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PRECLINICAL STUDY

BRCA1 genetic testing in 106 breast and ovarian cancer families from southern Italy (Sicily): a mutation analyses

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Abstract
Purpose To evaluate the contribution of germline BRCA1 mutations in the incidence of hereditary and familial Breast Cancer (BC) and/or Ovarian Cancer (OC) in patients from Southern Italy (in the region of Sicily) and to identify a possible association between the higher frequency of BRCA1 mutations and a specific familial profile.
Experimental design A consecutive series of 650 patients with BC and/or OC diagnosed between 1999 and 2005 were recruited from the Southern Italian region of Sicily, after interview at the ''Regional Reference Centre for the Characterization and Genetic Screening of Hereditary Tumors'' at the University of Palermo. Genetic counselling allowed us to recruit a total of 106 unrelated families affected with breast and/or ovarian cancer screened for mutations occurring in the whole BRCA1 gene by automatic direct sequencing.
Results Germline BRCA1 mutations were found in 17 of 106 (16%) Sicilian families. The HBOC profile had a major frequency (66%) of mutations ($P < 0.01$). A total of 28 sequence variants was identified. Seven of these were pathogenic, 5 unknown biological variant (UV) and 16 polymorphisms. We also identified a pathological mutation (4843delC) as a possible S*icilian founder mutation.*
Conclusions The present study is the first BRCA1 disease-associated mutations analysis in Southern Italian families. The early age of onset of such tumors and the association with the HBOC familial profile could be two valid screening factors for the identification of BRCA1 mutation carriers. Finally, we identified a BRCA1 mutation with a possible founder effect.

Keywords BRCA1 · Genetic testing · Breast cancer · Ovarian cancer

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Introduction

From 15 to 20% of breast and/or ovarian cancers (BC and/or OC) occur in patients with a strong familial history and are associated with a polygenic susceptibility of low-risk alleles, while 5–10% are hereditary tumors and are associated with mutations in specific genes, mainly in the Breast Cancer 1 gene (BRCA1) [1–3]. This gene codifies for a nuclear phosphoprotein that negatively regulates the cell cycle and is actively involved in the maintenance of genome stability [4].

The detection of BRCA1 gene alterations has become the molecular basis of *genetic testing*, which makes it possible to recognize subjects who are carriers of the germline mutation in this gene and at ''high risk'' of developing BC and/or OC [5, 6]. Women carriers of BRCA1 mutations may develop a BC in 56–80% and an OC in 10–30% of cases up to 70 years old. In addition, women affected by BC and carriers of a known BRCA1 mutation show a 40–60% risk of developing a second breast tumor, while males with BRCA1 mutations show a risk of less than 1% of developing BC [7–10]. BRCA1 mutations also appear to be associated with a higher risk of developing prostatic, colorectal and pancreatic tumors [11]. In the Italian population particularly, BRCA1 mutation carriers have a probability of about 40% of developing BC and/or OC up to the age of 70 [12, 13]. Unfortunately, these data do not include information regarding the Sicilian population and no data involving BRCA1 mutational screening in this specific population are yet available.

Although BRCA1 mutations span the whole gene, without any hot spot loci, a major incidence of specific BRCA1 gene mutations has been identified according to the ethnic group and geographical area [14]. The identification of some of these mutations with a *founder effect* has been reported in several countries, including Italy and this may have an important practical implication for genetic testing [15–18].

More than 600 pathogenic mutations in the BRCA1 gene have been identified in families presenting BC and/or OC and reported in the BIC (Breast cancer Information Core) database [19]. Of these, about 90% give rise to a functionally inactive truncated protein. In addition, many studies have recently reported that some genetic variants of unclassified clinical significance, the so-called unknown biological variant (UV), may play a specific role in the detection of the risk of developing BC and/or OC [20]. The discrimination of deleterious/high-risk variant from the neutral/low-risk one is a very important key-point for the assessment of a specific clinical significance of a variant and, consequently, conclusive results of genetic counseling. For this reason, many different methods have been widely used in a large number of laboratories including the cosegregation analysis, the measure of the influence of the UVs on the protein activity and the comparison of sequence conservation across species [21, 22].

The aim of this study is, first at all, to determine the contribution of germline BRCA1 mutations in the incidence of hereditary and familial BC and/or OC in patients from the Southern Italian region of Sicily. Furthermore, we hoped to identify a possible association between the higher frequency of BRCA1 mutations and a specific familial profile.

Patients and methods

Recruitment criteria and study plan

A consecutive series of 650 patients from the Southern Italian region of Sicily, with BC and/or OC diagnosed between 1999 and 2005, were recruited after interview at the ''Regional Reference Centre for the Characterization and Genetic Screening of Hereditary Tumors'' at the University of Palermo. Written informed consent was obtained from all patients, who then underwent *genetic counseling*. The patients received information regarding the aims and limits of genetic testing. Genetic counseling was conducted by an oncologist, a geneticist and a psychologist and included information regarding the personal and familial history of patients (Table 1), in order to make it possible for us to evaluate risk assessment and the genealogic tree. The latter was updated every year and investigated for at least three generations in those patients with breast/ovarian cancer or other types of tumors, in order to evaluate the presence within the family of other types of neoplasias related to BRCA1 alteration. All information regarding the proband and the affected relatives was verified and confirmed by means of analysis of their hospital records. All cancer diagnosis was confirmed by pathology reports.

After consideration of the various family relationships, a total of 106 unrelated families affected with breast and/or ovarian cancer proved to be eligible for inclusion in an ongoing study (Table 1) [23, 24] and were screened for mutations occurring in the whole BRCA1 gene by automatic direct sequencing. None of families met the strict criteria for other known syndromes involving breast cancer, such as ataxia-telangectasia, Cowden disease or Li-Fraumeni syndrome. All the material regarding each individual case (a personal data chart, interviews, blood samples) was filed under an individual personal code respecting the patient's privacy. For each family, we selected the youngest member with breast and/or ovarian cancer as the index case for BRCA1 mutation screening. When a BRCA1 mutation had been identified, the sequencing procedure was repeated a second time in order to confirm the result. In cases of positive testing, the patients were invited to follow preventative programs for the early diagnosis of BRCA1-associated tumors and to communicate the results to their first-degree relatives so that they might undergo genetic counseling. When

Table 1 Study recruitment criteria and distribution of families according to index cases among the overall series of 106 families enrolled in the study

Characteristics	Number	Frequency (%)
Personal history	75	
BC ≤ 40 years	43	57
OC ≤ 40 yrs	6	8
BC and OC (any age)	3	4
Bilateral BC	13	17
BC ≤ 40 yrs and bilateral	1	1
Male BC	7	9
Multiple tumors in the same pts (one of which BC or OC)	2	3
First degree family history	31	
Two BC cases (one ≤ 40 years)	12	39
Two OC cases (one ≤ 40 years)	3	10
Two carcinomas cases (one BC and/or OC at any age)	5	16
Three or more BC cases	9	29
Multiple tumors in the same family (one of which BC or OC)	2	6

this proved negative, the relatives of such patients were encouraged to take a regular part in the early diagnosis programs for their familial condition of cancer risk. If the result of genetic testing was unclear, the patient was informed of this fact in order to decide whether or not to proceed to other biomolecular investigations.

Patient and control population features

The group studied was made up of 7% males (7/106) and 93% females (99/106). Eighty-seven percent (92/106) of the patients were affected by breast cancer, 22% (20/92) of these with bilateral BC. Furthermore, 8% (9/106) involved women with OC while 5% (5/106) were women affected by both diseases.

Seventy-one percent (75/106) of the patients were included in the population study only because of their personal history of tumors, 15% (16/106) both because of personal and family history of first degree tumors and only 14% (15/106) were studied for family history of first degree tumors (Table 1). Sixty-two percent (56/91) of the patients with a personal tumor history had developed both breast and/or ovarian cancers at an early age (≤40 years).

Frequencies of previously unreported BRCA1 variants were also established on a control population composed of 50 healthy Sicilian donors over fifty without familial history for these tumors.

Mutational screening of the gene BRCA1

All the samples were analyzed anonymously. Genomic DNA was extracted from the whole peripheral blood of each proband according to the instructions contained in the QIAamp Blood Kit (Qiagen, Hilden, Germany). A 9700 thermal cycler (Applied Biosystems, Foster City, CA) was used to perform the polymerase chain reaction (PCR) of all the exons, the exon–intron boundaries and of the 12 overlapping fragments constituting exon 11 of the gene BRCA1 [25, 26]. The amplicons were checked by means of agarose gel electrophoresis at a concentration ranging from 1.5 to 2% and were stained with ethidium bromide. Direct sequencing of the PCR products was performed using a BigDye Terminator v3.1 Cycle sequencing Kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Each genetic variant was confirmed by direct sequencing analysis on two independent blood samples.

Mutation nomenclature and interpretation of sequence analysis results

All genetic variants were named according to the convention of BIC database [19] and the systematic nomenclature [27]. Sequence variants detected by molecular analysis were classified as deleterious mutations, polymorphisms and variants of unknown significance.

Mutations were considered *deleterious*, if they prematurely truncated the proteic product at least 10 amino acids before C-terminus. In addition, specific missense mutations and noncoding sequence mutations were interpreted as deleterious/high risk on the basis of data derived from the linkage analysis of high risk families, functional assays, biochemical analysis or demonstration of abnormal mRNA transcript processing. When there was clear evidence of presumed deleterious mutation, the mutations were reported as suspected deleterious, and were included in the positive group. Missense mutations and mutations occurring in analyzed intronic regions have not yet been determined as variants of *unknown significance*. Those variants which do not modify exon splicing, do not change amino acids or change them without any substantial clinical consequence and which have been identified with a frequency major or equal to 2% were considered as *polymorphisms.*

Immunohistochemistry

Immunohistochemical analyses were performed on serial sections of 2 μm as previously reported [28]. The slides were immunostained with the following primary monoclonal antibodies: anti-ER (clone 1D5, dilution 1:35, Dako Cytomation, Denmark), anti-PR (clone

PgR 636, dilution 1:50, Dako Cytomation, Denmark) and anti c-erB-2 (Polyclonal, dilution 1:250, Dako Cytomation, Denmark). Negative controls were carried out using non-immune sera instead of the primary antibody. For each of the markers studied, the positive tumor cells were quantified by two independent observers by evaluating at least 2000 cells from four different specimens of the same tumors and were expressed as percentage ratio of total number of tumor cells. Inter-observer variation was less than 5%; differences were discussed and a consensus was reached. Slides used for ER and PgR were scored as positive when at least 10% of the tumor cells showed nuclear staining c-erB-2 positive score were defined when the percentages of immunopositive cancer cells (any labeled cancer cell membrane) were >25%. All samples were evaluated blind without any knowledge of either clinical diagnosis or histological parameters.

Statistical analysis

The statistical association between mutation frequency and the other parameters was assessed using a χ^2-test. Differences were considered to be significant when the *P*-value was less than 0.05. Statistical analyses were carried out with the SPSS statistical software (SPSS Inc., Chicago, IL).

Results

BRCA1 mutational screening

Germline BRCA1 mutations were found in 17 of 106 (16%) Sicilian families. Families were grouped according to four profiles: Hereditary Breast Cancer (HBC, with ≥ 2 cases of female breast cancer); Hereditary Ovarian Cancer (HOC, with cases of ovarian cancer); Hereditary Breast and Ovarian Cancer (HBOC, with cases of breast and ovarian cancer); Male Breast Cancer (MBC, with at least one case of male breast cancer). According to the analysis of the different familial profiles, and also taking into consideration the II degree, the HBOC profile had a major frequency (66%) of mutations ($P < 0.01$) (Table 2).

No instances of HBOC families with male breast cancer cases were observed. A total of 28 sequence variants was identified (Table 3). Sixty-four percent (18/28) were missense mutations, 18% (5/28) were frameshift mutations, 7% (2/28) were nonsense mutations and 11% (3/28) were intronic variants. Five of eighteen (28%) missense mutations were unknown biological variants (UV). According to the mutation effects, 7 were pathogenic, 5 suspected deleterious UV and 16 polymorphisms. All the pathogenic and unknown variants in the BRCA1 gene were distributed throughout the whole gene.

Table 2 Associations between familial profile and pathological variables of 106 index cases and BRCA1 mutational status

	BRCA1 wt (%)	BRCA1 mutation (%)	*P*
Familial profile (n)			
HBC (83)	75 (90)	8 (10)	<0.01
HOC (6)	5 (83)	1 (17)	
MBC (7)	5 (71)	2 (29)	
HBOC (10)	4 (40)	6 (60)	
Breast cancer histotype (n)			
CDI (77)	66 (86)	11 (14)	NS
CLI (9)	8 (89)	1 (11)	
CM (1)	1 (100)	0 (0)	
CDI and CLI (9)	8 (89)	1 (11)	
Breast cancer grading (n)			
G1 (16)	15 (94)	1 (6)	NS
G2 (32)	30 (94)	2 (6)	
G3 (29)	19 (65)	10 (35)	
Ovarian cancer histotype (n)			
(6)	5 (83)	1 (17)	NS
(4)	2 (50)	2 (50)	
(1)	1 (100)	0 (0)	
Ovarian cancer grading (n)			
G1 (0)	0	0	NS
G2(4)	2 (50)	2 (50)	
G3 (3)	2 (67)	1 (33)	

HBC (Hereditary breast cancer), HBOC (Hereditary breast and/or ovarian cancer), HOC (Hereditary ovarian cancer), MBC (Male breast cancer)

Pathogenic mutation

Pathogenic mutations were detected in twelve families (11%) of our cohort. Six of these were HBOC, four were HBC, one HOC and one MBC (Table 4). All the families with carriers of a deleterious mutation had at least one member with early onset of BC and/or OC.

Seven different pathogenic mutations leading to non-functional truncated proteins were identified (Y101X, 633delC, 916delTT, R1443X, 4843delC, 5083del19 and 5149del4).

The Y101X mutation, detected once in the BIC database, was identified in two cases affected by OC (index case, 37 years) and BC (33 years) respectively, with HBOC profile (FAM49).

The 633delC mutation was identified in an HOC profile (FAM76) containing 3 cases of OC at ages 40 (grandmother), 45 (index case) and 29 (daughter). The other daughter was a healthy carrier of the same mutation.

Table 3 *BRCA1* sequence variants identified in 106 unrelated families: M, missense mutation; F, frameshift mutation; N, nonsense mutation; IVS intronic variant sequence; UV, unknown variant; P, polymorphism

Sequence variant designation	Systematic nomenclature	Exon	NT	Codon	Base change	AA Change	Mutation type	Mutation effect	N° pts	Times in BIC
I68K	c.223T>A	5	322	68	T to A	Ile/Lys	M	UV	1	1
Y101X	c.303T>G	7	422	101	T to G	Tyr/Stop	N	N	1	1
IVS 7-34 C/T	c.81-34C>T	7	–34	–	C to T	–	IVS	P	17	7
IVS 8-58 delT	c.548-58delT	8	663-58	–	DelT	–	IVS	P	10	7
Y179C	c.536A>G	8	655	179	A to G	Tyr/Cys	M	UV	1	16
633delC	c.514delC	8	633	172	DelC	Stop233	F	F	2	16
916delTT	c.797_798delTT	11	916	266	DelTT	Stop285	F	F	2	2
Q356R	c.1067A>G	11	1186	356	A to G	Gly/Arg	M	P	7	21
F486L	c.1456T>C	11	1575	486	T to C	Feu/Leu	M	P	1	17
A521T	c.1561G>A	11	1680	521	G to A	Ala/Thr	M	UV	2	2
N550H	c.1648A>C	11	1767	550	A to C	Asn/His	M	UV	1	34
D693N	c.2077G>A	11	2196	693	G to A	Asp/Asn	M	P	8	16
2201C/T	c.2082C>T	11	2201	694	C to T	Ser/Ser	M	P	38	11
V740L	c.2218G>C	11	2337	740	G to C	Val/Leu	M	UV	1	1
2430T/C	c.2311T>C	11	2430	771	T to C	Leu/Leu	M	P	23	25
P871L	c.2612C>T	11	2731	871	C to T	Pro/Leu	M	P	54	24
E1038G	c.3113A>G	11	3232	1038	A to G	Glu/Gly	M	P	66	32
S1040N	c.3119G>A	11	3238	1040	G to A	Ser/Asn	M	P	4	23
K1183R	c.3548A>G	11	3667	1183	A to G	Lys/Arg	M	P	52	31
R1443X	c.4327C>T	13	4446	1443	C to T	Arg/Stop	N	N	1	100
4427T/C	c.4308T>C	13	4427	1436	T to C	Ser/Ser	M	P	47	15
S1512I	c.4535G>T	15	4654	1512	G to T	Ser/Ile	M	P	1	52
5083del19	c.4964_4982del19	16	5083	1655	del 19bp	Stop1670	F	F	4	38
4843delC	c.4724delC	16	4843	1575	DelC	Stop1600	F	F	2	0
S1613G	c.4837A>G	16	4956	1613	A to G	Ser/Gly	M	P	57	34
M1652I	c.4956G>A	16	5075	1652	G to A	Met/Ile	M	P	6	38
5149del4	c.5030_5033del4	17	5149	1677	delCTAA	Stop1678	F	F	2	16
IVS 18 + 66G/A	c.5093 + 66G>A	18	5272	–	G to A	–	IVS	P	30	8

Table 4 BRCA1 alteration identified. In withe rows the index cases (12/106) with deleterious mutations, in gray the index cases (5/106) with BRCA1 missense mutations with unknown biological significance

Mutations in *BRCA* 1 gene	Family ID	Familial profile	Index case status (age)	Family History (BC/OC; age)	Hystotipe/ Grades	ER	PgR	c-ErB-2
R1443X	FAM 22	MBC	BCm (60)	2BC (36, 60)	IDC G2	–	–	–
5149del4	FAM 7	HBC	BC 50	3BC (35, 50, 51)	IDC e LC G3	–	–	+
5083del19	FAM 71	HBC	BC bil (40)		IDC G3	–	–	–
916delTT	FAM 43	HBC	BC bil (33)		IDC G3	–	–	–
5083del19	FAM 102	HBC	BC (39)		IDC G3	–	+	+
633delC	FAM 76	HOC	OC (45)	3OC (29, 40, 45)	CAp/m G3			
916delTT	FAM 46	HBOC	BC (32)	BC/OC (32/ 63)	IDC G3	–	–	+
4843delC	FAM 64	HBOC	BC (44)	2BC (40, 44)	IDC G2	–	–	+
5083del19	FAM 78	HBOC	BC bil (75)	BC/OC (75/39)	IDC G3	+	+	–
5083del19	FAM 106	HBOC	BC bil (42)	3OC (32, 42, 50)	IDC G3	–	–	+
4843delC	FAM 92	HBOC	OC (46)	BC/2OC (52/40, 42)	Cap G3			
Y101X	FAM 49	HBOC	OC (37)		CAp/m G2			
I68K	FAM 3	HBC	BC (32)		IDC G3	–	–	+
A521T	FAM 19	MBC	BCm (47)		IDC G2	+	+	+
A521T	FAM 20	HBC	BC (39)		IDC G3	–	–	+
Y179C & N550H	FAM 79	HBC	BC (35)		LC G2	+	+	–
V740L	FAM 90	HBC	BC (33)		IDC G2	+	+	–

N, nonsense; F, frameshift; M, missense. IDC (invasive ductal carcinoma), LC (lobular carcinoma). HBC (Hereditary breast cancer), HBOC (Hereditary breast and/or ovarian cancer), HOC (Hereditary ovarian cancer), MBC (Male breast cancer). CAp: Cystoadecarcinoma papillary; CAp/m: Cystoadecarcinoma mucinous and papillary; AE: Adenocarcinoma Endometroid; CCA: Clear Cell (mesonephroid) Adenocarcinoma

Two specific French mutations (R1443X and 916delTT) were found in 3 different families (FAM22, FAM43 and FAM46). The first one is the most common alteration reported in the BIC database while it is important to emphasize that the other one is reported only twice. In particular, the FAM43 family presented two bilateral breast cancer cases at ages of 33 (index case) and 40 as well as four other types of cancer (2 prostate, 1 pancreas and 1 gastric). The R1443X mutation was identified in a family with MBC profile (FAM22) which included a male breast cancer (index case) and a female breast cancer at ages 60 and 36 respectively.

The 5149del4 mutation was identified in a family (FAM7) with an HBC profile containing 3 sisters with BC at ages 35, 50 (index case), and 51.

The 4843delC mutation had not been reported either in the BIC or in the Human Gene Mutation Databases (http://www.archive.uwwcm.ac.uk/uwcm/mg) and is therefore considered novel. This pathogenic mutation was found in two unrelated families (FAM 64 and FAM 92) from the same restricted geographical area of the south-western area of Palermo, in the Italian Region of Sicily. This mutation is located in exon 16 and generates a stop codon at position 1600 of BRCA1 gene. The sequencing analysis showed that both mutation carriers had the same sequence variants (P871L, E1038G, K1183R, 4427T/C, S1613G, M1652I, IVS 18 + 66 G>A). Furthermore, both the carriers showed a strong family history. The sequencing analysis conducted, in fact, on the healthy carriers of the families showed that the I and II degree relatives affected by BC or OC had the same mutations (Fig. 1).

The frameshift mutation 5083del19 proved to be the mutation with the higher incidence (4/12) and three of the four carriers had a bilateral breast cancer.

Unknown biological variants

Five unknown biological variants (I68K, Y179C, A521T, N550H, V740L) were identified in five families (Table 3). All UV mutations carriers showed only a personal history of early-onset tumors or of male BC (Table 4).

None of the UV mutations were found in any of the 50 control populations.

The I68K mutation gives rise to the substitution of isoleucyne 68, characterized by the presence of a buried hydrophobic group, by lysine, which is a basic amino acid. This substitution modifies the BRCA1 interaction with the E2 ubiquitin ligase enzyme and it may have a pathogenic effect.

The genetic testing of the FAM79 index case identified in the gene BRCA1 sequence two missense mutations in *cis*, Y179C and N550H. Furthermore, these alterations were associated with the rare substitution of F486L, of low clinical significance or neutral (LCS/neutral).

The V740L and A521T mutations are rare unknown variants reported in the BIC database only once or twice respectively.

Polymorphisms

Six of eighteen (50%) missense mutations and all the three intronic variants (IVS 8–58 delT, IVS 7–34 C/T, IVS 18 + 66G/A) are reported as polymorphisms in the BIC database (Table 3). Of these, the most frequent, in our study, are: E1038G (62%), S1613G (54%), P871L (51%) and K1183R (49%).

Silent mutation

Three missense mutations (2201C/T, 2430T/C, 4427T/C) resulted as neutral variant since the nucleotide change did not influence the amino acids change and the BRCA1 protein function (Table 3).

Clinical–pathological features of breast and ovarian cancers

We obtained data on ER/PgR, c-erbB-2 immunostaining for all 14 BRCA1 mutation carriers affected by BC of the present series. Nine (64%) were ER/PgR negative, four (28%) were ER/PgR positive and one (7%) was negative only for ER. Eight (57%) tumors were c-erbB-2 positive (Table 4).

Both BRCA1 mutation carriers and non-carriers presented mainly an invasive ductal carcinoma (IDC). In addiction, the majority of BRCA1 positive patients had an advanced grade breast cancer. BRCA1 negative patients with OC presented mainly papillary cystoadenocarcinoma, while the majority of mutation carriers had mucinous and papillary cystoadenocarcinomas (Table 5).

Discussion

The present study on the molecular screening of the BRCA1 gene in patients affected by breast and/or ovarian cancers is the first conducted on a Sicilian population. Up till now, the Italian Consortium of Hereditary Breast and Ovarian Cancer has examined

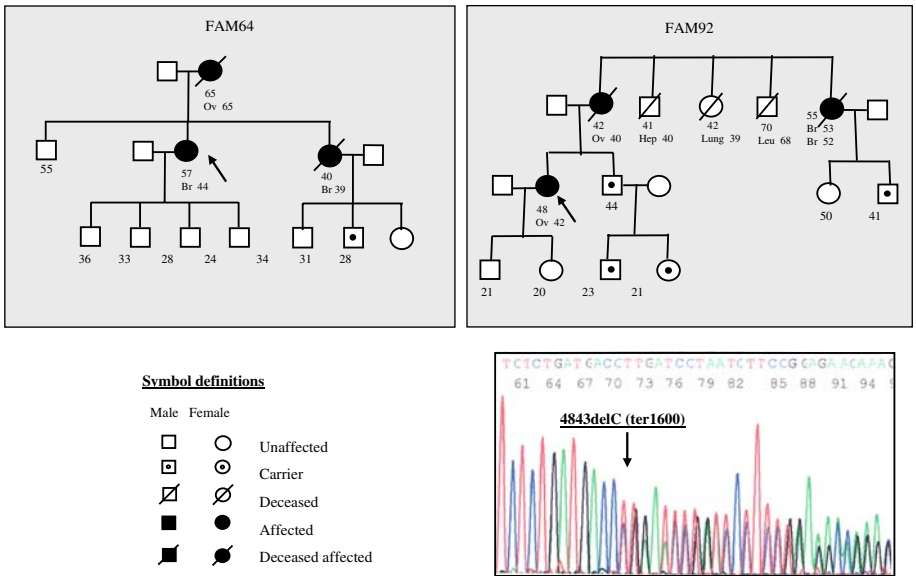

Fig. 1 Genealogical tree of two families with 4843delC mutation and electropherogram of mutation indicated by an arrow

1,758 families and has found that 14% of them prove to be BRCA1 pathogenic mutation carriers [14]. Unfortunately, these data have not included the incidence of BRCA1 mutations in a Sicilian population. In this study, the BRCA1 molecular screening conducted at the ''Regional Reference Center for the Characterization and Genetic Screening of Hereditary Tumors'' at the University of Palermo showed a frequency of BRCA1 mutations of 16%. In agreement with previous Italian reports, in fact, [14, 26], 17 of the 106 unrelated families included in this study proved to be carriers of pathological mutations or UV mutations.

The identification of BRCA1 alterations strictly depends on the adoption of specific criteria for the selection of patients affected by breast and/or ovarian cancer. Notwithstanding the fact that these criteria have been defined by ASCO [23, 24], many reports have shown a certain variability in their application which often reflects the variability of the mutation frequency [29, 30]. The results of this study show that early onset of these tumors and family history are two important selection criteria for the identification of Sicilian patient carriers of the BRCA1 mutation. Except for one index case of bilateral BC diagnosed at the age of 43, in fact, all the families who were carriers of a deleterious mutation had at least one member with early-onset BC and/or OC. The present results are in agreement with other studies [2, 10, 12, 31] which indicate that the frequency of BRCA1 mutations decreases as the age of cancer onset increases.

Bearing in mind these extremely important selection criteria, other groups have reported a mutation frequency of 15% [31] or higher [32], in agreement with our present study. Taking into account the overall familial history, we found a major incidence of BRCA1 mutations (66%) in families with an HBOC profile. A similar incidence was identified in a study involving 99 Italian index cases with breast and/or ovarian cancer [33]. Moreover, as reported by Malander et al. [34], we identified a higher frequency of BRCA1 mutations in

Table 5. Pathological features of BC and OC patients occurring in the study population according to the BRCA1 status.

Parameters	BRCA1 – N(%)	BRCA1+N(%)
Breast Cancer	84(86)	14(14)
Histopathologic type		
IDC	67(80)	12(86)
IDC+LC	8(9.5)	1(7)
LC	8(9.5)	1(7)
Grades		
G1	15(20)	0
G2	30(29)	5(36)
G3	20(26)	9(74)
Unknown	19(24)	0
Ovarian Cancer	11(71)	3(21)
Histopathologic type		
Cap	6(60)	1(25)
Cap/m	3(30)	2(50)
AE	0	1(25)
CCA	1(10)	0
Grades		
G1	0	0
G2	2(20)	2(50)
G3	1(10)	2(50)
Unknown	7(70)	0

IDC: Invasive Ductal Carcinoma; LC: Lobular Carcinoma; CAp: Cystoadecarcinoma papillary; CAp/m :Cystoadecarcinoma mucinous and papillary; AE: Adenocarcinoma Endometroid; CCA: Clear Cell (mesonephroid) Adenocarcinoma

OC patients when both familial and personal history were considered as selection criteria.

BRCA1 related cancers are linked to an aggressive tumoral phenotype and to a higher risk of development of the same type of neoplasia in relatives, often at an earlier age than that of the proband [31]. In this study, in fact, after the analysis of I and II degree familiarity, the relatives of 7/12 families carrying BRCA1 mutations showed an earlier onset age (data not shown).

Two of seven (28%) male BC patients included in this study were BRCA1 mutation carriers. In western countries the frequency rate ranges from 0 to 4% depending on the ethnic group [35–39]. Obviously, more additional cases should be studied in order to validate the frequency of the mutations identified in a male Sicilian population.

BRCA1-associated carcinomas have been reported to have typical characteristics in that they are more frequently of the ductal invasive type, present a poorly differentiated (G3) tumor, and are ER/PgR negative and c-erbB-2 positive [3, 29, 40–42]. In accordance with these data, all of these characteristics were also significantly more frequent in our own series.

In this BRCA1 genetic screening two different French mutations [43, 44], R1443X and 916delTT, were identified in one male and in two woman respectively. In particular, the R1443X is a French mutation reported for the first time in 1997 in northern France and subsequently listed 100 times in the BIC but only three times in Italy. In addition, in a study conducted on a French-Canadian population, the founder couple with the highest probability of having introduced the mutation into the Quebec population was identified [43]. The identification of BRCA1 mutations typical of a French population in Sicilian patients can be explained by the widespread allelic heterogeneity arising from the several different dominations that Sicily has undergone throughout the centuries.

In Italy, founder mutations have been identified in restricted geographical areas. The 5083del19 has been described in patients of Calabrian origin [18] and the 1499insA, probably a new founder mutation, in patients of Tuscany [26]. In our study, we found the 5083del19 mutation in 4 patients and all four families confirmed their Sicilian origin. Since this pathological mutation has been found by other research groups conducting BRCA1 molecular screening in patients originating from Central and Southern Italy [12, 18, 21, 29], we suggest that this mutation might well originate from the South of Italy. Only a haplotype analysis will make it possible to understand if all these families originating from Southern Italy have a common ancestor.

To our knowledge the 4843delC pathological mutation [45] has never before been described and reported in the BIC Database. This might well be a *Sicilian founder mutation*, since it was identified in two families who, although not related, came from the same small geographical area of the south west of Palermo, in the Italian region of Sicily. Moreover, the sequencing analysis conducted on the family members has shown that this is a high penetrance mutation. Finally, in both index cases the same sequence variants (seven SNPs) have been identified. Haplotype analysis will subsequently be performed in order to see whether these mutation carriers are likely to originate from a common ancestor in the Sicilian population. Further extension of this analysis to other families in the same geographical area will be able to confirm the link between the high penetrance of the mutation and the familial predisposition to breast and ovarian cancer. Founder mutation identification, therefore, in population-based studies with homogeneous ethnic background, would make it possible to use genetic testing in routine clinical practice for the rapid recognition of high-risk families in specific populations and could have an impact on public health.

The assessment of a clinical significance to a specific sequence variant require the following information: the type and site of the mutation, the presence of the same mutation in a control group, the co-segregation of the variant and disease within families, the co-occurrence with a deleterious mutation, the type of amino acid change, the conservation of the amino acid across species and the biochemical function [20]. For example, the I68K variant, detected also in this study, was studied by Morris et al. to predict pathogenicity and disease-association by biochemical/functional experiments [46]. They found that this substitution is located inside the Ring finger domain of the BRCA1 protein and brings about a folding alteration of the domain due to its E2-ligase binding. In addition, it has been demonstrated that the V740L and the A521T mutations, reported respectively once and twice in this study, are located in a strongly conservative site and are linked to BRCA1-associated cancer [47, 48]. In this study, we found sequence variants of unknown biological significance in the BIC database but potentially pathogenetic. Obviously this affirmation, based on the mutational analysis of a population of 50 control cases and on the conclusion that the majority of UV carriers developing BC at an early age have no family history of such tumors, should be confirmed by additional experiments. Two unclassified missense substitutions (Y179C and N550H) and a neutral/LCS missense substitution (F486L) have been identified in cis in a

single patient [49]. Tavtigian et al. has shown that these three variants are always found together, perhaps as a rare haplotype and are quite probably associated with the disease [50]. In other studies, on the contrary, these alterations have been found in trans with pathological mutations, indicating that each single variant does not represent a higher disease risk [20]. Based on our own experience, it might well be that the presence of both the substitutions in cis have a higher effect in the structural alteration of the protein and in the development of BRCA1-related tumors [49, 50]. More extensive information regarding cancer predisposition would be of critical importance to genetic counselors, since such UV mutations lead to informative genetic testing results.

In conclusion, the present study is the first BRCA1 disease-associated mutation analysis in Southern Italian families. We found a percentage of BRCA1 mutations (16%) among the Southern Italian population similar to those reported in other countries. In this consecutive series of Sicilian patients affected by breast and/or ovarian cancers, the early age of onset of such tumors and the association with the HBOC familial profile could be two valid screening factors for the identification of BRCA1 mutation carrier. We also identified a pathological mutation (4843delC) as a possible S*icilian founder mutation.* Further analysis will be conducted in order to confirm the link between the high penetrance of the mutation and the familial predisposition to breast and ovarian cancer with the aim of using genetic testing in routine clinical practice for the rapid recognition of high-risk families in specific populations.

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