

4843delC of the BRCA1 gene is a possible founder mutation in Southern Italy (Sicily)

A. Russo^{1*}, V. Calò¹, C. Augello¹, L. Bruno¹, V. Agnese¹, V. Schirò¹, F. Barbera¹, S. Cascio¹, E. Foddai^{1,3}, G. Badalamenti¹, C. Intrivici¹, M. Cajozzo⁵, G. Gulotta⁶, E. Surmacz², G. Colucci⁴, N. Gebbia¹ & V. Bazan¹

¹Department of Surgery and Oncology, Regional Reference Centre for the Biomolecular Characterization and Genetic Screening of Hereditary Tumors, University of Palermo, Palermo, Italy; ²Sbarro Institute for Cancer Research and Molecular Medicine, College of Science and Technology, Temple University, Philadelphia, PA, USA; ³Department of psychology, University of Palermo, Palermo; ⁴Division of Medical Oncology, National Institute of Oncology, Bari; ⁵Section of General and Thoracic Surgery, Department of GENURTO, Università di Palermo, Palermo; ⁶Department of GENURTO, Università di Palermo, Italy

Background: The frequency and the type of *BRCA1* mutations vary widely and might have different geographic and ethnic distribution. Most of these alterations are generally found in isolated populations as a consequence of the founder effect. The object of this study was to determine whether 4843delC, a deleterious mutation of the *BRCA1* gene, might be due to a founder effect originating in the Sicilian region of Italy. This mutation was described by us for the first time and identified in two unrelated Sicilian families with hereditary breast/ovarian cancer. The two families were from the same geographical area (south-western area of Palermo, Sicily). The homogeneity of the ethnic group of the two families and the Single Nucleotide Polymorphism (SNPs) analysis of probands led us to perform a study of the allelotype of the various members.

Patients and methods: The analysis of the haplotype of the probands and of several family members was conducted by means of a study of the highly polymorphic microsatellites within or flanking the *BRCA1* gene.

Results: This analysis revealed the presence of a common allele associated with the mutation.

Conclusions: We therefore conclude that 4843delC of the *BRCA1* gene is a possible founder mutation in the Sicilian population.

Key words: *BRCA1* gene, founder mutation, genetic counseling, haplotype analysis, haplotype frequency

introduction

Linkage and segregation studies have shown that high-penetrance germinal mutations in the gene *BRCA1* give rise to a genetic predisposition for the development of breast and ovarian carcinomas [1, 2]. Over 600 pathological mutations in this gene have been described in the Breast Cancer Information Core database South-western area of Palermo, Sicily (BIC, <http://research.nhgri.nih.gov/bic/>); these are uniformly distributed along all the coding regions and intronic sequences flanking each exon; thorough screening of the whole gene is therefore necessary in order to identify these alterations.

The frequency and the type of *BRCA1* mutations vary widely and might have different geographic and ethnic distribution. Most of these alterations are generally found in isolated populations as a consequence of the founder effect [3]. Examples of founder mutations are 185delAG and 5385insC of the gene *BRCA1* in the descendants of Ashkenazi Jews, with

an incidence estimated at 1% and 0.13%, respectively; the study of the haplotype has shown that such mutations are clearly linked to a common ancestor [4, 5].

Additional founder mutations of *BRCA1* gene have been described in several populations from other geographical areas, for example in Belgium and The Netherlands (2804delAA), in Sweden (1675delAA) and 5382insC, C61G and 4253delA recently identified in eastern Europe [6–9]. A regional founder effect has been demonstrated in Italy for *BRCA1* 5083del19 and will probably be demonstrated for the 1499insA *BRCA1* mutation in Tuscany (Northern Italy) [10, 11].

One hundred and six families from Sicily in Southern Italy, selected with strict criteria in order to identify those at high risk of being carriers of *BRCA1* mutations, underwent mutational screening. During these analyses, we observed a new *BRCA1* 4843delC mutation in two index cases affected by breast/ovarian carcinoma who were apparently not related to each other [12]. 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 of tumors. Up till now, no founder mutation has been identified in the population of Sicily.

*Correspondence to: Antonio Russo, MD Department of Surgery and Oncology, Regional Reference Centre for the Biomolecular Characterization and Genetic Screening of Hereditary Tumors, University of Palermo, Via Veneto 5, 90144 Palermo, Italy
Tel/Fax: +39-91-6554529; E-mail: lab-oncobiologia@usa.net

The aim of this study was to determine whether or not this mutation had a founder effect in the Southern Italian (Sicilian) population. Haplotype analysis of mutation carriers and noncarriers of two families was performed by means of the analysis of five highly polymorphic microsatellite markers (D17S932, D17S1232, D17S1326, D17S1320, and D17S1325).

materials and methods

patients

Two families with breast and ovarian cancer were recruited after interview at the ‘Regional Reference Center for the Characterization and Genetic Screening of Hereditary Tumors’ at the University of Palermo. All members were from the Southern Italian region of Sicily. Blood samples were obtained from two affected probands and from unaffected family members after obtaining written informed consent. All relatives underwent genetic counseling conducted by an oncologist, a geneticist and a psychologist and were asked to provide information regarding their personal and familial history so that we were able to evaluate risk assessment and the genealogical tree. The latter was updated every year and investigated for at least three generations in order to identify patients with breast/ovarian cancer or other types of tumors, and to evaluate the presence within the family of neoplasias related to *BRCA1*-associated tumors. All information regarding the proband and the affected relatives was verified and confirmed by means of analysis of their hospital records. All cancer diagnoses were confirmed by the pathologist’s reports. Eleven members of two families were enrolled for the study. Blood samples obtained from 50 healthy Sicilians served as controls; informed consent was obtained from all subjects before the collection of samples. All the material regarding each individual case (a personal data chart, interviews, blood samples) was filed under an individual personal code in order to respect the patient’s privacy.

mutation detection

Genomic DNA was extracted from whole peripheral blood according to the instructions contained in the QIAamp Blood Kit (Qiagen, Hilden, Germany). Direct sequencing of the PCR product of exon 16 of the *BRCA1* gene was performed using a BigDye Terminator v3.1 and then sequencing by ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) as described previously [13]. Each genetic variant was confirmed by direct sequencing analysis on two independent blood samples.

haplotype analysis

The *BRCA1* haplotype was analyzed by using five microsatellite repeat markers located on chromosome 17q within or near *BRCA1* (Figure 1). From centromere to telomere, the order reported in the National Center for Biotechnology Information (NCBI) Database (<http://www.ncbi.nlm.nih.gov>) is as follows: D17S1320, D17S932, D17S1323, D17S1326, and D17S1325. Primer sequences to amplify these markers can be retrieved on line from the NCBI. Standard PCR protocol was used. The amplified products were visualized by microsatellite analysis with an automated apparatus (ABI Prism 3100Avant, Applied Biosystems).

results

The 4843delC mutation in the *BRCA1* gene has never been previously reported, either in the BIC or in the Human Gene Mutation Databases (<http://www.hgmd.cf.ac.uk/ac/index.php>) and can therefore be considered novel. The 4843delC mutation previously described and identified in two unrelated families (FAM92 and FAM64) were from the same geographical area.

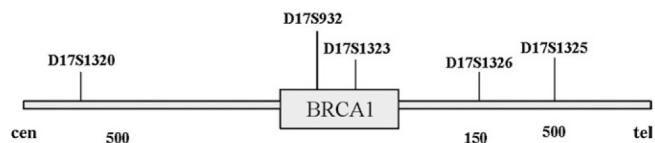


Figure 1. Physical map of five microsatellite repeat markers to *BRCA1* locus at 17q used in the genotyping of families and healthy controls. Distances (in kb) between microsatellite markers are taken from Baudi [11].

The proband of the FAM64 family, indicated by an arrow in pedigree, was a multiparous non-Ashkenazi woman with breast cancer at age 48. While neither of her children were a mutation carrier, her brother, nephews and her cousin proved to be healthy carriers (Figure 2). The proband of the FAM92 family, indicated by an arrow in pedigree, was a multiparous, non-Ashkenazi woman with ovarian cancer at age 44. Only her nephew, affected by megacariocytoma, was found to be a mutation carrier. The strong family history of breast/ovarian cancers and *BRCA1*-associated tumors in these families is due to the high penetrance of this mutation. The allele frequencies of the five microsatellite markers within or flanking the *BRCA1* gene were first determined in 50 healthy Southern Italian (Sicilian) subjects (Table 1). The two unrelated families (FAM92 and FAM64) with the 4843delC mutation in *BRCA1* were then typed with these markers. All the cases with the 4843delC mutation shared a haplotype of 4-2-8-2-3 at loci D17S932, D17S1320, D17S1323, D17S1326, D17S1325, respectively. Haplotype analysis for noncarriers of the mutation showed that none of these and none of the 50 healthy Sicilian controls had the 4-2-8-2-3 haplotype associated with the 4843delC mutation. Allelotype analysis highlighted the presence of a common allele in the affected individuals and healthy carriers, therefore suggesting the presence of a founder effect (Figures 1 and 2).

discussion

A large number of distinct mutations in the *BRCA1* gene are found in patients with hereditary breast/ovarian cancer. In contrast to several investigations indicating a wide spectrum of mutations along the whole gene, there are now emerging many studies reporting the presence of recurrent mutations peculiar to populations of different ethnic or geographic origins. This phenomenon may be the result of historical events leading to the accumulation of an ancient ‘founder’ effect initially occurring in homogeneous populations. The progressive expansion of these ethnic groups gives rise to an increase in the prevalence of the ‘founder mutation’ in a larger proportion of the population. Founder effects are well documented among Icelanders or Ashkenazi Jewish people [4, 5].

We found 4843delC in the *BRCA1* gene, a novel mutation in two families of 106 with familial and/or hereditary breast/ovarian cancer [3], highlighting the possibility that this might have a founder effect, and the individuals examined might share a common ancestor. To confirm this hypothesis, we performed allelotype analysis by using five highly polymorphic microsatellite markers.

Table 1. Allele frequency of microsatellite markers within and flanking the BRCA1 gene in 50 healthy Sicilian controls

D17S932		D17S1320		D17S1323		D17S1326		D17S1325	
Allele	Frequency (%)	Allele	Frequency (%)	Allele	Frequency (%)	Allele	Frequency (%)	Allele	Frequency (%)
1	4	1	2	1	1.5	1	1	1	1.5
2	2	2	45	2	1.5	2	31	2	3
3	4	3	5	3	5	3	1	3	1.5
4	21	4	29	4	60	4	1	4	23
5	10	5	9	5	3	5	1	5	31.5
6	19	6	8	6	8	6	20	6	10
7	26	7	2	7	10	7	18	7	10
8	12			8	1.5	8	18	8	4.5
9	2			9	1.5	9	2	9	4.5
				10	8	10	6	10	1.5
						11	1	11	3
								12	1.5
								13	4.5

FAM92

FAM64

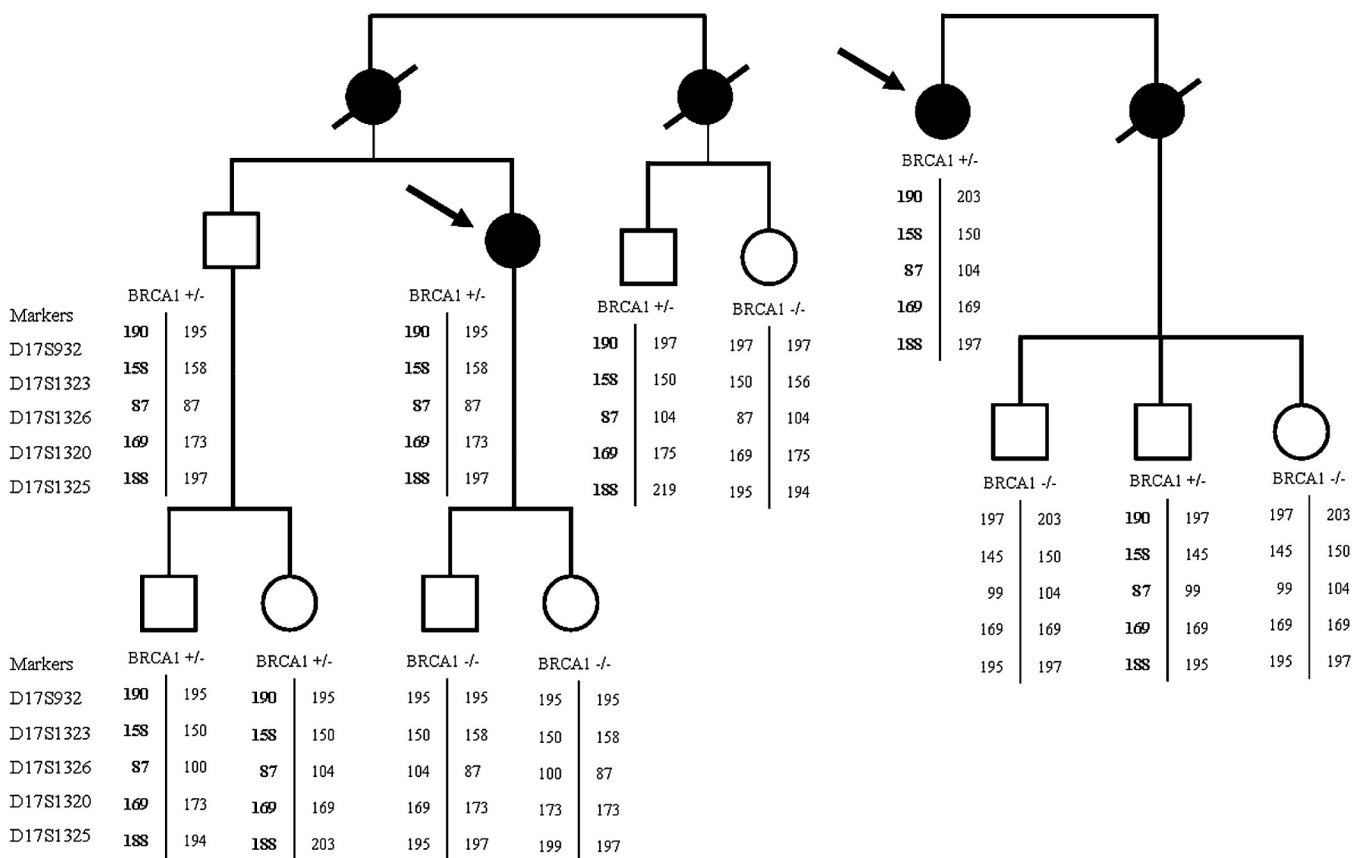


Figure 2. Pedigree of the two unrelated families (FAM92 and FAM64) where the 4843delC mutation has been identified.

The 4-2-8-2-3 haplotype at loci D17S932, D17S1320, D17S1323, D17S1326, D17S1325 shared by the patients and family members with the *BRCA1* 4843delC mutation was not detected in family members with *BRCA1* wild type. Furthermore, the frequencies of these common alleles (4-2-8-2-3) at loci D17S932, D17S1320, D17S1323, D17S1326, D17S1325 were 21.0%, 45.0%, 8%, 31% and 1.5%, respectively (Table 1).

A common haplotype was indeed detected among our population, strengthening the hypothesis of a ‘founder’ effect. These observations lend further support to the possibility that the patients with the 4843delC mutation share a common ancestral mutation.

Only the observation of a larger number of patients with hereditary breast and/or ovarian cancer syndrome (HBOC) of

Southern Italy (Sicily) will make it clear as to whether or not this alteration has a founder effect. It is important to reach a fuller understanding of the specific mutations of restricted ethnic groups in order to simplify genetic testing in the routine clinical practice of oncology.

In conclusion, the identification of this putative *BRCA1* founder mutation among Southern Italian (Sicilian) subjects may aid in the genetic screening of Sicilian patients with HBOC. Further investigation of this possible founder mutation in a larger group of Sicilian patients with breast cancer as well as population studies from the region is warranted for a better understanding of the origin and distribution of the mutation.

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