
3	3 (9)	0	3 (8)
4	2 (6)	1 (17)	3 (8)
Unknown	4 (12)	0	4 (10)
Number of nodes involved			
0	17 (50)	3 (50)	20 (50)
1–3	6 (18)	2 (33)	8 (20)
4–9	5 (15)	0	5 (13)
10+	2 (6)	1 (17)	3 (8)
Unknown	4 (12)	0	4 (10)
Distant metastasis			
Absent	30 (88)	6 (100)	36 (90)
Present	1 (3)	0	1 (3)
Unknown	3 (9)	0	3 (8)
Final TNM stage			
0	1 (3)	0	1 (3)
I	8 (24)	0	8 (20)
II	17 (50)	5 (83)	22 (55)
III	4 (12)	1 (17)	5 (13)
IV	1 (3)	0	1 (3)
Unknown	3 (9)	0	3 (8)

BRCA1−, patients in which no definite *BRCA1* mutations were detected; *BRCA1*+, carriers of deleterious *BRCA1* mutations.

age range 22–29 years, while the other 4 were 30 years or older at diagnosis.

The clinical and pathological features of the tested patients are reported in Tables 3–5. There were no significant differences in the frequency of axillary nodal involvement and distant metastasis between *BRCA1* mutation carriers and non-carriers, but the tumours of *BRCA1* mutation carriers appeared significantly less likely to be of small dimensions (Tis or T1) than the tumours of *BRCA1* non-carriers ($P=0.032$). Infiltrating ductal carcinoma was the predominant type in the cohort, regardless of mutation status. A diagnosis of medullary carcinoma was made in 1 of 6 women (17%) with mutations, but in none of those without mutations. Histological grade was reported for 26 tumours (65%), in particular, histological grade III disease was noted in 4 (100%) of the 4 mutation carriers for whom the data were available, compared with 10 (45%) of 22 non-carriers for whom data were available ($P=0.044$). Oestrogen receptor status was known for all the *BRCA1*-related and for 25 *BRCA1*-unrelated tumours: receptors were negative in 6 (100%) of 6 tumours of the former group, compared with 13 (52%) of the latter ($P=0.020$). Progesterone receptors were negative in 5 (83%) of 6 mutation carriers and in 12 (50%) of the 24 non-carriers for whom the data were available ($P=0.141$). Tumour proliferation rate was known for 30 patients: it was classified as high in 6 (100%) of the 6 mutation carriers and in 11 (46%) of 24 non-carriers ($P=0.017$).

4. Discussion

The indication for testing for *BRCA1* mutations in women with early-onset breast cancer and modest family history profiles is still a matter of debate. In fact, not all the studies on *BRCA1* mutations in young breast cancer patients performed thus far have reported a mutation frequency of at least 10%, which is the threshold generally adopted to select a population for mutation screening [11–16]. In particular, in a recent study, performed on 193 women diagnosed as having breast cancer before the age of 35 years, an overall mutation frequency of 6.2% was reported; the frequency appeared even lower in the subgroup without a family history of breast cancer [15]. A lower rate of mutation carrier was also observed in a population-based study performed in Britain by Peto and colleagues [16].

Notwithstanding the limitations related to the small sample size, partially balanced by the high sensitivity of the direct DNA sequencing technique, our results are in

Table 4
Pathological features of breast carcinomas occurring in the study population

Parameter	Patients (*n*)		
	BRCA1− (34) *n* (%)	*BRCA1*+ (6) *n* (%)	All (40) *n* (%)
Histological type			
IDC	26 (76)	5 (83)	31 (78)
Medullary	0	1 (17)	1 (3)
ILC	2 (6)	0	2 (5)
DCIS	1 (3)	0	1 (3)
Mucinous	2 (6)	0	2 (5)
Other/unknown	3 (9)	0	3 (8)
Histological grade			
1	3 (9)	0	3 (8)
2	9 (26)	0	9 (23)
3	10 (29)	4 (67)	14 (35)
Unknown	12 (35)	2 (33)	14 (35)
Oestrogen receptor status			
Positive	12 (35)	0	12 (30)
Negative	13 (38)	6 (100)	19 (48)
Unknown	9 (26)	0	9 (23)
Progesterone receptor status			
Positive	12 (35)	1 (17)	13 (33)
Negative	12 (35)	5 (83)	17 (43)
Unknown	10 (29)	0	10 (25)
Proliferation rate			
High	11 (32)	6 (100)	17 (43)
Low	13 (38)	0	13 (33)
Unknown	10 (29)	0	10 (25)

IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; DCIS, ductal carcinoma *in situ*; *BRCA1*−, patients in which no definite *BRCA1* mutations were detected; *BRCA1*+, carriers of deleterious *BRCA1* mutations.

Table 5
Comparison of clinical and pathological features between *BRCA1* carriers and non-carriers

Parameter	*BRCA1*− *n* (%)[a]	*BRCA1*+ *n* (%)[a]	*P* value
Tumour stage			
Early (Tis + T1)	14 (47)	0	0.032
Advanced (T2–4)	16 (53)	6 (100)	
Nodal involvement			
Absent	17 (57)	3 (50)	0.764
Present	13 (43)	3 (50)	
Distant metastasis			
Absent	30 (97)	6 (100)	0.656
Present	1 (3)	0	
Histological grade			
Low (1−2)	12 (55)	0	0.044
High (3)	10 (45)	4 (100)	
Oestrogen receptor status			
Positive	12 (48)	0	0.020
Negative	13 (52)	6 (100)	
Progesterone receptor status			
Positive	12 (50)	1 (17)	0.141
Negative	12 (50)	5 (83)	
Proliferation rate			
Low	13 (54)	0	0.017
High	11 (46)	6 (100)	

[a] Percentages are calculated after exclusion of cases where data were unknown.

contrast with such findings. In fact, among 40 women diagnosed with breast cancer at the age of 35 years or less, we identified 6 (15%) carriers of definite *BRCA1* mutations. Frameshift mutations, causing truncated protein products, were detected in 4 of those women, while the other 2 carried the missense mutation 300T→G, causing the amino acid change Cys61Gly in the zinc-finger domain of BRCA1 protein. Moreover, unclassified variants were detected in 3 patients of the cohort: if these alterations were demonstrated as predisposing to breast cancer, mutation frequency should be considered as high as 23%.

The higher mutation rate detected could be partially explained by the method of molecular analysis adopted. In fact, this is the only study on early-onset breast cancer to our knowledge, in which direct automated sequencing is performed as an exclusive technique for *BRCA1* screening. Previously reported studies were based on less sensitive methods such as the Protein Truncation Test and Single Strand Conformation Polymorphism analysis. The choice of sequencing only exons 2, 5, 6, 11, 13 and 20 was supported by our previous experience of sequencing all *BRCA1* exons, which failed to determine any mutations in exons other than these. This choice could have resulted in an underestimate of the real mutation frequency, that, however, does not affect our conclusions.

Although in patients with a family history suggestive of genetic predisposition (labelled as HBC or HBOC) the mutation rate appeared higher, in the subgroup with no family history of breast cancer, including 14 patients, we found 2 (14%) mutation-carriers. Such frequency would be sufficient to justify screening for *BRCA1* mutations in women with breast cancer diagnosed at the age of 35 years or less, regardless of family history. The detection of cancer predisposing mutations in family groups with one or few breast cancer cases is of particular relevance, since it can allow for the identification of healthy individuals with increased breast cancer risk who, on the basis of pedigree, would not be considered at risk. However, cancer risk associated with *BRCA1* mutations in individuals with modest family history is still uncertain [32,33].

The choice of age 35 years as the threshold for inclusion in the study was arbitrary, representing a compromise among age limits reported by other authors, but our findings have confirmed it as useful for selecting a group with increased probability of carrying *BRCA1* mutations. However, since our data are consistent with previous estimates and reports of an increase of mutation frequency with decreasing age of diagnosis [16,34], it is suggested that a lower threshold would select for a group with a higher probability of mutation.

There is increasing evidence of biological and clinical differences between *BRCA1*-associated and sporadic tumours of the breast. In fact, cancers occurring in women harbouring a mutation of *BRCA1* are more likely to be characterised by high histological grade, lack of hormone receptors and high proliferation rate; in addition, medullary carcinoma appears to be overrepresented in mutation carriers [21–24]. Our data are in agreement with such evidence; in fact, high histological grade, absence of oestrogen receptors and high proliferation rate were reported in all the *BRCA1*-related tumours for which these data were available, leading to a statistically significant difference if compared with *BRCA1*-unrelated cancers. Noteworthy, the only medullary carcinoma of the whole cohort was diagnosed in a woman carrying a deleterious mutation of *BRCA1*. Breast carcinomas arising in women without a definite mutation showed very heterogeneous phenotypes. Unfortunately, a part of the pathological data was missing, because the study was based on a review of clinical files, and not of pathological specimens. This situation, together with the low number of *BRCA1*-related breast cancer, might affect the statistical power and limit the reproducibility of our data. There is consolidated evidence that breast tumours occurring in young women are more likely to be hormone receptor-negative, rapidly growing and of high histological grade [18,20]. One possible reason is that early-onset breast cancer is more likely to be associated with *BRCA1* mutations than late-onset. In our cohort, after elimination

of *BRCA1*-associated tumours, the proportion of carcinomas with aggressive phenotype was 7–10% lower, supporting such a hypothesis. In a previous report by our group, out of 6 patients under the age of 35 years without a family history of breast/ovarian cancer, who had been diagnosed with breast cancer displaying high histological grade, the absence of steroid receptors and high proliferation rate, 3 (50%) were found to carry deleterious *BRCA1* mutations [25]. Although preliminary, these data support the hypothesis that the combination of age at onset and tumour phenotype could provide an efficient model for identifying individuals with an extremely high probability of carrying *BRCA1* mutations.

Based on these preliminary data, we are starting a larger, population-based study of early-onset breast cancer, with the aim of confirming both the frequency of *BRCA1* mutations in young breast cancer patients from our geographical area and the correlation between mutations and tumour features. Moreover, when an adequate follow-up is available, the prognosis of young breast cancer patients carrying *BRCA1* mutations will be investigated.

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