Introduction

Breast cancer (BC) is one of the main causes of cancer associated death in women[49] A great percentage (70%) of ovarian and breast cancer has a sporadic origin with an etiology not certainly knew, while the 15%-20% of these are hereditary, in which no one of the identified mutation has been associated to the susceptibility of such tumors [13].

About 5%-10% of these primary tumors are due to dominant autosomic transmission of various *susceptibility genes* identified in the last decade, among which the most implicated in breast cancer are the two highly penetrant predisposing genes BRCA1 [32]e BRCA2. They play an important role in the maintenance of the genomic integrity, in cellular proliferation and apoptosis.

Other genes that include CHEK2, PTEN, TP53, ATM, STK11/LKB1, CDH1, NBS1, RAD50, BRIP1 and PALB2 have been described to be high or moderate penetrance breast cancer susceptibility genes, all contributing to the hereditary breast cancer spectrum[15, 20,26, 38,44]

A lot of linkage studies showed that the tumor suppressor genes Brca1/2 predispose women to breast and ovarian cancer. *BRCA1* gene is implicated in about 30-45% cases of Breast Cancer, and in a great percentage with Ovarian cancer. A great part of the mutation caused by *BRCA1/2* is at high penetrance and it is estimated that gives to carrier women a cumulative risks of about 60–85%[37,37] to develop BC, often bilateral , and a risk of 15-54% to suffer from OC [3,11,13,42,46,51].

Moreover *BRCA 2* is also involved in the increasing risk to develop prostate and a pancreatic cancer [8- 19] .

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 . For this *"carriers"* patients it is possible to establish an appropriate clinical management for example the prophylactic surgery [9].

According to the hereditary tumor polygenic model unfavorable combination of polymorphic variants on the

susceptibility genes at low penetrance might explain the increased risk of hereditary tumor.

A different geographic and ethnic distribution is described in a lot of works as concern the mutation type and frequencies of Brca1/2.

The family affected by hereditary breast and ovarian cancer (HBOC) were distinguished by a high aggregation cases and by the arise of such tumor in earlier age. The genetic analysis of families with multiple cases of breast cancer with a mean age of diagnosis before age 50 years and/or ovarian cancer facilitated the discoveries of the breast-ovarian cancer susceptibility genes, BRCA1 and BRCA2.

In 2002 the American society of clinical oncology suggested the possibility to identify the risk of a family to develop an hereditary tumor. It depends by the earlier rising up of the tumor, by the bilateral location, by the coexistence of breast and ovarian cancer in the same patience, by the history family and the ethnic background. In this vision is really important to make a discrimination with an appropriate oncogenetic counseling between family at high risk and family at low risk.

Brca1/2 genes

Brca 1 is located in the chromosome 17, has 24 exons, 22 codificant, spans approximately 200kb of genomic DNA, and encodes a 1863 amino acid protein n , while Brca 2 localized on chromosome 13 has 27 exons, 26 codificant , and encodes a protein of 3418 amino acids. The 11 exon in Brca1 is very large in dimension and codifies for the 60% of the protein. Both genes pointed out an elevate structural homology and they have no similes to other known genes. These proteins had a nuclear localization and are expressed in a lot of tissues with a determinant role in repairing the DNA repair. They form complexes that will activate the repair of double strand breaks (DSBs) and initiate homologous recombination (HR). RAD51 is the key component of this mechanism. Co-localization of BRCA1 and BRCA2 with RAD51 at the site of recombination and DNA damaged induced foci strongly suggest that they are

involved in the detection and the repair of DSBs. Moreover they are also implicated in the maintenance of chromosome stability, possibly through their function in recombination.

BRCA1 is likely to participate as a sensor or transducer rather than directly as a repair factor and it has been suggested that BRCA1 functions as scaffold or platform to coordinate different activities needed for repair. The exact molecular function of BRCA1 in the DNA damage response remain elusive, even if a lot of biochemical activities have been performed on the transcriptional regulation, mRna polyadenylation, chromatin remodeling and ubiquitination [29].

BRCA2 interacts and regulates the function of RAD51, that has a catalytic activity. The interaction involves a substantial proportion of the total cellular pool of each protein, and this suggest that Brca2 regulate the availability and activity of RAD51 [52-29] .

One faulty copy of BRCA1 or 2 in the germline is sufficient for cancer predisposition, but the loss of the second allele is required for cancer development [12-32]. Little is known about the mechanisms by which the wild-type allele is lost. Although the association with inherited predisposition, somatic mutation in BRCA1 and 2 are rare in sporadic BC and OC [14,27].

Mutations

The identification of carriers of germline mutations largely relies on the mutation analysis of genomic DNA or transcribed mRNA. Various methods alone or in combination for the detection of mutations, showed that BRCA1 and BRCA2 are large genes with many coding exons. Mutations span the whole gene, without any hot spot loci. It is possible that mutation at the 5'extremity of Brca1 may be associated mostly with ovarian cancer instead that one at the 3'extremity. It was observed that the mutations occurred in the 4075-6503 portion of the 11 exon of Brca2 are associated with an increased risk to develop ovarian cancer and for this reason called Ovarian Cancer Cluster Region.

In the BIC (Breast Cancer Information Core) more than 3000 Brca1/2 sequence variants are described , about 600 of both Brca1/2 are considered deleterious. About 15% of all *BRCA1* and *BRCA2* alterations reported to the BIC database are intronic variants probably involved in splice sites and a subset of these variant are located in intronic sequence [41]. These alterations may be defined as variant of uncertain/unknown significance (VUS), pathological and polymorphism [41].

Some splice site mutations and large rearrangements do not change the reading frame, but cause a loss or gain of one to several exons, which is thought to compromise the gene function[41]. Deleterious missense mutations are retired within specific residues of functional element. It is still unknown the risk role of a large number of sequence variants. These 'variants of unknown significance' (VUS) include missense changes and small in-frame deletions and insertions, coding synonymous nucleotide substitutions that do not lead to amino acid shifts, as well as alterations in non-coding intervening sequences (IVS) or in untranslated exonic regions (UTRs). Up to 10-20% of the BRCA tests report the identification of a variant of uncertain significance.

Mutations called "*founder*" are genetic alteration originated from a forefather of the analyzed populations and kept during the evolution. A lot of these are recurrent and are found in isolated population as results of a founder effect. [8-9]. Such founder mutations in BRCA1 and BRCA2 have been described in French Canadian [45], Swedes [24], Icelandic women [48], Norwegians [2], Finns [22], Dutch women [39,40], Russians [17], Japanese women [23] and African Americans [16]. In the Southern Italy the Brca1-5083del119 and Brca2-8765delAG mutation are mostly identified. Another BRCA1-4843delC mutation could be a possible Sicilian founder mutation, although present evidence is scarce. [43].

To study with extremely confidence the message give by VUS, especially for their "uncertain significance", two important researcher groups, the ENIGMA (Evidence-based network for the Interpretation of Germline Mutant Alleles) and the WECARE (Women's Environment, Cancer, and Radiation

Epidemiology) tried to create large data sets to establish an international guidelines [4,21,41].

Clinical significance of VUS

Almost 1,800 distinct sequence variants in *BRCA1/2* are listed as having unknown clinical significance on the Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/).

VUSs in the *BRCA1* and *BRCA2* genes front a significant block, because patients and physicians do not understand if the VUSs predispose to cancer or are neutral respect to cancer risk. Consequently, carriers of VUSs and their family members cannot gain benefit of the risk assessment, prevention, and therapeutic measures that are obtainable to carriers of known deleterious truncating mutations. Additionally, carriers of VUSs are sometimes counseled to make prophylactic surgery because of the presence of the VUS, but in the absence of any knowledge of the cancer relevance of the VUS [5]. For these reasons, the determination of the clinical relevance of VUSs in *BRCA1* and *BRCA2* has become an important clinical issue [18,33,34].

A multitude of factors could provide significant confidence in determining the clinical significance of a VUS, for example the analysis of the co-segregation, the epidemiology, the co-occurrence with deleterious mutations, the evolutionary data, the amino acid substitution, loss of heterozygosis (LOS) in respective tumors and functional analysis [7,25,9,31,53].

For BRCA1,some authors developed a predictive algorithm that combines a measure of cross-species conservation, including nonmammalian *BRCA1* sequences, with a measure of the degree of chemical change in amino acids, 15 to identify 50 putative deleterious *BRCA1* missense mutations [1].

A lot of VUSs have been examined, as concern their effect on protein, with use of functional assays.

Works in this area have focused on *BRCA1* mutations in the two C-terminal BRCT domains,[6,34] which evaluate the activation of transcriptional activity of the BRCT domains with

use of mammalian and yeast-based models,18–20 and on the E3 ligase activity associated with the N-terminal BARD1- binding domain of BRCA1[35].

As concern BRCA2 function assays of homology-directed repair and centrosome amplification in response to ectopic expression of full-length, wild-type VUS containing BRCA2 protein have been performed [53].

Recently, crystal structures of the *BRCA1* BRCT and *BRCA2* DNA-binding domains have been used to predict that a number of VUSs predispose to cancer,[31,50] but genetic evidence suggests that some of these predictions are incorrect.

No one of the approaches described above have successfully been used to classify the clinical relevance of *BRCA1* or *BRCA2* VUSs, even if a lot of these revealed promise in this view.

Each of these approaches have particular strengths and limitations in addressing the general problem of causality of sequence variants .

Another real problem in the classification of VUS is the laboratory quality control measures and technical limitations of results. Confirmed the quality of the control procedures of the diagnostic laboratory activity, it should be made clear that a DNA alterations exists, but the clinical interpretation of the DNA alteration is unclear. This gap must be discuss to clarify the interpretation of the mutation, either pathogenic or not, to the patients questions [28].

Actually one of the most active research group operating in the VUS field is the IARC (Unclassified Genetic Variants Working Group). They try to collect as much information as possible from the various population groups with the use of databases and statistical programs.

Study of the principal method of VUSs assessment

Definition and localization of deleterious mutation

Sequence variants are listed on the basis of their predicted effect on the mRNA and amino acid level and defined as

deleterious mutations according to the following established (BIC) criteria:
1) all frameshift and nonsense variants with the exception of the neutral stop codon *BRCA2* c.9976A>T (BIC: K3326X) [30], and other variants located 3' thereof;
2) all VUS variants occurring in the consensus splice acceptor or donor sequence sites, either within 2 bp of exon-intron junctions or when experimentally demonstrated to result in abnormal mRNA transcript processing;
3) missense variants that have been conclusively demonstrated, on the basis of data from linkage analysis of high risk families, functional assays or biochemical evidence, to have a deleterious effect on known functional regions.

Analysis of control group

According to the selection criteria of the studied groups, a useful method for the study of VUS therefore involves the analysis of the variants in a control group in order to distinguish the percentage of the allelic frequency and to attribute a certain clinical significance. For these reasons it is important to consider both size and ethnic homogeneity.

Co-segregation

To evaluate causality from the co-segregation data, it is useful to apply the statistical model described in literature by [47]. For these calculations, it is assumed an allele frequency of the variant of 0.0001 and used the *BRCA* penetrance estimates that is based on the recent meta-analysis of 22 population-based studies [3] with pooling across age groups, if necessary, depending on the level of detail of the family history information. While family-based estimates might be more appropriate, it is better to use these estimates, since the criteria for testing differ markedly among testing centers and the use of the population data would be conservative. It is impossible to assess that a that a variant observed in the proband is a new mutation, although this could easily be incorporated into the model. Unfortunately the main problem of the segregation study regards the availability of family data and its size,

requiring sampling of additional individuals in the pedigrees which may be difficult to achieve [18].

Co-occurrence in trans

A lot of studies indicate that homozigosity for BRCA1 is embrionically letha. This result is reinforced by the clear deficit of BRCA1 homozygotes and compound heterozygotes compared with expected number, between a series of individual with a certain founder mutation [1]. Assumed that homozygosity for BRCA 1 and 2 pathogenic mutation is embryonically lethal, it is possible to classify a variant as neutral on the basis of a single analysis. If the variant is neutral, the probability of an individual with the variant also carrying (in trans) a deleterious mutation, can be approximately extimated as half the overall frequency of deleterious mutation in the population studied. However it is logical to assume that compounds in heterozygotes for deleterious mutations in BRCA2 are really rare in adults, since the Fanconi anemia phenotype usually cause to death in early childhood. A complication that occur in these data is the distinction between mutations occurring in *cis* and those in *trans [*18].

Personal and family history

A large number of family history information is available for BRCA1 and 2. The data of the Myriad Genetics laboratories are the most important source that gives scope and completeness of the genotyping. When individuals and families with pathogenic mutations are compared to individuals and families without mutations, specific characteristics of the BRCA ½ phenotype, such as age of onset of cancer and number of cancer of different types, are associated with exact likelihood of a pathogenic mutation being present [18].

Aims of the thesis

- To choose the appropriate study method for the frequency mutation in our cases.
- To study VUS of sicilian population by statistical algoritms and software to assess cancer risk and clinical relevance of BRCA1/2 variants.
- To compare our results with the data present in literature.
- To make a multi-parametrical analysis method to help clinicians to individuate classes of risk.

Materials and methods

Cases and controls

Family recruitment

Families with BC or OC get-together clinical criteria with an expectancy of a BRCA1/2 mutation of ≥ 10% or high heterozygote and lifetime risk were genetically counseled and invited to participate in a structured breast cancer surveillance program of the "Regional Reference Center for the Characterization and the Genetic Screening of Hereditary tumors" at the University of Palermo. The genetic counseling has been carried out in patient with a suspect for BRCA1 and 2 mutations. The medical genetist draw an accurate family tree for at least three generations in order to recruit information regarding familial and personal history. During the counseling it is also individuated the belonging ethnic group because of the presence to individuate some high frequency founder mutation and make known to the physician important information about the case sheet. The psychologist take care about the feeling of the patients and note the psychological profile to pursuit during the therapeutically plan . After having signed written informed consent, blood samples are collected from each proband. A number corresponding to the proband in the logbook for the molecular screening is assumed to maintain the privacy information.

After having signed written informed consent, blood samples are collected from each proband. A number corresponding to the proband in the logbook for the molecular screening is assumed to maintain the privacy information. For this study a number of 74 probands are recruited.

Control populations

The control population include unrelated, healthy, both female and male, blood donors, sharing the same ethnic background with the cancer families All donors were healthy during their donation, and none of them had a reported familial history of BC and/or OC at that time and an age >40 years. The 50 case controls for this study were random recruited between from 2009-2011. According to the data present in literature, all the

donors were examined by a standard questionnaire. Informed consent for the study was given by all participants.

Methods

Mutation analysis of the BRCA1/2 genes

Genomic DNA is isolated using the QIAamp® DNA Blood Mini Kit. The mutational screening is carried out using polymerase chain reaction (PCR) amplification of all amplicons of BRCA1 and 2. The sequencing of these multitude of fragments is carried out using the 3100 Genetyc Analyzer (Applied Biosystems/life Technologies).

The primers used for the screening, are built following the universal sequences of BRCA1 and 2 genes present on the online BIC database (http://www.nhgri.nih.gov/Bic/).

Mutation nomenclature is referred to the BIC reference sequences GenBank U14680.1 (BRCA1), NM_000059 (BRCA 2).

In term of quality results the complete molecular screening for BRCA1 and BRCA 2, of each patients take a quantity of time and consuming laboratory materials.

Results

BRCA1 and BRCA2 mutational screening

Germline BRCA1 mutations were found in 17 of 87 (23%) Sicilian probands.

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).

The most represented familial profile in the cohort is the HBC profile (70%). The HBOC and MBC profiles are both 14% and the HOC is 2%. According to the analysis of the different familial mutated profiles, the HBOC profile had a major frequency of mutations (41%). The HBC profile had a frequency of 36%, the MBC 18% and only 5% HOC. A total of 30 sequence variants was identified. For BRCA1 /2.

Fourthy-seven percent (14/30) were missense mutations, 27% (8/30) were frameshift mutations, 10% (3/30) were nonsense mutations and 17% (5/30) were intronic variants.

11/14 (78%) missense mutations were unknown biological variants (UV).

According to the mutation effects 15 were pathogenic, 11 suspected deleterious UV.

All the pathogenic and unknown variants in the BRCA1/2 gene were distributed throughout the whole gene.

BRCA ½ pathogenic mutation

Pathogenic mutations were detected in 22 families (%) of our cohort. Six of these were HBOC, four were HBC, one HOC and one MBC. All the families with carriers of a deleterious mutation had at least one member with early onset of BC and/or OC. Twelve different pathogenic mutations leading to non-functional truncated proteins were identified: Y101X, 633delC, 916delTT, R1443X, 4843delC, 5083del19 and 5149del4, 6079del4, Q2042X, 9254del5, 6310del5, V211T (fig1).

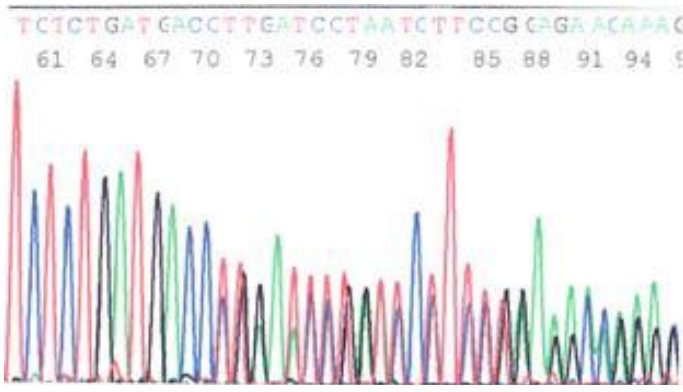
The Y101X mutation, detected once in the BIC database, was identified in one cases affected by OC (index case, 37 years) and BC (33 years) respectively, with HBOC profile (FAM49). The 633delC mutation was identified in an HOC profile (FAM76) containing 3 cases of OC at ages 40 (grandmother), 45 (index case) and 29 (daughter). The other daughter was a healthy carrier of the same mutation.

Table 1 Study recruitment criteria and distribution of families according to index cases among the overall series of 87 families enrolled in the study

Characteristics of patients	**Number**	**Frequency (%)**
BC ≤40 years	38	44
BC >40 years	40	46
BC and OC (any age)	4	5
OC ≤40 years	5	6
OC >40 years	0	0
Male BC	12	14
Bilateral BC	12	14
Personal hystory	54	62
Family history	9	10
Personal/family hystory	24	28

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.

Fig 1. Example of 4843delC non-functional truncated proteins



When there was clear evidence of presumed deleterious mutation, the mutations were reported as suspected deleterious, and are 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 [Tab2].

Gene	Sequence variant designation	Systematic nomenclature	Exon	NT	Codon	Base change	AA Change	Mutation type	Mutation effect	N° pts	Times in BIC
BRCA1	C61G	c.181T>G	5	300	61	T to G	Cys/Gly	M	-	1	
BRCA1	Y101X	c.303T>G	7	422	101	T to G	Tyr/Stop	N	N	1	1
BRCA1	Y179C	c.536A>G	8	655	179	A to G	Tyr/Cys	M	UV	1	16
BRCA1	633delC	c.514delC	8	633	172	delC	Stop233	F	F	2	16
BRCA1	916delTT	c.797_798delTT	11	916	266	delTT	Stop285	F	F	2	2
BRCA1	A521T	c.1561G>A	11	1680	521	G to A	Ala/Thr	M	UV	2	2
BRCA1	N550H	c.1648A>C	11	1767	550	A to C	Asn/His	M	UV	1	34
BRCA1	V740L	c.2218G>C	11	2337	740	G to C	Val/Leu	M	UV	1	1
BRCA1	A622V		11	1984	622	A to V	Ala/Val	M	UV	1	
BRCA1	R1443X	c.4327C>T	13	4446	1443	C to T	Arg/Stop	N	N	1	100
BRCA1	5083del19	c.4964_4982del19	16	5083	1655	del 19bp	Stop1670	F	F	4	38
BRCA1	4843delC	c.4724delC	16	4843	1575	delC	Stop1600	F	F	2	0
BRCA1	5149del4	c.5030_5033del4	17	5149	1677	delCTAA	Stop1678	F	F	2	16
BRCA2	A22T	-	2	-	-	A to T	-	M	UV	1	-
BRCA2	Y42C	c.353A>T	3	353	-	42	A to T	M	UV	1	140
BRCA2	T200I	-	-	-	-	-	-	-	-	-	-
BRCA2	V211I	c. 859V>I	7	859	211	V to I	V to L	M	M	1	7
BRCA2	R2034C	c.6328C>T	11	6328	2034	C to T	C to T	M	UV	1	96
BRCA2	Q2042X	c.6352 C>T	11	6352	2042	C to T	C to T	N	N	1	3
BRCA2	A2466V	c.7625C>T	14	7625	2466	C to T	C to T	M	UV	1	49
BRCA2	P2639A	c.8143C>G	17	8143	2639	C to G	C to G	M	UV	1	1
BRCA2	T3013I	c.9266 C>T	23	9266	3013	C to T	C to T	M	UV	1	53
BRCA2	IVS 2-7T>A	c.296-7delT	I-2	296-7	-	del T	del T	F	UV	1	4
BRCA2	IVS11-19delAT	c.7070-19delAT	I-11	7070-19	-	delAT	delAT	F	UV	1	1
BRCA2	IVS14+6G/A	c.7663+6G>A	I-14	7663+6		G to A	G to A	M	UV		12
BRCA2	IVS24-16T/C	c.9485-16T>C	I-24	9485-16	-	T to C	T to C	M	UV	1	4
BRCA2	IVS25-12T/G	c.9730-12T>G	I-25	9730-12	-	T to G	T to G	M	UV		2

Tab.2 Positive group of proband at the BRCA1/2 molecular screening

Discussion

The present work on the molecular screening of the BRCA1 and BRCA2 gene in patients affected by breast and/or ovarian cancers is in line with the first conducted in Sicilian population in 2007 [Russo et al.]. Up till now, the Italian Consortium of Hereditary Breast and Ovarian Cancer has examined 1,758 families and has found that 14% of them prove to be BRCA1 pathogenic mutation carriers . 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 University of Palermo showed a frequency of BRCA1 mutations of 16%. In agreement with previous Italian reports, in fact, 17 of the 84 unrelated families included in this study proved to be carriers of pathological mutations or UVS mutations. The identification of BRCA1/2 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, many reports have shown a certain variability in their application which often reflects the variability of the mutation frequency. Two important selection criteria in our study are the early onset of these tumor and the family history of our cases. 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 which indicate that the frequency of BRCA1/2 mutations decreases as the age of cancer onset increases.

The result of a test for a cancer susceptibility gene wants to be interpreted in the context of the individual's specific mutation and history. The identification of a in the past described pathogenic mutation, that correlates with an increased risk of developing cancer, profiles a positive test. The entire family is thus classified as carrier of a known mutation. A negative test should be interpreted differently, depending upon whether a family alteration had been previously identified. In a family where an affected member tests positive for a specific mutation, relatives who test negative are

considered "real negatives" and might be reassured that their risk is similar to the rest of the population.

On the other hand, in the case that no BRCA1 or BRCA2 alteration has been previously identified in the family, a negative test can be defined as "non-informative". It might be possible, in fact, that there is a mutation in BRCA1 and BRCA2 areas that cannot be identified using current methods ("false negative"), or that mutation of another gene, known or not yet identified, is involved. Besides, it might be the case that the family risk is due to multiple, low penetrance genes or that the familial "aggregation" is due to shared environmental risk factors, or to chance. It is also possible that the case is sporadic, despite the subject belonging to a high-risk family.

The identification of variants of uncertain significance, such as previously undescribed missense mutations that are not predicted to result in a loss of protein function, results in an inconclusive test outcome. At present, the approaches to determine the clinical significance of these mutations are difficult to implement and usually not feasible as part of a study. The approaches to determine the clinical significance of these mutations are difficult to implement and usually not feasible as part of a study. For our study, the most practical and clinically useful method is to determine whether the mutation segregates with cancer in family members, but this requires to test additional members in the pedigree which, in some circumstances, could be difficult to achieve.

REFERENCES

1. Abkevich V, Zharkikh A, Deffenbaugh AM, Frank D, Chen Y, Shattuck D, Skolnick MH, Gutin A, Tavtigian SV (2004) Analysis of missense variation in human BRCA1 in the context of interspecific sequence variation. J Med Genet 41:492–507

2. Andersen T.I.,. Borresen A.L, Moller P. A common BRCA1 mutation in Norwegian breast and ovarian cancer families? Am. J. Hum. Genet. 59, 486–487 (1996)

3. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B,Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, TuliniusH, Thorlacius S, Eerola H, Nevanlinna H, Syrjakoski K, KallioniemiOP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG,Easton DF (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 72:1117–1130.

4. Borg A, Haile RW, Malone KE, Capanu M, Diep A, Torngren T,Teraoka S, Begg CB, Thomas DC, Concannon P, Mellemkjaer L, Bernstein L, Tellhed L, Xue S, Olson ER, Liang X, Dolle J, Borresen-Dale AL, Bernstein JL (2010) Characterization of BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance in unilateral and bilateral breast cancer: the WECARE study. Hum Mutat 31:E1200–E1240.

5. Burke W, Daly M, Garber J, Botkin J, Kahn MJ, Lynch P, Mc- Tiernan A, Offit K, Perlman J, Petersen G, et al (1997) Recommendations for follow-up care of individuals with an inherited predisposition to cancer:

II. BRCA1 and BRCA2—Cancer Genetics Studies Consortium. JAMA 277:997–1003

6. Chapman MS, Verma IM (1996) Transcriptional activation by BRCA1. Nature 382:678–679

7. Chenevix-Trench G, Healey S, Lakhani S, Waring P, Cummings M, Brinkworth R, Deffenbaugh AM, Burbidge LA, Pruss D, Judkins T, Scholl T, Bekessy A, Marsh A, Lovelock P, Wong M, Tesoriero A, Renard H, Southey M, Hopper JL, Yannoukakos K, Brown M, Easton D, Tavtigian SV, Goldgar D, Spurdle AB (2006) Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. Cancer Res 66:2019–2027.

8. Consortium BCL (1999) Cancer risks in BRCA2 mutation carriers. The Breast Cancer Linkage Consortium. J Natl Cancer Inst 91:1310–1316

9. Domchek S, Weber BL (2008) Genetic variants of uncertain significance: flies in the ointment. J Clin Oncol 26:16–17.

10. EastonDF, Deffenbaugh AM, Pruss D, Frye C, Wenstrup RJ, Allen-Brady K, TavtigianSV, Monteiro AN, Iversen ES, Couch FJ, Goldgar DE. 2007. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. AmJHumGenet 81:873– 883.

11. Finch A, Beiner M, Lubinski J, Lynch HT, Moller P, Rosen B, Murphy J, Ghadirian P, Friedman E, Foulkes WD, Kim-Sing C, Wagner T, Tung N, Couch F, Stoppa-Lyonnet D, Ainsworth P, Daly M, Pasini B, Gershoni-Baruch R, Eng C, Olopade OI, McLennan J, Karlan B, Weitzel J, Sun P, Narod SA (2006) Salpingo-

oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 mutation. JAMA 296:185–192.

12. Friedman L.S., E.A.Ostermeyer, C.I.Szabo, P.Dowd, E.D.Lynch, S.E.Rowell, M.C.King. (1994) Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families, Nat Genet 8 399-404

13. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M. (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 62:676–689

14. Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y: BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 1994, **266:**120-122.

15. Gasco M., Yulug I.G., Crook T., TP53 mutations in familial breast cancer: functional aspects. (2003) Hum. Mutat. 21, 301–306

16. Gao Q., Neuhausen S., Cummings S., Luce M., Olopade O.I. (1997) Recurrent germ-line BRCA1 mutations in extended African American families with early-onset breast cancer. Am. J. Hum. Genet. 60, 1233–1236

17. Gayther S.A., Harrington P., Russell P., Kharkevich G., Garkavtseva R.F., Ponder B.A. (1997) Frequently occurring germ-line mutations of the BRCA1 gene in

ovarian cancer families from Russia. Am. J. Hum. Genet. 60, 1239–1242

18. Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ, Breast Cancer Information Core (BIC) Steering Committee (2004) Integrated evaluation of DNA sequence variants of unknown clinical significance: application to *BRCA1* and *BRCA2.* Am J Hum Genet 75:535–544

19. Hahn SA, Greenhalf B, Ellis I, Sina-Frey M, Rieder H, Korte B, Gerdes B, Kress R, Ziegler A, Raeburn JA, Campra D, Grutzmann R, Rehder H, Rothmund M, Schmiegel W, Neoptolemos JP, Bartsch DK (2003) BRCA2 germline mutations in familial pancreatic carcinoma. J Natl Cancer Inst 95:214–221

20. Hemminki A., Markie D., Tomlinson I., Avizienyte E., Roth S., Loukola A., Bignell G., Warren W., Aminoff M., Hoglund P., Jarvinen H., Kristo P., Pelin K., Ridanpaa M., Salovaara R., Toro T., Bodmer W., Olschwang S., Olsen A.S., Stratton M.R., De la Chapelle A., Aaltonen L.A., (1998) . A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 391, 184–187

21. Howard HJ, Horaitis O, Cotton RG, Vihinen M, Dalgleish R, Robinson P, Brookes AJ, Axton M, Hoffmann R, Tuffery-Giraud S (2010) The Human Variome Project (HVP) 2009 forum ''towards establishing standards''. Hum Mutat 31:366–367.

22. Huusko P., Paakkonen K., Launonen V., Poyhonen M., Blanco G., Kauppila A., Puistola U., Kiviniemi H., Kujala M., Leisti J., Winqvist R. (1998)Evidence of founder mutations in Finnish BRCA1 and BRCA2 families. Am. J. Hum. Genet. 62, 1544–48

23. Inoue R., T. Fukutomi, T. Ushijima, Y. Matsumoto, T. Sugimura, Nagao M.,(1995) Germline mutation of BRCA1 in Japanese breast cancer families. Cancer Res. 55, 3521–3524

24. Johannsson O., Ostermeyer E.A., Hakansson S.,. Friedman L.S, U. Johansson, G. Sellberg, K. Brondum-Nielsen, Sele V., Olsson H., King M.C., Borg A. Founding BRCA1 mutations in hereditary breast and ovarian cancer in southern Sweden. Am. J. Hum. Genet. 58, 441–450 (1996).

25. Karchin R, Monteiro AN, Tavtigian SV, Carvalho MA, Sali A (2007) Functional impact of missense variants in BRCA1 predicted by supervised learning. PLoS Comput Biol 3:e26.

26. Ko L.J., C. Prives, p53: puzzle and paradigm. (1996) Genes Dev. 10, 1054–1072

27. Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C, Seal S, Barfoot R, Collins N, Bignell G, Patel S, Hamoudi R, Larsson C, Wiseman RW, Berchuck A, Iglehart JD, Marks JR, Ashworth A, Stratton MR, Futreal PA: **BRCA2 mutations in primary breast and ovarian cancers.** *Nat Genet* 1996, **13:**238-240.

28. Lindor Noralane M., Guidugli L., Wang X.,Vallè M.P., Monteiro A.N.A., Tatvigian S., Golgar D.E., Couch F.J. A Review of a Multifactorial probability-Based Model for Classification of BRCA1 and BRCA2 Variants of Uncertain Significance (VUS). 2012. Human mutation, vol 33 No1,8-21.

29. Lorick K.L., Jensen J.P., Fang S., Ong A.M., Hatakeyama S., Weissman A. M. (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent

ubiquitination. Proc. Natl. Acad. Sci. USA 96, 11364–11369

30. Mazoyer S, Dunning AM, Serova O, Dearden J, Puget N, Healey CS, Gayther SA, Mangion J, Stratton MR, Lynch HT, Goldgar DE, Ponder BA, Lenoir GM. 1996. A polymorphic stop codon in BRCA2. Nat Genet 14:253–254.

31. Mirkovic N, Marti-Renom MA, Weber BL, Sali A, Monteiro AN (2004) Structure-based assessment of missense mutations in human BRCA1: implications for breast and ovarian cancer predisposition. Cancer Res 64:3790–3797.

32. Miki Y, Swensen J, Shattuck-Eidensen D. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266: 66-71.

33. Monteiro AN, Couch FJ (2006) Cancer risk assessment at the atomic level. Cancer Res 66:1897–1899

34. Monteiro AN, August A, Hanafusa H (1996) Evidence for a transcriptional activation function of BRCA1 C-terminal region. Proc Natl Acad Sci USA 93:13595–13599.

35. Morris JR, Pangon L, Boutell C, Katagiri T, Keep NH, Solomon E (2006) Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility. Hum Mol Genet 15:599–606

36. Narod S (2002) What options for treatment of hereditary breast cancer? Lancet 359:1451–1452.

37. Narod SA, Boyd J (2002)Current understanding of the epidemiology and clinical implications of BRCA1 and

BRCA2 mutations for ovarian cancer. Curr Opin Obstet Gynecol 14:19–26

38. Nelen M.R., Padberg G.W., Peeters E.A.,. Lin A.Y, Helm B. van den, Frants R.R., Coulon V., Goldstein A.M.,. Reen van M.M, Easton D.F., Eeles R.A., Hodgsen S., Mulvihill J.J., Murday V.A, Tucker M.A., Mariman E.C., Starink T.M., Ponder B.A., Ropers H.H., Kremer H., Longy M., C. Eng, 1996 Localization of the gene for Cowden disease to chromosome 10q22-23. Nat. Genet. 13, 114–116.

39. Peelen T., Vliet van M., Petrij-Bosch A., Mieremet R., Szabo C., Ouweland van den A.M., Hogervorst F., Brohet R., Ligtenberg M.J., Teugels E., Luijt van der R., Hout van der A.H.,. Gille J. J, Pals G., Jedema I., Olmer R., Leeuwen van I., Newman B., Plandsoen M., der Est M van., Brink G., Hageman S., Arts P.J.,. Bakker M.M, Devilee P. et al., (1997)A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. Am. J. Hum. Genet. 60, 1041–1049

40. Petrij-Bosch A., Peelen T., Vliet M. van, , Olmer R. van Eijk, Drusedau M., Hogervorst F.B., Hageman S., Arts P.J., Ligtenberg M.J., Meijers-Heijboer H., Klijn J.G., Vasen H.F., Cornelisse C.J., Veer van't L.J., Bakker E., Ommen van G.J., Devilee P. (1997)BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. Nat. Genet. 17, 341–345

41. Radice P, De Summa S, Caleca L, Tommasi S (2011) Unclassified variants in BRCA genes: guidelines for interpretation. Ann Oncol 22(Suppl 1):i18–i23.

42. Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Kwan E, Jack E, Vesprini DJ, Kuperstein G, Abrahamson JL, Fan I, Wong B, Narod SA (2001)

Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 68:700–710.

43. Russo A, Calò V, Agnese V, Bruno L, Corsale S, Augello C, Gargano G, Barbera F, Cascio S, Intrivici C, Rinaldi G, Gulotta G, Macaluso M, Surmacz E, Giordano A, Gebbia N, Bazan V. (2006).BRCA1 genetic testing in 106 breast and ovarian cancer families from Southern Italy (Sicily): a mutation analyses. Breast Cancer Res Treat. 2007 Nov;105(3):267-76

44. Sigal A., Rotter V., Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Res. 60, 6788–6793 (2000).

45. Simard J., Tonin P., Durocher F., Morgan K., Rommens J., Gingras S., Samson C., Leblanc J.F., Belanger C., Dion F.et al., Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. Nat. Genet. 8, 392–398 (1994)

46. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA (1997) The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 336: 1401–1408.

47. Thompson D, Easton DF, Goldgar DE (2003) A full-likelihood method for the evaluation of causality of sequence variants from family data. Am J Hum Genet 73:652–655

48. Thorlacius S., Olafsdottir G., Tryggvadottir L., Neuhausen S., Jonasson J. G.,. Tavtigian S.V, Tulinius H., Ogmundsdottir H.M.,. Eyfjord J. E. (1996) A single BRCA2 mutation in male and female breast cancer

families from Iceland with varied cancer phenotypes. Nat. Genet. 13, 117–119

49. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, Wickens M, Carmichael J (2010) Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 376:235–244

50. Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP (2002) BRCA2 function in DNA binding and recombination from a BRCA2-DSS1- ssDNA structure. Science 297:1837–1848

51. Walsh T, Casadei S, Coats KH, Swisher E, Stray SM, Higgins J, Roach KC, Mandell J, Lee MK, Ciernikova S, Foretova L, Soucek P, King MC (2006) Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA 295:1379–1388.

52. Welcsh P.L., King M.C., (2001) BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. Hum. Mol. Genet. 10, 705–713

53. Wu K, Hinson SR, Ohashi A, Farrugia D, Wendt P, Tavtigian SV, Deffenbaugh A, Goldgar D, Couch FJ. Functional evaluation and cancer risk assessment of BRCA2 unclassified variants. Cancer Res 65:417–426 2006

Abstract

Background: *BRCA1* and *BRCA2* are considered the two major breast cancer (BC) susceptibility genes. Most related mutations to the pathogenesis of breast cancer are frameshift and nonsense mutation, causing the production of a truncated and non functional protein. About 8% of all *BRCA1* and *BRCA2* alterations reported to the BIC database are intronic variants probably involved in splice sites and a subset of these variant are located in intronic sequences. These alterations may be defined as variant of uncertain/unknown significance (VUS), pathological and polymorphism.

Aim: The molecular screening of *BRCA* genes could allow to earlier individuate subjects with different BC risk and to develop target programs of clinical surveillance. In particular we focused our attention on the study of clinical significance of VUS and polymorphisms of *BRCA* genes.

Methods: Seventy-four patients with breast and/or ovarian cancer were screened for germline mutations in *BRCA1* and in *BRCA2* at the " Regional Reference Center for the Characterization and Genetic Screening of Hereditary Tumors" at the University of Palermo. We performed a molecular analysis of the complete coding sequence and the exon-intron boundaries of *BRCA* genes, in our cohort of patients and in a control population consisting of index cases without a family history of cancer, using automatic direct sequencing.

Results and Conclusions: During *BRCA1/2* molecular screening of this group of patients, we identified different intronic variants both in *BRCA1* and *BRCA2* gene. The intronic variant IVS7–34 C>T was classified as a polymorphism in BIC; two (IVS 14+6G/A, IVS11–19delAT) as probably deleterious because of their involvement in splicing process and the remaining four variants were classified as VUS in BIC database. The *BRCA2* IVS11+80del4 variant was found in a patient in association with a pathological mutation. This study may contribute to better understand clinical significance of intronic variants in the *BRCA1/2* genes.

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- Bruno, L; Calò, V; Schirò, V; La Paglia, L; Agnese, V; Calcara, D; **Cimino, S**; Fanale, D; D'Andrea, A; Corsini, LR; Amodeo, V; Rizzo, S; Terrasi, M; Bronte, G; Bruno, D; Piazza, D; Symonds, ES; Federico, M; Fiorentino, FP; Grassi, N; Pantuso, G; Frazzetta, M; Bazan, V; Russo, A BRCA1 and BRCA2 variants of uncertain clinical significance and their implications for genetic counseling. oncology, 77(Suppl. 1), 136.

- Calo, V; Bruno, L; La Paglia, L; Schirò, V; Agnese, V; Calcara, D; **Cimino, S;** Fanale, D; D'andrea, A; Corsini, LR; Amodeo, V; Rizzo, S; Terrasi, M; Bronte, G; Bruno, D; Piazza, D; Fiorentino, FP; Grassi, N; Pantuso, G; Frazzetta, M; Symonds, CE; Federico, M; Bazan, V; Russo, (2009) A BRCA1 and BRCA2 germline mutations in sicilian breast and/or ovarian cancer families and their association with familial profiles. Oncology, 77(Suppl. 1), 136-137.

- Amodeo, V; Terrasi, M; D'Andrea, A; Insalaco, L; Fanale, D; La Paglia, L; Corsini, L R; Perez, M; Federico, M; Bronte, G; Rizzo, S; **Cimino, S**; Bruno, L; Calò, V; Agnese, V; Messina, M; Symonds, C E; Fiorentino, F P; Grassi, N; Pantuso, G; Frazzetta, M; Bazan, V; Russo, A (2009). EGF Induces STAT3-Dependent VEGF Expression in HT-29 colon cancer cells. Oncology, 77(Suppl. 1), 132.

- Fanale, d; Corsini, LR; D'Andrea, A; Terrasi, M; La Paglia, L; Amodeo, V; Bronte, G; Rizzo, S; Insalaco, L;

Perez, M; **Cimino, S;** Bruno, L; Calò, V; Agnese, V; Symonds, CE; Federico, M; Grassi, N; Pantuso, G; Frazzetta, M; Bazan, V; Calvo, EL; Iovanna, JL; Russo, A (2009). Analysis of germline gene copy number variants of patients with sporadic pancreatic adenocarcinoma reveals specific variations. Oncology, 77(Suppl. 1), 143-143.

- Corsini, LR; Fanale, D; D'andrea, A; La Paglia, L; Calcara, D; Amodeo, V; Terrasi, M; Insalaco, L; Perez, M; **Cimino, S**; Bruno, L; Calò, V; Agnese, V; Schirò, V; Bronte, G; Rizzo, S; Federico, M; Symonds, CE; Grassi, N;Pantuso, G; Frazzetta, M; Bazan, V; Russo, A Downregulated expression of Cdc25A gene in MCF-7 breast cancer cell. Oncology, 77(Suppl. 1), 141.

- Perez, M; Marganese, N; Calò, V; Bruno, L; La Paglia, L; **Cimino, S**; Corsini, LR; Terrasi, M; Fanale, D; Amodeo, V; Insalaco, L; Napoli, L; Di Gaudio, F; Di Piazza, F; Miraglia, MC; Bazan, V; Russo, A (2010). VUS variants in BRCA genes of hereditary breast/ovarian cancer. Paper presented at XII NATIONAL GOIM.