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Y179C, F486L and N550H are BRCA1 variants that may be associated with breast cancer in a Sicilian family: results of a 5-year GOIM (Gruppo Oncologico dell'Italia Meridionale) prospective study

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Background: Over 600 different pathogenic mutations have been identified in the BRCA1 gene. Nevertheless, numerous missense mutations of unknown biological function still exist. Understanding of biological significance of these mutations should help in genetic counselling to carriers and their families.

Patients and methods: A total of 104 patients with breast and/or ovarian cancer whose genetic counselling answered the criteria of the American Society of Clinical Oncology (ASCO 2003), were prospectively screened for mutations in all coding exons of the BRCA1 gene by automatic direct sequencing.

Results: During these mutational screening procedures one case presented three mutations classified in the Breast Cancer Information Core Database as unknown variants. These were 655A/G found in exon 8 of BRCA1, 1575T/C and 1767A/C found in exon 11 of the same gene. The identification of the three unknown variants in the proband (16SIRIO) and in her mother and sister indicates that such alterations exist in *cis*.

Conclusions: Our results suggest that the charge and stechiometry variations determined by the changes in the amino acids Y179C, F486L and N550H might produce an effect on the conformation of the protein and, consequently, on its function.

Key words: breast cancer, BRCA1, germinal mutations, unknown variant

introduction

The 5%–10% of cases of breast/ovarian carcinoma are associated with hereditary genetic mutations and a high penetrance of the oncosuppressor BRCA1 [1]. Genetic alterations in this gene lead to a higher predisposition to breast and ovarian cancer and confer a significantly higher risk of endometrial, pancreas, cervix and prostate gland cancers [2]. The BRCA1 gene consists of 22 exons encoding a nuclear 1863 amino acid phosphoprotein. BRCA1 protein contains several functional domains, which interact directly or indirectly with a variety of molecules, including tumour suppressors, oncogenes, DNA damage repair proteins, cell cycle regulators, transcriptional activators and repressors [3, 4].

Over 600 different pathogenic mutations identified in the gene BRCA1 have been reported in the Breast Cancer Information Core Database (BIC) [5]; most of these are frameshift or nonsense mutations that give rise to the formation of truncated proteins; others instead are missense substitutions or intronic variants, including those involved in splicing, which have been linked with pathogenesis.

Recently, many studies have focused on the interpretation of the unknown biological significance of missense mutations in the gene BRCA1, defined as unknown variant (UV), with the aim of determining whether they had a pathogenic or a neutral role [6, 7]. Moreover, these functional and biochemical alterations within the different protein domains must be acquired in order to understand their role in cancer predisposition and aid in genetic counselling.

During genetic screening of BRCA1 in Sicilian patients with breast/ovarian cancer (BC/OC), selected according to hereditary breast/ovarian cancer syndromes (HBOC) criteria, the patient 16SIRIO presented three UVs in the gene. We therefore examined other members of her family to assess whether the UVs were present in *cis* and if their segregation was associated with other neoplasias diagnosed in these subjects.

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patients and methods

A consecutive series of 650 patients resident in Sicily with breast/ovarian cancer diagnosed between 1999 and 2004 were prospectively recruited at the 'Regional Reference Centre for the Characterisation and Genetic Screening of Hereditary Tumours' at the University of Palermo. Written informed consent to genetic counselling was obtained from all patients recruited. This protocol was conducted by an oncologist, a geneticist and a psychologist and included information regarding personal and family history in order to evaluate risk factors and genealogical tree. The latter was updated every year and investigated retrospectively for at least three generations in patients with breast/ovarian cancer or other types of tumours related to BRCA1 mutations.

After considering different family relationships, a total of 104 patients, selected during genetic counselling according to the criteria of the American Society of Clinical Oncology (ASCO) [8], were screened for mutations in all coding exons of the BRCA1 gene by automatic direct sequencing. In cases of positive testing, the patients were invited to follow preventative programmes for the early diagnosis of BRCA1-associated tumours and to communicate the results to their first-degree relatives so that they might also undergo genetic counselling. Even when genetic testing proved negative, the relatives of such patients were encouraged to take a regular part in the early diagnosis programmes because of their familial cancer risk. If the genetic testing was unclear, the patient was informed in order to decide whether or not to proceed to other types of biomolecular investigation.

The genomic DNA was extracted from peripheral blood according to the instructions contained in the QIAamp DNA mini Kit (Qiagen, Hilden, Germany). A 9700 thermal cycler (Applied Biosystem, Foster City, CA) was used to perform the polymerase chain reaction (PCR) of all the exons and, after dividing exon 11, of the 12 fragments of the gene BRCA1. The fragments of amplicons were analysed by agarose gel electrophoresis at a concentration ranging from 1.5% to 2%. Direct sequencing of the PCR products was performed with a 3100 Avant Analyzer automatic sequencer (Applied Biosystems, Foster City, CA).

The DNA samples from the 16SIRIO family members were screened for BRCA1 mutations in exons 8 and 11. Screening of the 50 DNA controls with any familial cancer history, sequenced for the positions 655A/C, 1575T/C and 1767A/C, were performed in order to verify that the three variants were present together only in the 16SIRIO family.

results

During mutational screening of the gene BRCA1 in a group of 104 Sicilian patients with hereditary and familial ovarian and/or breast cancer selected in the Regional Reference Centre for the Characterisation and Genetic Screening of Hereditary Tumours (University of Palermo, Italy), one case presented three missense mutations classified in the BIC database as unknown variants. These were 655A/G found in exon 8 of BRCA1 and 1575T/C and 1767A/C found in exon 11 of the same gene (Table 1). Furthermore, the same patient showed the intronic variation IV7-34 C>T, which was reported several times in the BIC both as a polymorphism and as an UV; in our negative controls, this intronic variant was detected in 70% of the cases (35/50), both in homozygotes and in heterozygotes.

The patient, indicated by an arrow in the geneological tree, was a nulliparous, non-Askenazi woman aged 35, with a ductal infiltrating carcinoma of the left breast (T1NoMx), intermediate grade (G2), positive to the test for oestroprogestinic receptors (40%) and for MIB-1 (10%) and negative for Erb-B2 (Table 2).

During genetic counselling, the patient's geneological tree was reconstructed together with her individual and family clinical history, retrospectively for three generations. The patient was included in our study because she was diagnosed with breast cancer at an early age (<40 years old) and also because of the tumour history in the first generation. The patient's mother had previously been diagnosed with a tumour of the uterus (spinocellular, cheratoblastic and endocervical infiltrating carcinoma) at the age of 42. All members of the family, including the proband's father, mother and sister, underwent analysis of exons 8 and 11 of the gene BRCA1. Direct automatic sequencing of DNA extracted from the lymphocytes showed exactly the same UVs: Y179C, F486L and N550H in the mother and the sister (Figure 1). Furthermore, the patient's father presented the same polymorphic intronic variant IV7-34 C>T as the proband, while the sister and the father showed S694S polymorphism (Table 1). The identification of the three UVs both in the mother and in the sister of the proband indicates that such alterations exist in *cis*. None of the control cases presented any of the three UVs.

discussion

The BRCA1 germinal mutations are responsible for the majority of hereditary breast and/or ovarian cancers and they have also recently been associated with a high risk for several other types of tumours, such as those involving the prostate gland, the pancreas and the endometrium [9, 10].

Since carriers of these mutations have a high risk of developing these tumours at an early age, the American Society of Clinical Oncology recommended genetic testing to be performed to patients before the age of 40 years [11].

The patient 16SIRIO developed breast carcinoma at an early age and analysis of her genealogical tree indicated that her mother had been affected by endometrial carcinoma at a similar age. This suggested that the patient was probably a carrier of

Table 1. Details of the five sequence variants of BRCA1 gene detected in the 16SIRIO family

Sequence variants	Exon/intron	Nucleotide no.	Base change	No. times in BIC	Amino acid change	Effect
Missense mutation	Exon 8	655	A>G	35	Y179C	UVs probably related to BC
Missense mutation	Exon 11	1575	T>C	36	F486L	when present simultaneously
Missense mutation	Exon 11	1767	A>C	34	N550H	
Intronic variant	Intron 7	IVS7-34	C>T	7	–	Neutral
Silent mutation	Exon 11	2201	C>T	12	S694S	Neutral

a BRCA1 mutation. Genetic tests on the patient 16SIRIO showed the presence of three UVs: 655A/G, 1575T/C and 1767A/C, which were also found in the patient's mother and younger sister, whereas none of these UVs were found in any of the control cases. Furthermore, both the proband and her father presented the intronic variation IVS7-34C/T, frequently reported in the BIC both as a polymorphism and also as an UV. This variant was found in 70% (35/50) of our control cases, both in homozygotes and in heterozygotes, indicating therefore that it was a benign nucleotide alteration. Moreover, both the patient's sister and father showed the nucleotide variation 2201C/T, which does not involve any amino acid variation (S694S).

Table 2. Proband's clinical features

Age at initial diagnosis	35
Family history of breast cancer	Negative
Family history of ovarian cancer	Negative
Laterality of breast cancer	Left
Histology of breast cancer	Ductal infiltrating
Tumour stage	T1
Number of nodes involved	0
Histological grade	2
Oestrogen receptor status	Positive
Progesterone receptor status	Positive
Erb-B2	Negative
MIB-1	Positive (10%)

The analysis of the control cases with the same ethnic background was important for determining whether the changes detected in the BRCA1 genomic sequence are variants associated with disease or benign variants that are typically found in the Sicilian population.

It is known that the conformation and therefore the final structure of a protein is the result of its amino acid composition and consequently of the physico-chemical properties and stechiometry of the amino acids. The three UV identified in this report are mentioned several times in the BIC and determine important amino acid changes: in fact, 655A/G gives rise to the

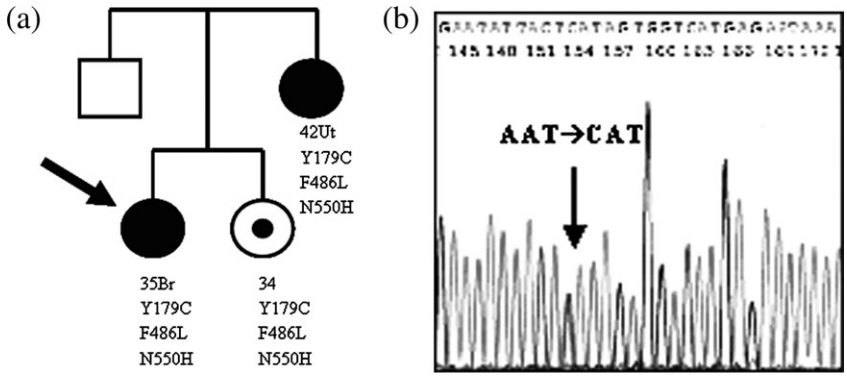

Figure 1. (**A**) Geneological tree with 16SIRIO proband indicated by arrow. Black circles indicate women affected by cancer. White square indicates man without any mutation. Br, breast cancer; Ut, uterine cancer. Numbers following abbreviations indicate age at diagnosis. (**B**) Electropherogram of exon 11 sequence (1767A/C) of BRCA1 gene in the proband.

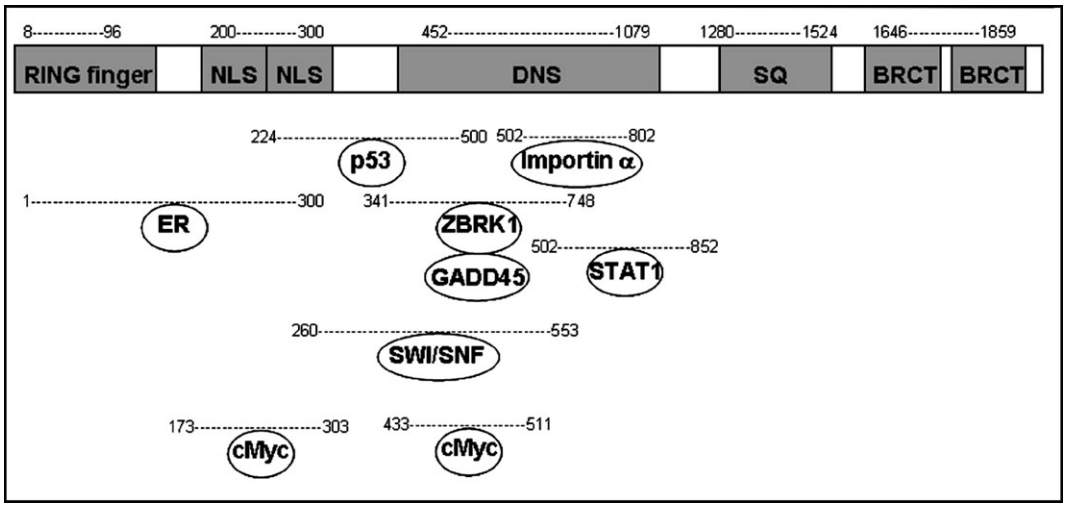

Figure 2. Schematic diagram of BRCA1 polypeptide showing main functional domains (in grey). In the circles are indicated some of the proteins interacting with BRCA1, whose interaction domains include the amino acids Y179, F486 and H550.

substitution of thyrosine 179 characterised by the presence of an aromatic ring, by cisteine, which possesses oxydrilic lateral chains (Y179C, n. 35 times in the BIC); 1575T/C brings about the substitution of phenylalanine 486, which also possesses an aromatic ring, by leucine, which has aliphatic lateral chains (F486L, n. 36 times in the BIC); and 1767A/C leads to the substitution of the amino acid not charge asparagin 550 by the amino acid basic histidine (N550H, n. 34 times in the BIC).

Several studies have been conducted to understand the pathogenicity of BRCA1 unknown variants. The studies were based on the alignment of the orthologous sequences and on *in vitro* embryonal engineering (Xenophus and Mouse), where UVs have been inserted in *trans* compared with deleterious mutations of the same gene. These analyses demonstrated that if Y179C, F486L and N550H are considered separately, they can be classified either as neutral alterations or as changes with only low clinical significance [12–14].

Combining these results and considerations with the study of the 16SIRIO family, we suggest that three amino acid changes identified might alter the charge and stechiometry of protein, and in consequence its function. The latter might be related to the change in BRCA1 interactions with proteins whose binding domains include the amino acids Y179, F486 and N550, for instance, TP53, SW1/SNF, ZBRK1/GADD45, importin alpha, STAT1, ER and cMYC. These factors are involved in DNA damage repair, cell cycle regulation and transcriptional regulation [3, 4]. In particular, cMyc oncogene interacts with two BRCA1 domains that include Y179 and F486 (Figure 2).

An accurate follow-up of this family, and particularly of the proband's sister, might confirm the hypothesis that the three amino acid alterations could play a role in determining breast and/or ovarian cancer susceptibility. It might also be interesting to study the segregation of the three variants in the carriers affected by disease compared with those detected in the healthy members of the family.

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