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Hereditary and familial breast and ovarian cancer:
spectrum of related tumors

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LIST OF PUBLICATIONS

This dissertation is based upon the following publications:

F Marroni, G Cipollini, B Peissel, E D'Andrea, **M Pensabene**, P Radice, MA Caligo, S Presciuttini, G Bevilacqua. Reconstructing the genealogy of BRCA1 founder mutation by phylogenetic analysis. Annals of Human Genetics (in press)

A Contegiacomo, **M Pensabene**, C Condello, I Capuano, I Spagnoletti, E De Maio. Tumori Eredo-familiari. In: AR Bianco. Manuale di Oncologia Clinica. 4th ed. Italy: McGraw-Hill. 2007. p. 25-45

C Condello, R Gesuita, **M Pensabene**, I Spagnoletti, I Capuano, C Baldi, F Carle, A Contegiacomo. Distress and family functioning in oncogenetic counseling for hereditary and familial breast and/or ovarian cancers. Journal of Genetic Counseling 2007;16:625-634

A Contegiacomo, **M Pensabene**, I Capuano, L Tauchmanova, M Federico, D Turchetti, L Cortesi, P Marchetti, E Ricevuto, G Cianci, V Barbieri, S Venuta, V Silingardi on behalf of the Italian Network on Hereditary Breast Cancer. Comment on 'Cancer Genetic Counseling" by P. Mandich et al. (Ann Oncol 2005; 16: 171). Annals of Oncology 2005;16(7):1208-1209

A Contegiacomo, **M Pensabene**, I Capuano, L Tauchmanova, M Federico, D Turchetti, L Cortesi, P Marchetti, E Ricevuto, G Cianci, S Venuta, V Barbieri, V Silingardi on behalf of the Italian Network on Hereditary Breast Cancer. An Oncologist-based Model of Cancer Genetic Counseling for Hereditary Breast and Ovarian Cancer. Annals of Oncology 2004;15(5):726-732

F Podo, F Sardanelli, R Canese, G D'Agnolo, PG Natali, M Crecco, ML Grandinetti, R Musumeci, G Trecate, S Bergonzi, T De Simone, C Costa, B Pasini, S Manuokian, GB Spatti, D Vrgnaghi, S Morassut, M Noiocchi, R Dolcetti, A Viel, C De Giacomi, A Veronesi, F Coran, V Silingardi, D Turchetti, L Cortesi, M De Santis, M Federico, R Romagnoli, S Ferrari, G Bevilacqua, C Bartolozzi, MA Caligo, A Cilotti, C Marini, S Cirillo, Marra V, Martincich L, Contegiacomo A, **Pensabene M**, I Capuano, GB Burgazzi, A Petrillo, L Bonomo, A Carriero, R Mariani-Costantini, P Battista, A Cama, G Palca, C Di Maggio, E D'Andrea, M Mazzocchi, GE Francescutti, C Zuiani, V Lordero, I Zunnui, C Gustavano, MG Centurioni, A Bozzelli, P Panizza, A Del Maschio. The Italian multi-centre project on evaluation of MRI and other imaging modalities in early detection of breast cancer in subjects at high genetic risk. J Exp Clin Cancer Res 2002;21(3 Suppl):115-124

G Aceto, P Di Fulvio, L Stuppia, S Veschi, A Contegiacomo, **M Pensabene**, A Cama, MC Curia, G Palka, R Mariani-Costantini, P Battista. Gene Symbol: BRCA1. Disease: Breast/Ovarian Cancer. Hum Genet (on-line) 2002;110:294-295

All the references to the above paper are referred in the body text. The articles are attached at the end of the dissertation.

ABSTRACT

This dissertation is focused on the evaluation of cancer spectrum related to hereditary and familial breast cancer. In BRCA1 mutation carriers, mean cumulative risk at age 70 years is 57% (95% CI, 47% to 66%) for breast cancer and 40% (95% CI, 35% to 46%) for ovarian cancer. Moreover, in BRCA2 mutation carriers mean cumulative risk at age 70 years is 49% (95% CI, 40% to 57%) for breast cancer and 18% (95% CI, 13% to 23%) for ovarian cancer. Various studies reported contradictory data concerning risk of cancer at sites different than breast and ovary in both of carriers of mutations in BRCA1 and BRCA2 genes.

We selected families referred to "Screening and follow-up for hereditary and familial cancers" Unit of University "Federico II" in Naples for oncogenetic counseling. Families were analyzed in order to evaluate the cancer spectrum related with inherited and familial breast and ovarian cancer. We examined 104 pedigrees for a total of 4100 individuals (2117 females, 1983 males), all of Caucasian ethnicity. Based on family history of breast cancer and/or ovarian cancer and on clinical characteristics at diagnosis, pedigrees were classified according to Modena model in: hereditary with clustering (41 families; 39%), hereditary without clustering (27 families; 26%) and familial (36 families; 35%).

A total of 587 independent events of cancer have been detected in the 104 families on study. In particular among the three major categories in which individuals have been grouped, 294 cases (17.6%) of tumors were registered in the category of hereditary with clustering constituted of a cohort of 1670 individuals, 103 cases (9.8%) of tumors in the category of hereditary without clustering constituted of a cohort of 1053 individuals and 190 cases (13.8%) of tumors in the familial category constituted of a cohort of 1377 individuals.

In the hereditary with clustering group a high frequency emerges for cancer of ovary (2%), uterus (1,4%), prostate (1,4%) and lung (0,9%). A moderate frequency emerges for colon-rectum (0,8%) and stomach (0,7%) cancers. In the hereditary without clustering group a similar association has not been revealed except for colon-rectum cancer ((0,8%). In the familial group a high frequency has been registered for cancers of ovary (1,3%), uterus (2%), and colon-rectum (1,3%). A moderate frequency has been registered for prostate cancer (0,9%).

We also determined frequency of tumors in families with mutations of BRCA1/2 genes. In the 10 families with BRCA1 mutations, 76 events of cancers have been detected in a total of 486 individuals. It emerges a clustering with ovarian (4.9%), uterine (1.2%) and bladder cancer (0.8%). In the 6 families with BRCA2 genotype, 33 events of cancers have been registered in a total of 185 individuals. It emerges a clustering with ovarian (2.8%), uterine (2,8%), colon-rectum (1%) and prostate cancers (2,6%).

At least, the statistical analysis have not revealed a typical cancer spectrum, because differences of statistical value have not been gained for any specific site other than breast in our series among risk categories and sex.

1 BACKGROUND

1.1 Breast Cancer

Breast cancer is the most commonly diagnosed cancer among women after non-melanoma skin cancer and is the second leading cause of cancer death after lung cancer. From 2002-2004 the age-adjusted rate of invasive breast cancer was approximately 127.8 per 100,000 women per year and the age-adjusted death rate was 25.5 per 100,000 women per year in all races. The median age at diagnosis for cancer of the breast was 61 years of age. Data from the Surveillance, Epidemiology and End Results (SEER) program report also that white women in United States have a 12.28% lifetime risk of developing breast cancer, whereas African American women have a 9.6% lifetime incidence (SEER – http://seer.cancer.gov/statfacts/html/breast.html).

In Italy, it has been estimated that every year overall 36,634 new female breast cancers are diagnosed. The cumulative risk (0-74 years) of developing breast cancer is estimated to be 9.02% in Italian women, that is 1 case every 11 women (Italian Network of Cancer Registries; Istituto Superiore di Sanità).

Male breast cancer is a rare event, representing 1% of all breast cancer. In Italy and in Western populations, the incidence of male breast cancer is about 1 case every 100,000 individuals, with a diagnosis in men aged 58-63 years.

Breast cancer represents a very interesting tool for clinical, molecular and translational research.

Multiple factors are associated with an increased risk of developing breast cancer, including age, family history, exposure to reproductive hormones, dietary factors, benign breast diseases and environmental factors.

In the last years increasing interest is devoting to the interaction between environmental and genetic factors. Family history has been recognized to be an important risk factor for developing breast cancer. Individuals with a first-degree family member affected with breast cancer have a relative risk of 2.1 (95% CI= 2.0-2.2) (Pharoah et al. 1997). Moreover, risk varies with the age at which the affected relative was diagnosed, the number of affected and unaffected family members and, finally, the closeness of the relationship (Coldiz et al. 1993; Johnson et at. 1995).

1.2 Hereditary breast cancer

In the mid-1990s, developments in the molecular genetics of cancer led to the identification of predisposing hereditary breast and/or ovarian cancer genes. Studies of linkage analysis showed the existence of autosomal dominant predisposition to breast cancer and led to the identification of several highly penetrant genes as the cause of inherited cancer in many breast cancer-prone families.

Overall, 5–10% of primary breast cancers are inherited and 15-20% are familial (Antoniou et al. 2003; Pharoah et al. 2002). Hereditary and familial forms are identified by the individual and family history. In familial forms, members of some families are prone to developing breast cancer in the absence of identifiable carcinogenic exposure. Affected individuals in these families may represent clustering of sporadic occurrences, multifactorial inheritance, the presence of low penetrance genes or common habits and similar life-style. Close relatives are at moderately increased risk of developing that type of malignancies. However, the average age of onset is usual similar to that observed in the general population.

The family features that suggest hereditary breast cancer predisposition include the following: a) multiple cases of breast and ovarian cancer in different generations have been present in a family, suggesting an autosomal dominant transmission (vertical transmission) according to the Lynch criteria; b) an early onset age at diagnosis of breast cancer; c) two or more primary cancers in the same individual. These could be multiple primary cancers of the same type (e.g., bilateral breast cancer) or primary cancer of different types (e.g., breast and ovarian cancer in the same individual); d) male breast cancer. The presence of both breast and ovarian cancer in a family increases the likelihood that a cancer-predisposing mutation is present.

About 84% of hereditary breast cancers derive from BRCA1 and BRCA2 mutations that sustain the hereditary breast/ovarian cancer (HBOC) syndrome with an autosomal dominant pattern of transmission, an incomplete penetrance and a variable expressivity (Antoniou et al. 2003).

Other known susceptibility genes such as ATM, PTEN, p53 and STK11 are involved in hereditary breast cancer syndromes with a well defined cancer spectrum. Unknown low penetrance genes also seem to be involved in other less frequent hereditary breast cancers (Antoniou et al. 2003).

Mutations in each of these genes produce different clinical phenotypes of specific cancers and, in some instance, other non-malignant abnormalities leading to different hereditary syndromes known to involve breast as site of tumors in their cancer spectrum, such as Li-Fraumeni syndrome, Cowden's syndrome, Ataxia Teleangectasia (AT) and Peutz-Jeghers syndrome. *Table 1* shows the most frequent hereditary syndromes with breast cancer as the main site of cancer of the spectrum. All genes known to be associated with a hereditary predisposition to breast cancer are tumors suppressor gene (Robson and Offit 2007).

1.3 BRCA1 and BRCA2 genes

In 1990, Hall et al. first mapped BRCA1 to long arm of chromosome 17 (17q21) through linkage studies of hereditary breast cancer (Hall et al.1990). The gene was subsequently cloned and found to be novel (Miki et al. 1994; Stolnick et al.1994). It consists of 22 exons coding a protein of 1863

aminoacids. BRCA1 is a nuclear protein of about 220 kDa, containing a zinc- and a DNA-binding "ring finger" motif at its N-terminal domain, and a BCRT (BRCA carboxy terminal) at its C-teminal domain, as a sequence of nuclear localization as recognizable motifs (Hall 1990; Narod 1991; Miki 1994). In *figure 1*, the structure of BRCA1 gene and sites of its interaction with other proteins have been shown.

A separate locus named BRCA2, mapped on the long arm of chromosome 13 (13q12-13), was associated with hereditary breast and ovarian cancer (*figure 2*). This gene does not share structural homology with BRCA1 gene, but encodes for a protein of 3418 aminoacids with biochemical functions similar to BRCA1.

Table 1. The most frequent syndromes and genes known to be associated with a hereditary predisposition to breast cancer

Gene	Chromosomal location	Transmission	Syndrome	Breast cancer risk (%)*	Cancer spectrum other than breast cancer
High penetrance					
BRCA1	17q21	AD	HBOC	39-87	Ovary, prostate, colon, pancreas cancers
BRCA2	13q12-13	AD	HBOC	26-91	Ovary, prostate, pancreas, ductal-gall cancers, melanoma
p53	17p13	AD	Li-Fraumeni	56-90	Soft-tissue sarcoma, osteosarcoma, leukemia, brain, adrenocortical and colon cancers
PTEN	10q23	AD	Cowden	25-50	Thyroid, endometrium, genito-urinary cancers
STK-11	19	AD	Peutz-Jeghers	45-54	colorectal, small bowel, uterine, testicular, ovarian sex cord, pancreatic cancers
Low to moderate penetrance					
ATM	11q22-23	AR	Ataxia-Teleangectasia	NA	Leukemia, lymphoma
CHEK2	22q11	AD	Li-Fraumeni variant	24	Prostate, colon cancers
BRIP1	17q22	?	Fanconi's anemia	NA	Undefined in heterozygotes
PALB2	16p22	?	None known	NA	Undefined in heterozygotes

*by age of 70 years. Abbreviations: AD= autosomal dominant; AR= autosomal recessive; HBOC= hereditary breast and ovarian cancer; NA= not available
Modified by Robson and Offit 2007
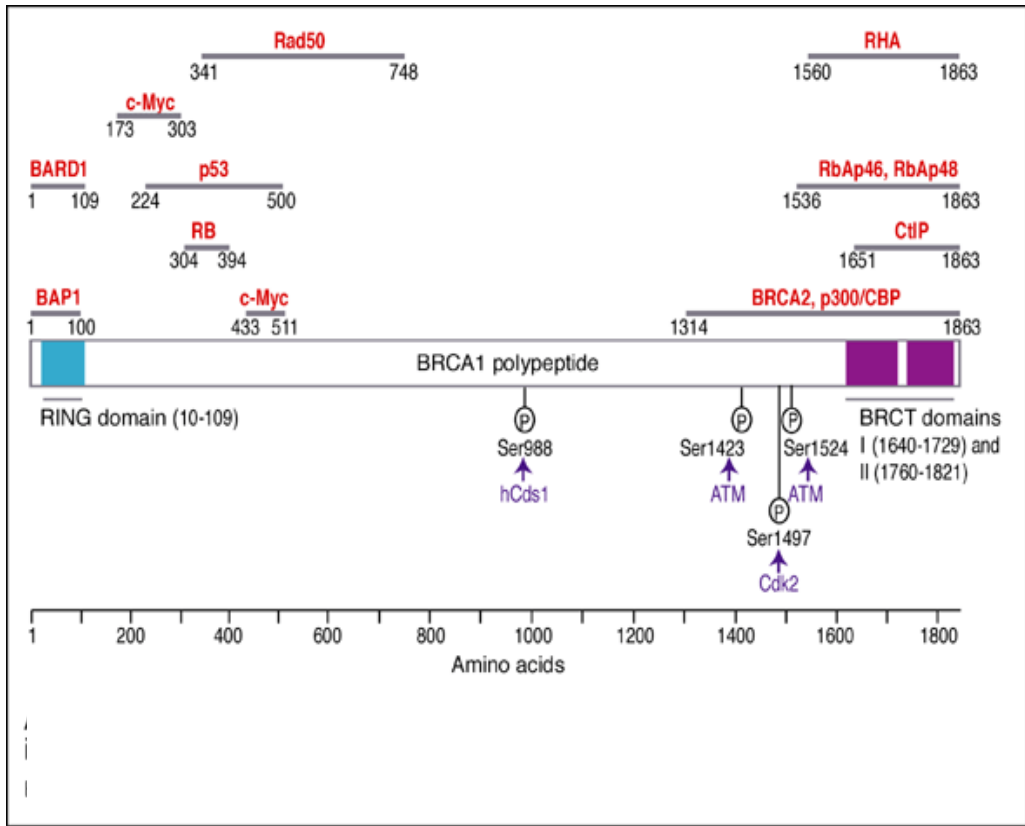
Figure 1. A schematic diagram of the BRCA1 polypeptide and sites of its interaction with other proteins. Published in Expert Reviews in Molecular Medicine by Cambridge University Press (2001)
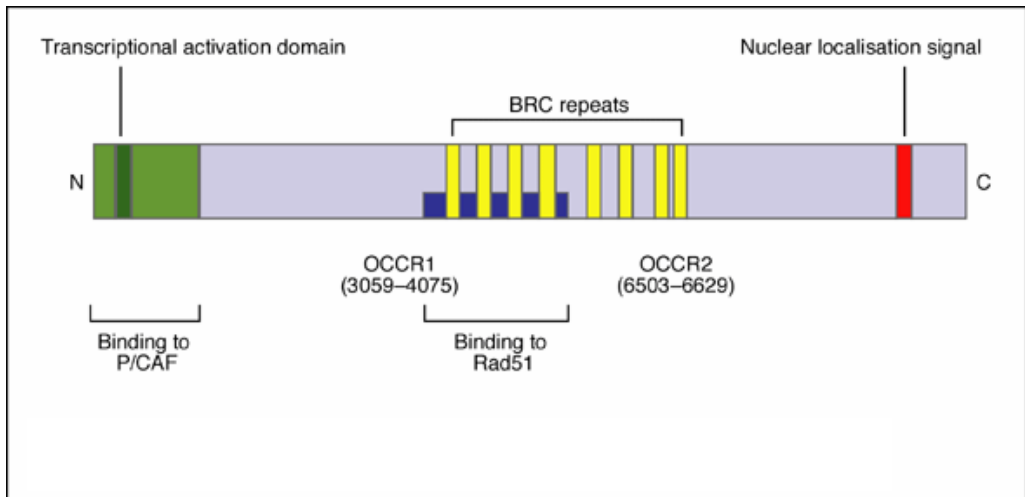
Figure 2. Functional domains of the BRCA2 protein. Published in Expert Reviews in Molecular Medicine by Cambridge University Press (2001)

1.4 Normal function of BRCA1 and BRCA2 genes

BRCA1 and BRCA2 genes belong to a class of genes known as tumor suppressor genes. Like many other tumor suppressor genes, they prevent cells from growing and dividing too rapidly or in an uncontrolled way. Both of BRCA1 and BRCA2 appear to serve as an important regulator of cell-cycle "checkpoint control" mechanisms involving cell-cycle arrest, cell death (apoptosis) and DNA repair (*figure 3*).

Figure 3. The role of BRCA1 and BRCA2 protein in DNA repair. Published in Expert Reviews in Molecular Medicine by Cambridge University Press (2001)

The role of both BRCA proteins are now emerging as central gatekeepers of genomic stability.

Protein encoded by BRCA1 gene seems to have a fundamental role in the cell cycle control. Levels of BRCA1 increase during DNA synthesis and mitosis.

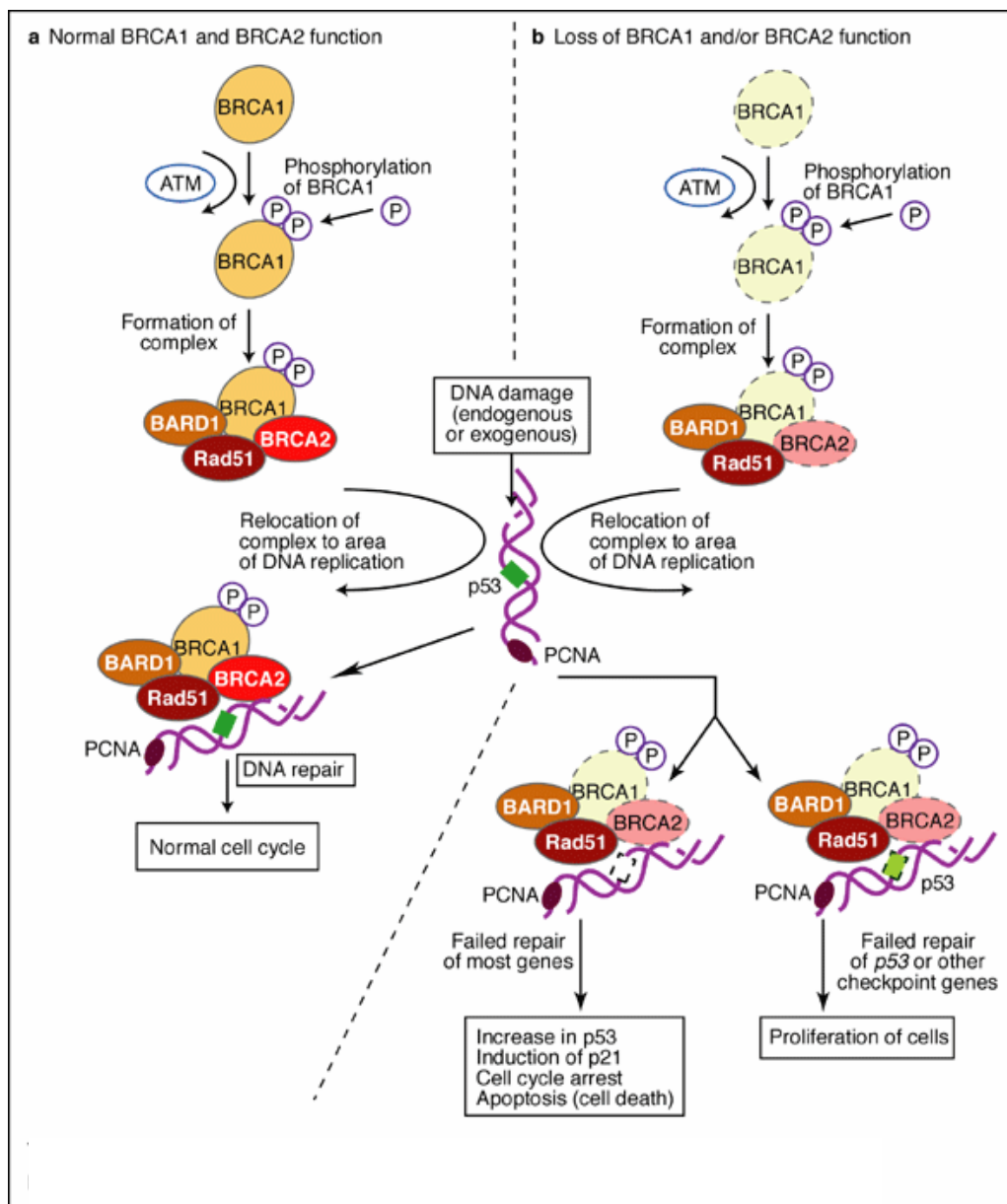BRCA1 gene is expressed in numerous organs, including breast, ovary and thymus and testis. BRCA1 expression in mice suggests a role of such gene in the differentiation of epithelial tissues according with the role of estrogen hormones able to stimulate the activity of the promoter of BRCA1. The normal function of BRCA1 protein appears to function to suppress the signaling of mammary epithelial cells by estrogen receptor. BRCA protein can play a pivotal role in control the sex steroid-regulated pathways inducing breast cancer development.

The BRCA1 protein is directly involved in the repair of damaged DNA. In the nucleus of many types of normal cells, the BRCA1 protein interacts with several other proteins, including a protein called RAD51, to mend breaks in DNA. These breaks can be caused by natural and medical radiation or other environmental exposures, but also occur when chromosomes exchange genetic material in preparation for cell division. By repairing DNA, BRCA1 and other proteins that interact with it play a role in maintaining the stability of a cell's genetic information.

Research suggests that the BRCA1 protein also regulates the activity of other genes and plays a critical role in embryonic development. Embryos of mouse, knock-out for BRCA1, show several abnormalities of nervous system and die after the seventh day of life. The BRCA1 protein probably interacts with many other proteins, including other tumor suppressors and proteins that regulate cell division.

Like BRCA1, BRCA2 is expressed in numerous tissues. It is involved in the same biochemical processes of BRCA1 such as cell-cycle control, transcription of genes and stability of genomic DNA.

1.5 Mutations of BRCA1 and BRCA2 genes

To date, in each of the BRCA genes approximately 3,400 sequence variants, listed online at the Breast Cancer Information Core - BIC - database (http://research.nhgri.nih.gov/bic), have been identified by extensive mutational analysis. Most of the BRCA1 and BRCA2 mutations are frameshift or nonsense that give rise to truncated non functioning proteins whereas other mutations are missense substitutions or intronic variants, including those involved in the splicing process.

Genes responsible for hereditary breast cancer conformed to the two-hit Knudson's hypothesis, which states that a point mutation might be inherited in one allele of a candidate gene at a putative susceptibility locus and that loss of

heterozygosity (LOH) or another genetic alteration might occur in the other allele of that locus later in life, leading to cancer (Knudson and Strong 1972).

The most of BRCA1 and BRCA2 mutations are nonsense or frameshift, leading to a clearly deleterious mutations with impacts on protein functions. Many of these mutations change one of the protein building blocks (amino acids) used to make the BRCA1 and BRCA2 proteins, resulting in a protein truncation and/or loss of important functional domains (e.g., protein cannot perform its normal DNA repair function). In some cases, large segments of DNA are missing from the gene. Other mutations cause the production of an abnormally short protein that does not function properly. Researchers believe that the defective BRCA protein is unable to help repair damaged DNA or fix mutations that occur in other genes. These defects accumulate and may allow cells to grow and divide uncontrollably and form a tumor.

A large number of genetic alterations are still classified as variants of unknown significance (UVs), such as intronic or missense alterations. Many efforts are being made to find the polymorphic or pathogenetic role of such mutations. The classification of a sequence alterations as a variants of unknown significance is a moving target. Some intronic variants have to be evaluated in order to understand their pathogenetic or polymorphic effects on the mRNA splicing process. Classifying these variants of unknown clinical significance as neutral or disease-causing is very important for genetic counseling and for the implications in terms of cancer risk.

Some specific mutations have been observed in defined ethnic group, because a likely founder effect. The most common in the United States are the three mutations in the Ashkenazi Jewish: two in BRCA1 (185delAG and 5382insC) and one (6174delT) in BRCA2 (Garber and Offit 2005). Founder mutations in numerous other populations, including those from Iceland (Thorlacius et al. 1998), Poland (Gorski et al. 2004) and in Dutch kindreds (Petrij-Bosch et al. 1997), have been identified, too.

Founder mutations have been described in geographically restricted areas of Italy; a regional founder effect has been demonstrated in Italian population for the mutations BRCA1 5083del19 and BRCA2 8765delAG, BRCA2 6696delTC. (Cipollini et al. 2004; Ottini et al. 2003). Recently, the BRCA1*1499insA mutation has been characterized as a new founder mutation by aplotype analysis and, applying a phylogenetic method, investigators have shown its origin in individuals living in Tuscany about 30 generations ago. (Marroni et al. in press).

1.6 BRCA1 and BRCA2-associated breast and ovarian cancers

BRCA1-associated breast cancers are usually high-grade, poorly differentiated and infiltrating ductal carcinomas. Atypical medullary carcinomas, a phenotype characterized by abundant lymphocytic infiltrate and a smooth margin, have also been observed more frequently. They frequently show a basal-like phenotype, characterized by estrogen receptor (ER),

progesteron receptor (PgR) and HER2/neu negativity and the expression of basal cytokeratins such as 5, 6 and 14. They also overexpress cyclin E and p53, and underexpress p27 (Lakhani et al. 1998, Narod et al. 2004).

BRCA2-associated breast cancers do not have a distinct phenotype or behavior compared with sporadic breast cancers (Lakhani et al. 1998, Narod et al. 2004; Garber and Offit 2005).

BRCA1 ovarian cancer usually are serous and papillary, less frequently endometrioid or clear cell are met. Borderline tumors of the ovary have been associated with hereditary breast and/or ovarian cancer syndrome, too.

Results with respect to survival in BRCA1-associated breast cancer are inconsistent, with some studies reporting a worse, others an identical survival as compared to age-matched patient with sporadic breast cancer. Brekelmans et al. demonstrated no significant differences between BRCA1-associated and sporadic breast cancer respect to ipsilateral breast cancer recurrence, disease-free survival (DFS) and BC-specific survival, while a trend towards a worse survival was found for ductal BRCA1-associated tumors. The classic factors such as tumors size and nodal status are of prognostic value both for sporadic and BRCA1-associated breast cancer (Brekelmans et al. 2006). BRCA2-associated breast cancers have a similar prognosis respect to sporadic ones. Rennert et al. shows that rates of survival for women with breast cancers associated with either BRCA1 or BRCA2 mutation are similar to those in women without these mutations. Other data have supported similar outcomes for carriers and non carriers of mutations, at least when adjuvant chemotherapy is used (Rennert et al. 2007; Robson et al. 2004).

1.7 Cancer genetic counseling

Scientific developments in the field of the genetics of cancer have led to new scenarios in the setting of prevention, diagnosis and management of hereditary and familial cancers. Given the necessity to identify and manage adequately the genetic and familial risk in oncology, ad hoc clinical services have been implemented in many countries delivered to support the individuals in any decision-making process concerning their own risk trough cancer genetic counselling.

As public awareness of cancer susceptibility genes has grown markedly in recent years, the demand has also grown for genetic services to assess familial cancer risk and genetic testing. Almost all centres provided services not only to cancer patients and their families for genetic testing but also to individuals concerned with risk. The most of genetic services from Europe and USA provided medical evaluation, cancer risk assessment, genetic counseling and pedigree analysis other than genetic testing (Epplein et al. 2005).

Genetic counseling was defined by the American Society of Human Genetics as "a communication process which deals with human problems associated with the occurrence or risk of occurrence of a genetic disorder in a family" (American Society of Human Genetics 1975). Genetic counseling in

oncological setting (cancer genetic counseling) should also provide sufficient information to enable the user to make a fully informed choice of action, particularly as regards prevention, in case of a familial cancer risk or of the identification of a mutation in a family. It is aimed to risk assessment, to promote awareness, to genetic testing for susceptibility genes, to manage patients or their family members at high risk offering adequate preventive measures.

As leading organization representing cancer specialists involved in patient care and clinical research, the American Society of Clinical Oncology (ASCO) affirms its commitment to integrating cancer risk assessment and management, including molecular analysis of cancer predisposing genes, into the practice of oncology and preventive medicine (ASCO 1996). In particular, the ASCO endorses some principles, such as indications of genetic testing, special issues in testing children for cancer susceptibility, counseling about medical management after testing, regulation of genetic testing, protection for insurance and employment discrimination, coverage of services, confidentiality and communication of familial risk, educational opportunities in genetics, special issues relating to genetic research on human tissue. Another important aspect concerns that the oncologists, involved in the management of at risk subject within oncogenetic counseling process, include the discussion of possible risks and benefit of prevention modalities (ASCO 2003). Within prevention setting, the knowledge of typical cancer spectrum related to hereditary breast cancer syndromes is relevant in individualizing management on cancer risk profile.

Familial cancer clinics are continuing to develop across Europe with considerable similarity in the organization of the activities provided, including breast cancer risk assessment, mutation testing and management within counseling. In the most of European centers, genetic counseling is led by medical specialists with expertise in the cancer genetics. Nevertheless, formal training in the field of hereditary cancers and cancer genetics is established in UK and Netherlands but is not available in France, Germany and Italy. Similarities among centers include provision of a multidisciplinary team, with access to psychological support, albeit with varying degrees of integration. Surveillance and management protocols are generally based on recommendations relied largely on opinion of experts rather than the draft of international guidelines (Hopwood et al. 2003).

Nevertheless, in Europe as other countries around the world, the most of heath care services are far from reimbursement of genetic counseling services. In this field the ASCO considered the need for further standardization of International Classification of Diseases (ICD-9), clinical modification codes for reimbursement of genetic counseling to ensured coverage for testing, counseling, screening, surveillance, and preventive therapy for individuals at increased risk of hereditary cancer (ASCO 2003).

In Italy, since 1999 to 2005 the Ministry of Research supported a national project entitled the "Development of a National Network for the Study of Hereditary Breast Cancer", in which the Unit of Naples designed and promoted

a multistep model of oncogenetic counselling (Contegiacomo et al. 2004; Contegiacomo et al. 2005). This model is conducted in different steps to aim awareness in subjects identified at hereditary or familial risk. The model is designed to entail a global approach to the patient affected by cancer and to disease-free at-risk subjects by a multidisciplinary team involvement. This is achieved through the identification of at-risk subjects and the definition of the breast cancer forms sustained by known susceptibility genes. Moreover, the model favours the management of at-risk subjects through prevention measures. Moreover, it could foresee also a more adequate oncological management for subjects already affected with cancer in adjunction to their specific follow-up.

1.8 Molecular genetic testing for BRCA1 and BRCA2 genes

Genetic testing for the molecular analysis of BRCA1 and BRCA2 genes has been available to the public since 1996. Mutation screening methods vary in their sensitivity. Methods widely used in research laboratories, such as single-stranded conformational polymorphism (SSCP) analysis and conformation-sensitive gel electrophoresis (CSGE), miss nearly a third of the mutations that are detected by DNA sequencing. In addition, large genomic rearrangements are missed by most of the techniques, including direct DNA sequencing. Such rearrangements are believed to be responsible for 10% to 15% of BRCA1 inactivating mutations.

Table 2. ASCO guidelines for genetic testing of cancer susceptibility genes

Indications for genetic testing	▪ offered when the individual has personal or family history features suggestive of a genetic cancer susceptibility condition ▪ the test can be adequately interpreted ▪ the results will aid in diagnosis or influence the medical or surgical management of patient or family member at hereditary risk of cancer ▪ only in the setting of pre- and post-test counseling
Counseling pre- and post-test	The oncologist discusses risk and benefits of genetic testing and preventive measures in pre and post-test counseling
Regulation of genetic testing	Regulation of laboratories that provide genetic testing. Oversight of the reagents, interlaboratory comparison of reference sample, standardization of report
Protection from insurance and employment discrimination (USA)	Federal laws prohibit insurance and employment discriminations on the basis of genetic susceptibility to cancer
Coverage of services	All individuals at significant increased risk of hereditary cancer have access to appropriate genetic counseling, testing, screening, surveillance and all related medical and surgical interventions

The estimated sensitivity of genetic testing for BRCA1/2 is about 85.4% (95% CI, 78.7% to 90.5%). The undiscovered mutations proportion of about 15% includes any mutations on susceptibility genes other than BRCA1 and BRCA2 (Berry et al. 2002; Eng et al. 2001).

In 1996 the American Society of Clinical Oncology (ASCO) approved recommendations for cancer genetic counseling and genetic testing (ASCO 1996). In tables 2 and 3, ASCO guidelines for genetic testing of susceptibility genes and advantages and limits of genetic testing are summarized.

Genetic testing has to be offered when individual has personal or family history features suggestive of a genetic cancer susceptibility condition, the test can be opportunely interpreted and the results will aid in diagnosis or influence the medical or surgical management of patient or family members at hereditary risk of cancer. Moreover, genetic testing has to be done only in the setting of pre- and post-test counseling (ASCO 2003).

Table 3. Potential benefits and burdens of genetic testing

Benefits	- To decrease distress in the case of negative test - To avoid intensive surveillance or other preventive measures in the case of negative test - Opportunity to reduce cancer risk through chemoprevention and prophylactic surgery in case of positive genetic test - Opportunity to involve other family members in the case of positive test - Elimination of uncertainty about hereditary susceptibility to cancer in the case of a positive test
Burdens	- Perception of any risk of developing cancer in the case of negative test - Risks and costs of increased screening or prophylaxis - Difficulty to communicate own genetic test results to family member in the case of positive test - Guilt about transmission of genetic risk to siblings in the case of positive test - Social discrimination in the case of positive test - Psychological distress, including anxiety, depression, reduced self-esteem in the case of positive test

1.9 Models for prediction of breast cancer risk and the likehood of BRCA1 and BRCA2 mutations

Different predictive models are available to assess risk for developing breast cancer. Family history is the main determinant of risk, but some of these models incorporate personal factors, such as reproductive history. Two models for predicting breast cancer risk, such as the Claus model and the Gail model, are widely used in research studies and in clinical practice of counseling (Claus et al. 1991 and 1994; Gail et al. 1989). In addition, several other models

exist to predict an individual's likelihood of having a BRCA1 or BRCA2 mutation (Domchek et al. 2003).

In fact, probability models have been developed to estimate the likehood that an individual has a mutation in BRCA1 and/or BRCA2 gene. The BRCApro model and the Myriad mutation prevalence tables are the most widely used. In *table 4*, strengths and limits of the two models have been shown (Robson and Offit 2007).

Table 4. BRCApro Model and Myriad Mutation Prevalence Tables: strengths and limitations

	BRCApro Model	Myriad Mutation Prevalence Tables
Strengths	▪ Estimates probability of a mutation in both BRCA1 and BRCA2 genes ▪ Provides mutation probabilities for both affected and unaffected individuals ▪ Is frequently updated ▪ Considers Ashkenazi Jewish heritage	
	▪ Provides other breast cancer risk information, such as the Gail and Claus empiric risks of developing breast cancer during one's lifetime ▪ Provides a printout of pedigree and risk calculations	
Limitations	▪ Analysis based on large, high-penetrance families ▪ Considers only first- and second-degree relatives ▪ Requires CancerGene software* and data entry for each family ▪ Requires information on all unaffected family members ▪ Incomplete validation in nonwhite populations	▪ Family history data obtained from test requisition forms and thus possibly limited ▪ Biased ascertainment of data ▪ Empirical model with incomplete validation ▪ It does not include unaffected family members

*developed by the University of Texas Southwestern Medical Center at Dallas, USA
Modified by Robson and Offit, NEJM 2007

Frank et al. for Myriad Genetics laboratories developed a probabilistic method to calculate the cumulative BRCA1 and BRCA2 likehood that breast cancer will be sustained by genes alterations. This method allows the estimation of the a priori probability, expressed as percentage, of being carrier of mutations in BRCA1 and BRCA2 genes on the basis of age of onset of breast and ovarian cancer in proband and the positive family history for breast and/or ovarian cancers in first and second degree family members (Frank et al. 1998).

The worldwide utilized model is the BRCApro designed and validated by Parmigiani et al., 1998 and subsequently updated (Berry et al. 2002). It allows to assess the probability of mutation in BRCA1 and BRCA2 on the basis of the penetrance of mutations of BRCA1 and BRCA2 genes in the population

on exam, inserting information regarding first and second degree family members such as race, number of subjects in the family, age of all subjects, breast cancers, ovarian cancers and other cancers and age at diagnosis. The model has been implemented with CancerGene software (CaGene version 3.3, supplied by Assistant Professor D. Euhus, UT Southwestern Medical Center at Dallas, Texas, USA). CancerGene is an "utility" that allows to construct pedigree and gives risk assessment automatically.

The BRCApro software has been specifically implemented for penetrance estimates of BRCA1 and BRCA2 genes in the Italian population (Marroni et al. 2004).

Debate on what is the most suitable and efficacious predictive model is open regarding the ability of predicting mutations expressed as sensibility and specificity (Kang et al. 2006).

Table 5. Hereditary and familial breast cancer according with the Modena Study Group Model adopted within the Italian Network for "Hereditary Breast and Ovarian Cancer"

Risk categories		Selection criteria					
		I	II	III	Criteria other than familial clustering		
		At least 3 BC or OC in two different generations	I/II degree among the affected members (male interposed)	At least one BC < 40 yrs or bilateral	BC<35 yrs	BC and OC	Male BC
Hereditary							
with clustering	HBC HBOC	X	X	X			
	SHBC SHBOC	X X	X	X			
without clustering	EOBC				X		
	BOC					X	
	MBC						X
Familial							
	FBC FBOC	X					
	SFBC SFBOC		X	X			
	SFBC+ SFBOC+		X	X			

Abbreviations: BC= breast cancer; OC= ovarian cancer; HBC= hereditary breast cancer; HBOC= hereditary breast and ovarian cancer; SHBC= suspected hereditary breast cancer; SHBOC= suspected hereditary breast and ovarian cancer; EOBC= early-onset breast cancer; BOC= breast and ovarian cancer in the same subject; MBC= male breast cancer; FBC= familial breast cancer; FBOC= familial breast and ovarian cancer; SFBC= suspected familial breast cancer; SFBOC= suspected familial breast and ovarian cancer; SFBC+= strongly suspected familial breast cancer; SFBOC+= strongly suspected familial breast and ovarian cancer
Modified by Cortesi L. et al. 2006

In Italy, the model of the Modena Study Group has been adopted within the Italian Network for "Hereditary breast and/or ovarian cancer". It is an epidemiologic model elaborated on the basis of the Lynch criteria. Hereditary and familial risks are clinically defined according to the Modena criteria (*table 5*), including familial clustering for breast and/or ovarian cancers, 1st and 2nd degree affected family members, age of onset of breast cancer less than 40 years and bilateral breast cancers (Federico et al. 1999). Breast cancer before the age of 35, male breast cancer and synchronous breast and ovarian cancers are all definitions of a hereditary risk without familial clustering (Cortesi et al. 2006).

1.10 Cancer related to mutations in BRCA1 and BRCA2 genes

The absolute risk of cancer by the age of 70 years conferred by a BRCA1 mutation is reported to be between 45% and 87% for breast cancer and between 36% and 66% for ovarian cancer (Ford et al. 1994; Struewing et al. 1997; Antoniou et al. 2000; Satagopan et al. 2001).

BRCA2 mutation carriers are known to be at high cumulative lifetime risk by age 70 for breast and ovarian cancer, reported to be 45% and 11%, respectively (Ford et al. 1998; Antoniou et al. 2000).

Recently, Chen and Parmigiani reported a meta-analysis of BRCA1 and BRCA2 penetrance. Mean cumulative risk at age 70 years were 57% (95% CI, 47% to 66%) for breast cancer and 40% (95% CI, 35% to 46%) for ovarian cancer in BRCA1 mutation carriers. Moreover, mean cumulative risk at age 70 years were 49% (95% CI, 40% to 57%) for breast cancer and 18% (95% CI, 13% to 23%) for ovarian cancer in BRCA2 mutation carriers (Chen and Parmigiani 2007). Mutations in BRCA1 and BRCA2 particularly increase the risk of early onset breast carcinoma. Whereas a woman's likehood of developing breast cancer before age 50 is normally only 2%, the risk is 33-50% for a woman with a mutation in one of the two genes. (Struewing et at. 1997; Easton et al. 1995). In women with breast cancer, mutations in BRCA1 have been associated with a 64% cumulative risk of controlateral breast cancer by age 70 (Ford et al. 1994).

The possibility of variation in cancer risk among the various studies involving families ascertained for breast cancer clustering suggest allelic heterogeneity. Moreover, the possibility of variation in risk within families and over the years, suggest the presence of modifying factors with a genetic and an epigenetic nature (Easton et al. 1995). Non genetic factors, such as menstrual and reproductive histories, contraceptive and hormone use, exercise and body weight, environmental and occupational exposure might explain some portion of the variation in breast cancer incidence, significantly influencing the penetrance even of high-penetrance mutations (King et al. 2003).

Various studies reported contradictory data concerning risk of cancer at sites different than breast and ovary in both of carriers of mutations in BRCA1 and BRCA2 genes.

First, the Cancer Genetics Studies Consortium reported an increased life-time cumulative risk for ovarian cancer (44%), colon-rectal cancer (6%) and prostate cancer (8%) in BRCA1 mutation carriers (Burke et al. 1997).

In a second study conducted in families ascertained for BRCA1 mutations, the Breast Cancer Linkage Consortium reported an increased relative risk for several cancers, including pancreatic cancer (RR = 2.26; 95% CI = 1.26–4.06), cancer of the uterine body (RR=2.65, 95% IC 1.69-4.16) and cervix (RR=3.72, 95% IC 2.26-6.10) and prostate cancer under 65 years of age (RR=1.82; 95% IC 1.01-3.29) (The Breast Cancer Linkage Consortium 2002).

The Breast Cancer Linkage Consortium observed also an increased risks for several other cancers in BRCA2 mutation carrier. In particular, statistically significant increases in risks were observed for prostate cancer (RR= 4.65; 95% CI= 3.48–6.22), pancreatic cancer (RR= 3.51; 95% CI= 1.87–6.58), gallbladder and bile duct cancer (RR= 4.97; 95% CI= 1.50–16.52), stomach cancer (RR =2.59; 95% CI=1.46–4.61) and malignant melanoma (RR= 2.58; 95% CI =1.28–5.17). The relative risk for prostate cancer for men below the age of 65 years was 7.33 (95% CI = 4.66–11.52). (The Breast Cancer Linkage Consortium 1999).

Bermejo and Hemminki confirmed the association of BRCA1 and BRCA2 mutations with ovarian, pancreatic, prostate and stomach cancers at a population level. In families with bilateral breast cancer or two breast cancers before age 50 years, there is concern about early onset pancreatic cancers. Prostate cancers are, also, in excess in these families but the risk is only moderate. Most cases of ovarian cancer in families with male breast cancer, and in families with at least two breast cancers diagnosed before age 50 years, are probably attributable to BRCA1/2 mutations. Other, non-BRCA1/2 related effects are probably involved in the clustering of early onset pancreatic cancer in families with two breast cancers under the age of 50 years, in the aggregation of ovarian cancer in families with breast and ovarian cancers, and in the increased incidence of early prostate cancer in families with male breast cancer (Bermejo and Hemminki 2004).

The reasons for these tissue-specificity differences between the BRCA1 and BRCA2 gene is not clear.

The relationship between BRCA1 mutations and colon cancer development remains puzzling. Recently, Garber wrote that the colon cancer risk in BRCA1 mutation carriers is one less thing to worry about on the basis of studies published (Garber et al. 2004). An increased risk of colorectal cancer in BRCA1 carriers may yet be demonstrated, but it seem more and more likely that it will be a small increase, if that, or limited to a particular subset of carriers. Intensified targeted colorectal cancer screening and prevention should be directed only to the subset of BRCA1 mutation carriers who have remarkable personal and familial colorectal cancer history or other risk

factors. Moreover, effects of modifying factors, such as diet and exposure to other environmental factors should be considered at all. Recently, epigenetic modifications of DNA are reported as responsible for reversible and clonally heritable alterations in transcription state producing a phenotype equivalent to that resulting from an inactivating germline mutation (Garber et al. 2004; Niell et al. 2004).

The various studies published in this field didn't report childhood cancers in hereditary breast cancer families. In the most of studies, an earlier onset diagnosis than sporadic cancers has been reported only for breast, prostate and pancreatic cancer.

Recent findings suggest a relation among the cancer spectrum and the position of the mutation at the level of the BRCA gene (Thompson et al. 2001; Lubinski et al. 2004). Moreover, the ethnic background of family appears to contribute to the phenotypic variation observed in families with BRCA2 mutations (Lubinski et al. 2004). Risch et al. reported an increased risk of breast cancer associated with downstream location of mutations in the BRCA1 coding sequence and a peak in ovarian cancer risk associated with mutations in the middle of the coding sequence. Several studies have reported a higher risk of ovarian cancer for BRCA2 mutation carriers in the Ovarian Cancer Cluster Region (OCCR), while an increased risk of breast cancer seems to be restricted to non-OCCR, particularly those in region 3' of the OCCR.

No data are available on the correlation between the site of the mutation and a specific cancer spectrum (Risch et al. 2006; Antoniou et al. 2003; Thompson et al. 2001). Thompson et al. reported a cumulative risk of prostate cancer by age 80 years for non-OCCR mutations of BRCA2 gene being 33.6% major than the OCCR risk (Thompson et al. 2001).

Estimating the risk of cancer at different body sites in individuals who carry a germline mutation in a cancer susceptibility gene has relevant clinical implications. The knowledge of cancer risk conferred by mutations of BRCA1 and BRCA2 genes can help the practitioner and patient in making adequate choices regarding prevention measures such as surveillance, chemoprevention and prophylactic surgery. Moreover, the identification of hereditary forms of breast cancer could influence the management and follow-up of those subjects already affected by cancer.

2 AIMS OF THE STUDY

BRCA1 and BRCA2 genes sustain hereditary breast and ovarian cancer syndrome. Mutation carriers have an increased life-time risk of developing breast and ovarian cancers. Controversial data are available about cancer risk in sites other than breast and ovary in this setting.

The aim of this study is to assess the frequency of cancers other than breast in high-risk breast and ovarian cancers families, considering in particular the subset of hereditary and familial breast cancer families and BRCA1 and BRCA2 mutated/non mutated groups. Families have been ascertained at "Screening and follow-up for hereditary and familial cancers" Unit at "Federico II" University in Naples, Italy. Families have been selected on the basis of clinical criteria fulfilled for hereditary and familial breast cancer according to the Modena Study Group criteria proposed within the Italian Network for "Hereditary Breast and/or Ovarian Cancer".

In details, the aims of the study are:
- to evaluate the frequency of breast cancer and ovarian cancer;
- to evaluate the frequency of male breast cancer;
- to evaluate the frequency of cancers at other sites different than breast;
- to consider the age of onset of each cancers per site;
- to evaluate the frequency of other cancers in the subsets of families at hereditary risk with and without clustering and at familial risk;
- to evaluate the frequency of other cancers in the subsets of families with mutations of BRCA1 and BRCA2 genes;
- to compare the incidence of cancers in families selected at our clinical unit to the incidence in general population on the basis of data from the Italian Cancer Registry.

3 MATERIALS AND METHODS

3.1 Identification of families at risk by oncogenetic counseling

Families were ascertained from subjects who referred for cancer genetic counseling to the *Screening and Follow-up for Hereditary and Familial Cancer Unit* at "Federico II" University in Naples, Italy, between 2000 and 2007. Subjects who referred to counseling were: 1) cancer-affected subjects with a personal history suggesting a genetic risk (e.g., early onset breast cancer, male breast cancer, breast and ovarian cancer in the same subject and multiple cancers beside breast and ovarian cancers in the same subject), 2) cancer-affected subjects with a family history of breast and/or ovarian cancer, and 3) disease-free subjects in families clustering breast and/or ovarian cancers. All subjects derived from Italy and were of Caucasian ethnicity.

Cancer genetic counseling was led by the oncologists of the multidisciplinary team, according to the multistep model (*figure 4*) that was previously designed and validated within the Italian Network for "Hereditary Breast and/or Ovarian Cancer" (Contegiacomo et al. 2004; Contegiacomo et al. 2005). This counseling model entails risk identification, risk definition and risk management of subjects with suspected hereditary breast cancer and their family members.

At proband intake the family history of at least three generations is acquired by pedigree construction including both of the maternal and paternal lines and the individual clinical history is registered and the consanguinity is eventually reported.

For each subject we defined the risk profile (hereditary, familial and personal) by predictive models that are widely used (Domchek et al. 2003). Hereditary and familial risks are clinically defined according to the Modena criteria, including familial clustering for breast and/or ovarian cancers, first and second degree affected family members, age of onset of breast cancer less than 40 years and bilateral breast cancers (Federico et al. 1999). Breast cancer before the age of 35 years, male breast cancer and synchronous breast and ovarian cancers are all definitions of a hereditary risk without familial clustering (Cortesi et al. 2006).

We assessed the a priori genetic risk of BRCA1/2 mutations by the Frank criteria and BRCApro model, the latest specifically implemented for penetrance estimates in the Italian population (Frank et al. 1998; Frank et al. 2002; Berry et al. 2002). For the BRCApro model, carrier probabilities were calculated entering information on the proband's first and second-degree relatives into CancerGene software (CaGene version 3.3, supplied by Assistant Professor D. Euhus, UT Southwestern Medical Center at Dallas, Texas, USA and adapted to the penetrance estimates of BRCA1 and BRCA2 genes in Italian population) (Marroni et al. 2004).

When an a priori hereditary risk ≥10% according with Frank criteria and/or BRCApro model has been assessed and/or when an hereditary risk has been identified clinically by Modena criteria, genetic testing for BRCA1 and

BRCA2 genes has been offered to affected subjects according to the American Society of Clinical Oncology policy statement (ASCO 2003) and the Italian guidelines for genetic testing (Santi et al. 1998).
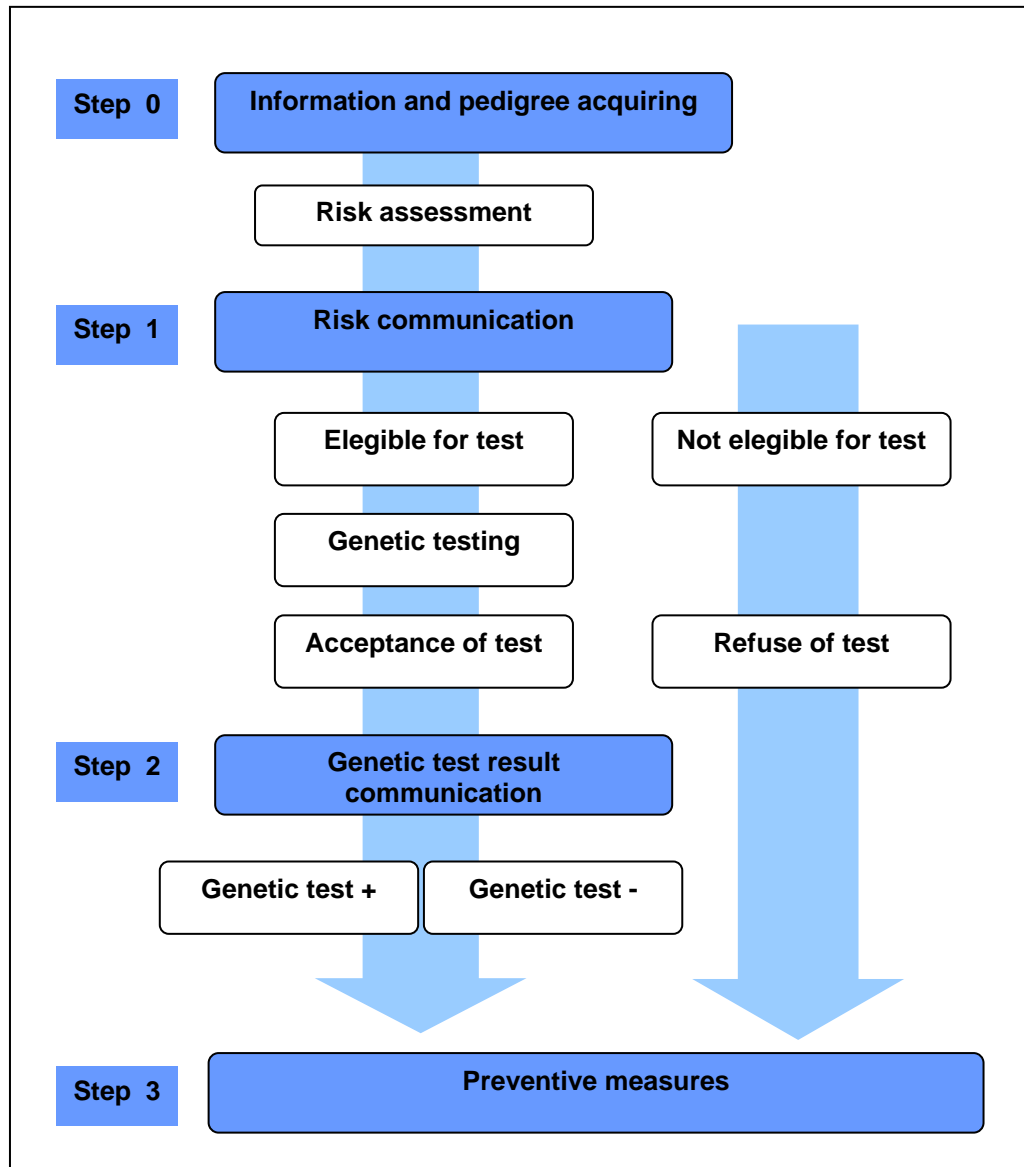
Figure 4. The multistep oncogenetic counseling model designed at the "Screening and follow-up for hereditary and familial cancer" unit – University Federico II of Naples (modified from Contegiacomo et al. 2004)

When a disease-free subject requested counseling, it was necessary that the affected family member, generally the youngest, underwent genetic testing to maximize the likelihood of obtaining a useful and informative test result, if a hereditary risk was suspected. When someone with a cancer diagnosis and a

family history of cancer has been tested and found to have a BRCA1 or BRCA2 mutation, other family members can undergo counseling and be tested for that specific mutation which has been identified in the family.

3.2 Mutational analysis for BRCA1 and BRCA2 genes

Mutational analysis for BRCA1 and BRCA2 genes has been performed at *Section of Genetic Oncology, Division of Surgical, Molecular and Ultrastructural Pathology*, University of Pisa. The genomic DNA was extracted from lymphocytes of peripheral blood sample, according to the instructions contained in the recommended protocol. Direct automatic sequencing of DNA was performed for both of BRCA1 and BRCA2 genes by 3100 ABIPRISM automated sequencer (Applied Biosystems, Foster City, CA). We used gene sequencing to analyze fully the coding regions and adjacent non coding regions of both of BRCA1 and BRCA2 genes in subjects who met hereditary criteria according to Modena model and/or Frank and/or BRCApro models.

In some cases mutational analysis for BRCA1 and BRCA2 genes has been performed by single strand conformation polymorphism (SSCP) and protein truncation test (PTT) other than sequence analysis. Genetic testing for BRCA1 and BRCA2 gained a sensibility of about 80-85%. All the analysis that demonstrated mutations were repeated for verification.

When the sequence analysis revealed an alteration with a likely pathogenetic role, further molecular analysis have been made in order to evaluate the effects of mutations on the mRNA maturation. RNA was isolated from the lymphocytes of peripheral blood (TriReagent; Molecular Research Center, http://www.mrcgene.com) and analyzed by reverse trascriptase polimerase chain reaction (RT-PRC) using SuperScript sspl (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols.

All mutations and genetic variants were named using the convention of Beaudet and Tsui with nucleotide numbering starting at the first transcribed base of BRCA1 and BRCA2 according to GenBank entries. A mutation was considered deleterious if it led to premature truncation of the BRCA1 protein product at least 10 aminoacids from C terminus or premature truncation of the BRCA2 protein product at least 270 amino acids from the C terminus. All the mutations identified were compared to the Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic).

3.3 Pedigree analysis for the evaluation of tumors related to hereditary and familial breast cancer

For each pedigree of the families on study data of the composition of family are recorded. They regard basic follow-up information such as date of pedigree acquiring and last updating, the number of all individuals for each pedigree, the number of males and females. In families where the identification of the hereditary line is possible data are recorded, such as number of

individuals, males and females of that specific branch. Moreover, for each family risk category is specified according to Modena model. For each proband the likehood of BRCA1 and BRCA2 mutations is recorded according with Frank and BRCApro models.

Data about the probands consist of the healthy or affected condition, the site and date of cancer diagnosis, the own genetic test result for BRCA1 and/or BRCA2 genes, when performed.

For probands and all first and second degree family members, data of identification and hereditary data are recorded. Data of identification concern pedigree code, generation and consecutive number. Personal information include sex, parity, date of birth, date of death, status (alive or died) at last follow-up and date and types of all cancers diagnosis. Data concerning hereditary regard line of family in which he/she is located, the branch of family in which hereditary can be attributed and the degree of relationship respect to the proband.

For affected subjects data concerning cancer, including site, date of diagnosis, residence at diagnosis and histological confirmation, are reported. Second and multiple cancers have been considered as independent events. All cancers were coded according to the 9th revision of the International Classification of Diseases (ICD-9) (World Health Organization 2002). When possible, cancers were confirmed by pathological report, clinical records or death certificate in order to maximize accuracy of tumor recording.

In subjects tested for BRCA1 and/or BRCA2 genes, mutation status (positive, negative, unknown) is reported. BRCA1/2 sequence variants considered as polymorphisms were recorded as negative and included in the no-mutation group.

As data on some pedigrees were incomplete or ambiguous, specified conventions were used for entry information (Kang et al. 2006).

The study had received approvals from the local ethical committee of the institution and all probands gave their informed consent at each step of counseling and for research use of data. All participants have given informed consent and have understood that as a result of participation personal details will have been recorded and stored in coded format on our database.

3.4 Statistical analysis

The statistical analysis has been performed at *Center of "Epidemiologia, Biostatistica ed Informatica Medica" at Università Politecnica delle Marche* in Ancona. The principal aim of this study was to estimate the frequency of cancer, expressed as cancer incidence, related to hereditary and familial breast cancer. Moreover, cancer incidence in the subset of families with carriers of mutations in BRCA1 and/or BRCA2 genes are estimated.

We considered first and second degree family members, excluding subjects with a degree of relationship major to the forth and non consanguineous ones, such as spouses.

We first constructed a cohort of the individuals belonging to the following risk categories according to Modena model (see *table 5*, on page 19 of the text): a) hereditary with clustering; b) hereditary without clustering; c) familial.

To compute incidence rates for individuals not affected with breast and/or ovarian cancer, follow-up was deemed to commence on their date of birth or on January 1, 1930, date at first cancer recording in our database, and to cease on the date of their first cancer, their date of death or loss to follow-up, their 85th birthday, or on June 2007, date of the last follow-up. For subjects with breast and ovarian cancer, entry into the cohort was assumed to begin at first diagnosis of breast cancer or ovarian cancer or on 1930, whichever occurred later. Exit was chosen as for the rest of the cohort. Follow-up before 1930 was ignored to minimize errors in classification of tumors and because reliable population-specific incidence rates were available for Italian country from that date, but often not before.

Furthermore, for the purpose of this study maternal or paternal lines that were judged not-hereditary or proved not harbour the mutation were excluded in order to avoid unnecessary dilution. In most cases this was possible due to verified mutations in relatives of the proband. However, when nobody was tested outside the proband, we based our assumptions of the origin of the mutation on the prevalence of breast and/or ovarian cancer within a line of family. In some families, because the affected member died and ethical limitations on the ascertainment of a germline mutations in a deceased individual, it makes impossible to understand mutation status. In families selected through cases of early onset breast cancer, male breast cancer, double cancer site, lacking a positive family history for breast and/or ovarian cancer both main branches of the pedigree were included in the study. For immigrants to Italy, only those individuals that reside within Italy were included into the study.

Crude incidence rates and standardized incidence rate of cancers have been computed by the appropriate age-, sex-, period-, site-, and population-specific incidence rates. The standardized incidence rates of tumors in our sample have been compared with the incidence rates in the general population from the Italian Cancer Registry (Verdecchia et al. 2002). The incidence rates of tumors (expressed as 100,000 individuals/year) have been estimated for decades starting from 1930 to 2007. for sex an risk categories (hereditary with clustering, hereditary without clustering and familial). Moreover, standardized incidence rates have been evaluated for site other than breast. Confidence intervals for the incidence rates (CI=95%) of cancer have been calculated according with the Poisson distribution. The statistical value has been gained when confidence intervals didn't overlap.

4 RESULTS AND DISCUSSION

4.1 Identification of families at hereditary and familial risk for breast cancer

Since January 1, 2000 to June 30, 2007 a total of 254 families requested oncogenetic counseling at *Screening and follow-up for hereditary and familial cancers* Unit at *Federico II* University in Naples, Italy. Among families referred to counseling we selected 104 pedigrees for this study in order to evaluate tumors associated to hereditary and familial breast and/or ovarian cancers. All probands gave their consent to study entry. In *table 6* the characteristics of pedigrees, selected for the evaluation of associated tumors in hereditary and familial breast cancer, are shown.

Table 6. Characteristics of 104 pedigrees selected for the evaluation of associated tumors in hereditary and familial breast cancer

Characteristic	No.	%
Pedigree		
Total	104	100
Individuals*	4100	100
Probands	104	2.6
I-IV degree family members of proband	3996	97.4
Females	2117	51.6
Males	1983	48.4
Caucasian ethnicity	4100	100
Individuals affected with cancer	533	13
Unaffected individuals	3567	87
Average number (range) of generations	5 (3-6)	
Average number (range) of individuals/pedigree	81 (10-257)	
Proband		
Females	100	96.2
Males	4	3.8
Affected with cancer	80	76.9
Unaffected	24	23.1
Tumor in proband		
Monolateral breast cancer	52	65
Bilateral breast cancer	13	16.2
Male breast cancer	3	3.8
Breast and ovarian cancer	3	3.8
Breast and other cancer	2	2.5
Ovarian cancer	2	2.5
Other sites (no.)**	5	6.2
Average age (range) at diagnosis of breast cancer	45 (23-79)	
Average age (range) at diagnosis of ovarian cancer	56 (38-65)	

*including probands and I-IV degree family members, excluding non consanguineous
** Hodgkin linfoma (1), colon (1), melanoma (1), prostate (1), stomach (1)

Pedigrees accounted a total of 4100 individuals (2117 females, 1983 males), including probands and their I-IV degree family members and

excluding non consanguineous such as spouses. All the individuals were of Caucasian ethnicity. Pedigrees have been constructed for at an average number of 5 generations (range 3-6), including both of the maternal and paternal lines. In evaluating related tumors, the branch of family, in which a clustering of breast and/or ovarian cancers has been registered suggesting a familial or hereditary form, has been only considered. Families ranged in size from 10 to 257 individuals, with the median size of families being 81 individuals/pedigree. Probands were affected with cancer in 80 cases (76.9%) and unaffected in 24 cases (23.1%). Probands were affected prevalently with female monolateral breast cancer in 52 cases (65%). The average age at diagnosis of breast cancer in proband was 45 (range 23-79). In 7 cases, probands were affected with breast cancer synchronous or metachronous with ovarian or other cancers. Moreover, in 5 cases (6.2%), probands had diagnosis of cancer at site other than breast and/or ovary.

4.2 Classification in risk categories

The 104 families on study have been classified in risk categories applying the criteria of the Modena model, as *figure 5* shows.
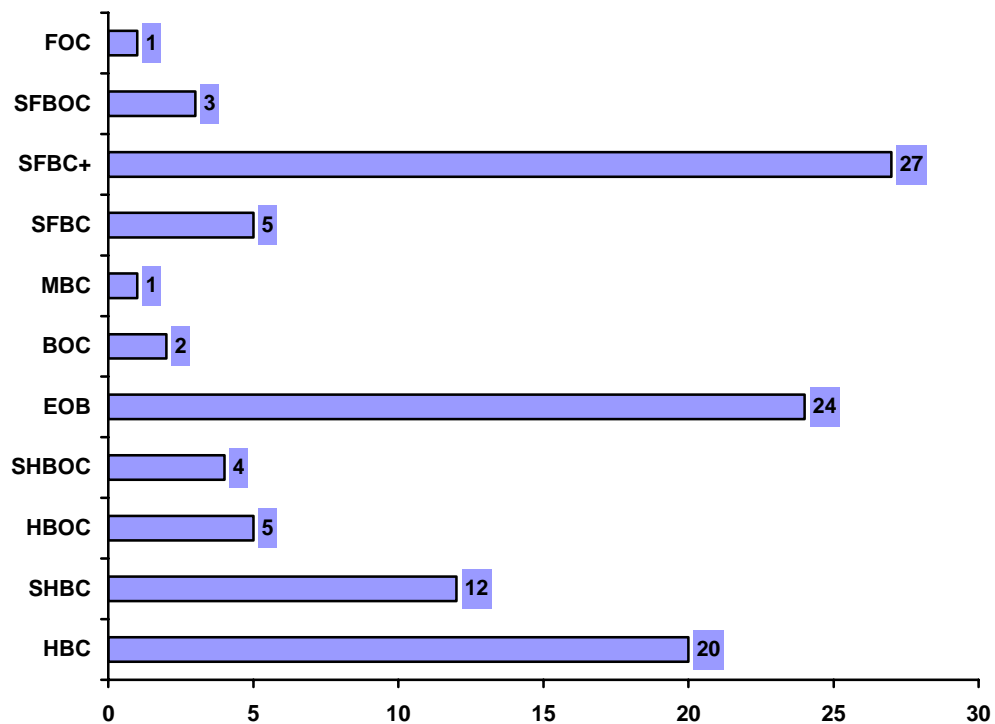
Figure 5. Classification of the 104 families according to Modena criteria
Abbreviations: HBC= hereditary breast cancer; HBOC= hereditary breast and ovarian cancer; SHBC= suspected hereditary breast cancer; SHBOC= suspected hereditary breast and ovarian cancer; EOBC= early-onset breast cancer; BOC= breast and ovarian cancer in the same subject; MBC= male breast cancer; FBOC= familial breast and ovarian cancer; SFBC= suspected familial breast cancer; SFBOC= suspected familial breast and ovarian cancer; SFBC+= strongly suspected familial breast cancer

The 104 families have been also grouped in the three major categories of the Modena model, such as hereditary with clustering (39.4%), hereditary without clustering (26%) and familial (34.6%) (*figure 6*).
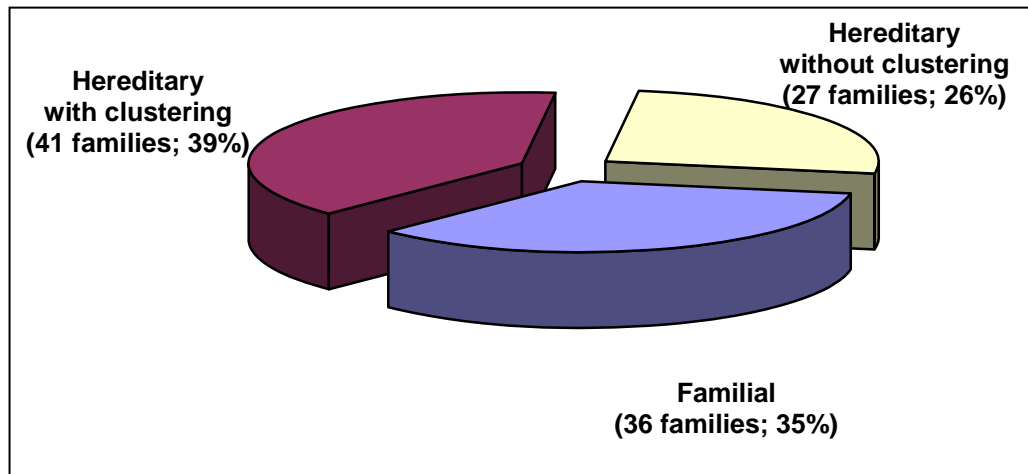

Figure 6. Families grouped in three major categories (hereditary with clustering, hereditary without clustering, familial) according to the Modena model

Applying the models of Frank and BRCApro Italia for each family on study we assessed the a priori genetic risk of BRCA1/2 mutations in order to consider eligibility to genetic testing. In *figure 7* cases eligible to genetic testing for BRCA1 and BRCA2 genes have been shown for each of the three predictive models used. The figure displays also the percentage of cases eligible to genetic test by the criteria of the Modena model compared with the Frank and BRCApro Italia models. For the Modena model the violet bar indicates the families that are eligible to genetic testing for BRCA1 and BRCA2 genes belonging to the hereditary with/without clustering categories, while the bordeaux bar indicates the families that are not eligible for genetic testing belonging to the familial category. For the Frank and BRCApro models violet bars represent the families that are eligible for genetic testing of BRCA1 and BRCA2 genes because of an a priori probability of BRCA1/2 mutations ≥ 10%, while the bordeaux bars indicate the families that are not eligible to genetic testing because of an a priori probability of BRCA1/2 mutations <10%. Moreover, the classification of the 104 families with the three selected predictive methods allows to verify the correspondence among the epidemiological model of the Modena Study Group and the probabilistic models of Frank and BRCApro Italia. As it emerges, a concordance of 62% and 63% of cases classified as eligible to genetic testing has been revealed between Modena model and Frank or BRCApro Italia models, respectively.

Considering the moderate level of correspondence among the three models and the open debate on what is the most suitable and efficacious model in predicting mutations (Kang et al. 2006), we decided to integrate them in risk

assessment in order to not excluding families from genetic testing. Moreover, the critical examination of pedigree by the oncologist according to the criteria of the genetics seems to be the most suitable method in selecting family to genetic testing.
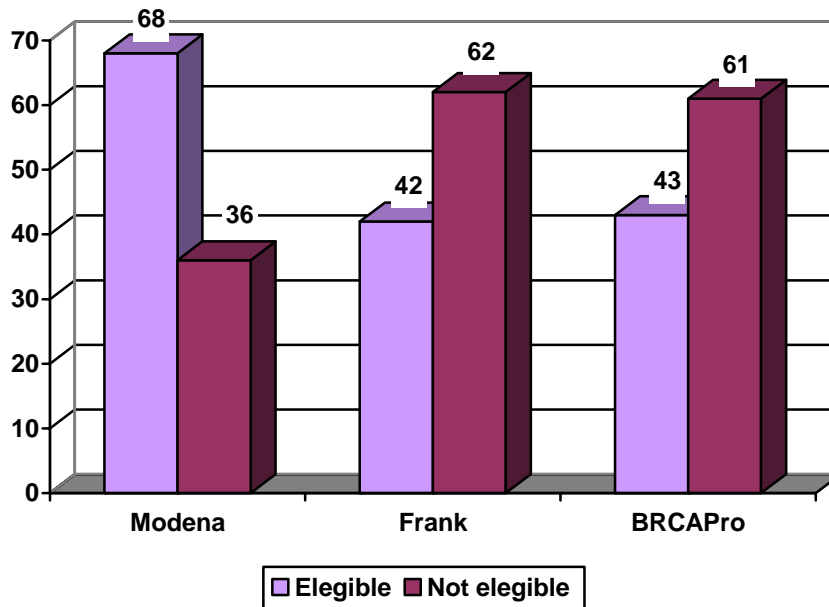
Figure 7. Classification of the probands belonging to the 104 families on study as eligible or not eligible to genetic testing for BRCA1 and BRCA2 genes according to Modena, the Frank and the BRCApro Italia model, comparatively

4.3 Results of genetic test for BRCA1 and BRCA2 genes

Among 68 subjects eligible for genetic testing because at hereditary risk according to Modena model, 44 affected subjects from different families have been screened for germline mutations in BRCA1 and/or BRCA2 genes. In 43 subjects tested for BRCA1 gene, a total of 10 (23.2%) distinct mutations have been detected. All of them were frameshift. In 30 subjects tested for BRCA2 gene, a total of 6 (0.2%) distinct mutations have been revealed (4 frameshift and 2 splice site). Furthermore, genetic testing revealed other sequence variants in 2 cases of which one in BRCA1 gene (variation 561-34 C>T in exon 7) and one in BRCA2 gene (variation 8133-15 T> C in intron 17). Both of these mutations are classified as not pathogenetic in the Breast Cancer Information Core (BIC) Database (http://research.nhgri.nih.gov/bic).

In *table 7*, the results of BRCA1 and BRCA2 genetic test have been shown for the 16 families in which mutations have been observed for BRCA1 and BRCA2 genes. Primary cancer in proband, risk category according to Modena

model, other tumors clustered in the family, mutated BRCA1 or BRCA2 gene, site and type of mutation and the effect on protein products have been displayed for each family, too.

Table 7. Families with mutations of BRCA1 and BRCA2 genes

N.	Proband's cancer	Risk category (Modena)	Other tumors clustered in family (n.)	Site	Type of mutation	Effect on protein
Mutations of BRCA1 gene						
1	BC	HBC	Colon (2) ; Stomach (1)	Exon 20	Ins C 5382	Truncated protein
2	BC	HBC	-	Exon 20	Ins C 5382	Truncated protein
3	BC	EOBC	Bladder (2); Head and neck (1)	Exon 20	Ins C 5382	Truncated protein
4	BC	HBOC	Ovary (3); uterus (1); Stomach (2); Kidney (3)	Exon 20	Ins C 5382	Truncated protein
5	BC	EOBC	Ganglioneuroblastoma (1); Leukemia (1)	Exon 20	Ins C 5382	Truncated protein
6	OC	HBOC	-	Exon 11	Ins A 1499	Truncated protein
7	BC	HBC	-	Exon 11	DelAG 1254	Truncated protein
8	BC	SHBOC	BOC (1)	Exon 1-2	Del exon 1-2	Truncated protein
9	BC	SFBOC	Prostate (1)	-	G→A 5272	Truncated protein
10	BC	EOBC	Ovary (1); Peritoneum (1) Bone sarcoma (1)	Exon 12	G→ T 4236	Truncated protein
Mutations of BRCA2 gene						
11	BC	EOBC	-	Exon 11	Del TC 6696	Truncated protein
12	BC	HBOC	-	Exon 11	Del TC 6696	Truncated protein
13	BC	HBC	Colon (2), Myeloma (1)	Exon 11	G→T 2722	Truncated protein
14	BC	MBC	Prostate (1)	Exon 10	Del AG 1724	Truncated protein
15	BOC	HBOC	Prostate (1); Uterus (1); Ovary (1)	Intron 13 Exon 7	IVS13-2 A>T G→A 859	Splicing alteration
16	BC	EOBC	-	Intron 22	IVS-del3insA	Splicing alteration

See text for details. Abbreviations: BC= breast cancer; OC= ovarian cancer; HBC= hereditary breast cancer; HBOC= hereditary breast and ovarian cancer; SHBOC= suspected hereditary breast and ovarian cancer; EOBC= early-onset breast cancer; BOC= breast and ovarian cancer in the same subject; MBC= male breast cancer; SFBOC= suspected familial breast and ovarian cancer

In 14 cases the mutations have involved the exonic sequences of the genes and have produced a truncated protein that is unable to perform its normal functions. In 2 cases the mutations have concerned the intronic sequences of BRCA2 gene leading to the alteration of the splicing mechanism and to the production of a non functioning protein, too. In particular, in one family (see family #15 in table 7 on page 33) whose pedigree has been shown in *figure 8*,
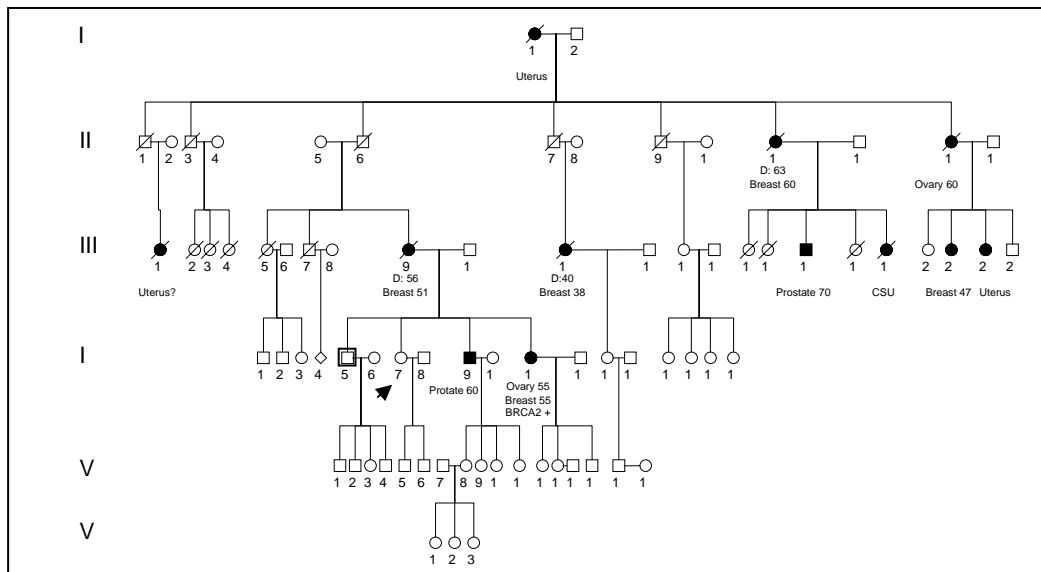
Figure 8. Pedigree of family with double BRCA2 mutation (859/G>A in exon 7 and IVS13-2 A>T regarding the splicing site in intron 13)

direct automatic sequencing of DNA extracted from the lymphocytes showed two sequence alterations on BRCA2 genes. The first sequence alteration (IVS13-2A>T) concerned the splicing site in intron 13, probably involved in an altered maturation of mRNA. The second sequence alteration concerned the variation 859/G>A, corresponding to the latest base in exon 7. It entails the substitution of Valine (Val) to Isoleucine (Ile) in the 811 position of the aminoacid sequence. The mutation at the splicing site in intron 13 showed an altered mRNA maturation with a transcription of a sequence skipping of exon 14 and an anomalous stop codon in exon 15. The variation in exon 7 led to an altered mRNA maturation with the transcription of a sequence lacking exon 7 and the subsequent anomalous stop codon in exon 9. Both of the mutations led to the expression of a truncated non functioning BRCA2 protein in its carboxy-terminal region (figure not shown). The analysis of a second blood sample confirmed the mutations. The Breast Cancer Information Core (BIC) Database (http://research.nhgri.nih.gov/bic) reported at least 30 different alterations of exon 7 of the BRCA2 gene, involving distinct mutations, polymorphisms and variant sequences. The intronic alteration (IVS13-2A>T), which was found in our patient, has not been reported as a polymorphism or as an unknown variant

(UV) in the BIC database yet. Most of BRCA2 sequence variants have been well characterized as clearly deleterious and known to be unequivocally involved in the pathogenesis of breast cancer. A large number of genetic alterations are still classified as variants of unknown significance. Some intronic variants have to be evaluated in order to understand their pathogenetic or polymorphic effects on the mRNA splicing process. Classifying these variants of unknown clinical significance as neutral or disease-causing is very important for genetic counseling and for the implications in terms of cancer risk. Different studies have reported new pathogenetic alterations charged to BRCA1 and BRCA2 genes. In our patient both of the identified BRCA2 mutations have been shown to be involved in the splicing mechanisms with an effect on mRNA splicing fidelity and expression. The RT-PCR analysis on the sample confirmed the pathogenetic role of both of the mutations leading to a non functioning BRCA2 protein. Recent findings suggest a relation among the cancer spectrum and the position of the mutation at the level of the BRCA gene (Thompson et al. 2001). Several studies have reported a higher risk of ovarian cancer for BRCA2 mutation carriers in the Ovarian Cancer Cluster Region (OCCR), while an increased risk of breast cancer seems to be restricted to non-OCCR, particularly those in region 3' of the OCCR. (Antoniou et al. 2003, Thompson 2001). The two mutations detected outside the OCCR in our patient could explain the development of breast cancer other than ovarian cancer. Moreover, they could explain the development of prostate cancer in the proband's brother. Then it could be interesting to test this subject.

The most frequent alteration concerns the Ins C 5382 in exon 20 of BRCA1 gene. Such mutation is the most common alteration in the Ashkenazi Jewish from the United States (Garber and Offit 2005).

Other two mutations are of particular interest, being proven founder allele. The first is the variation Del TC 6696 in exon 11 of BRCA2 gene that we previously reported as novel mutation (Aceto et al. 2002) (see family #6 in table 7 on page 33). The second mutation is the variation Ins A 1499 in exon 11 of BRCA1 gene, that has been revealed in a previous haplotype analysis as a founder allele, probably originated in Tuscany (Italy). We applied a phylogenetic method for estimating the age of the mutation Ins A 1499 in exon 11 of BRCA1 gene. A chromosome segment of about 25 cM, including 37 short tandem repeats (STRs) around the *BRCA1* gene (DeCode map), was typed in 50 subjects (28 mutation carriers) from 14 unrelated families. The time to the most recent common ancestor (MRCA) of the mutation carriers was estimated by the length of the shared haplotype between all possible pairs of individuals. A function relating the length of the shared haplotype to the time to the MRCA was obtained by a computer simulation. This approach gives results comparable with those of other existing mutationdating methods, but does not depend on population-specific parameters such as allele frequencies, provides narrower confidence intervals (CI), and allows to build an extended genealogical tree of all mutation carriers. The 1499insA mutation shared by the investigated subjects was present in an individual living about 30 generations

ago (95% CL 22-56), or 750 years (95% CL 550-1,400) (Marroni et al. in press).

4.4 Tumors related to hereditary and familial breast cancer

After removing those individuals with inconsistent follow-up in the relevant period of the analysis, the final cohort comprised 4100 individuals, including probands and their I/IV degree family members. Individuals were affected with cancer in 533 cases and unaffected in 3567 cases. A total of 587 independent events of cancer have been detected in the 104 families on study. In particular among the three major categories in which individuals have been grouped according with the Modena model, 294 cases (17.6%) of tumors were registered in the category of hereditary with clustering constituted of a cohort of 1670 individuals, 103 cases (9.8%) of tumors in the category of hereditary without clustering constituted of a cohort of 1053 individuals and 190 cases (13.8%) of tumors in the familial category constituted of a cohort of 1377 individuals.
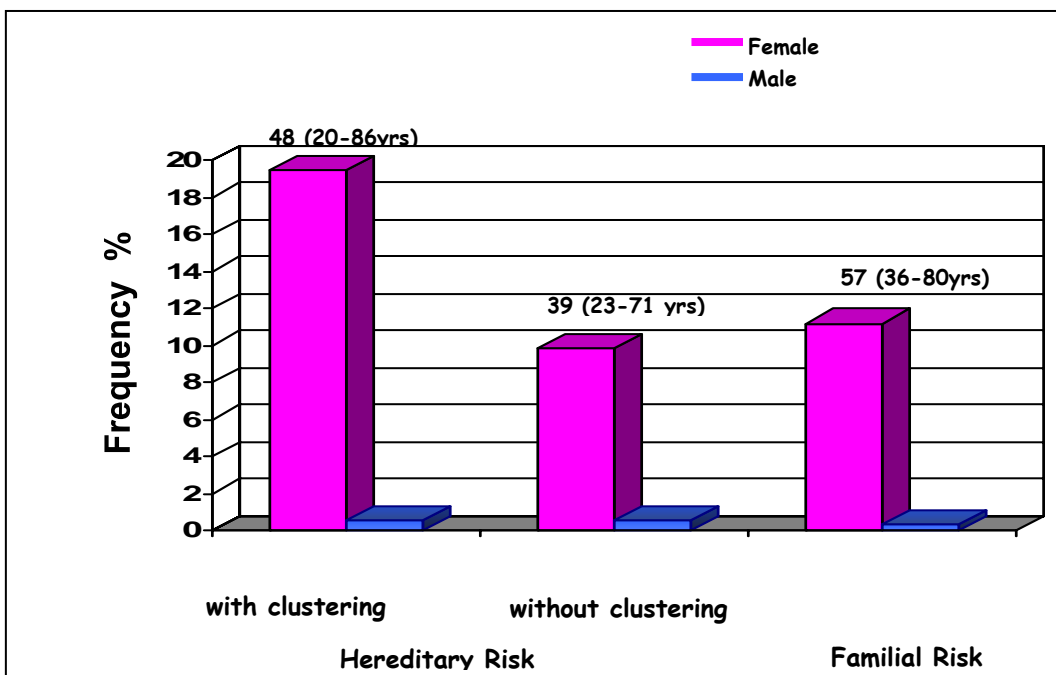
Figure 9. Frequency and age at diagnosis of breast cancer in the three risk groups according with the Modena model and sex

In *figure 9* the frequency of breast cancer has been reported for the three risk categories, considering sex and mean age at diagnosis of breast cancer. In hereditary with clustering group breast cancer were detected in 173 females (19.48%) and in 4 males (0.51%) with a mean age at diagnosis of 48 years

(range 20-86). In hereditary without clustering group breast cancer were detected in 51 females (9.8%) and in 3 males (0.56%) with a mean age at diagnosis of 39 years (range 23-71 yrs). In familial group breast cancer were detected in 79 females (11.2%) and in 2 males (0.29%) with a mean age at diagnosis of 57 years (range 36-80 yrs). The age at diagnosis of breast cancer is earlier in the hereditary without clustering group compared with the other two groups in which the age at diagnosis around 50 years is similar than sporadic one.
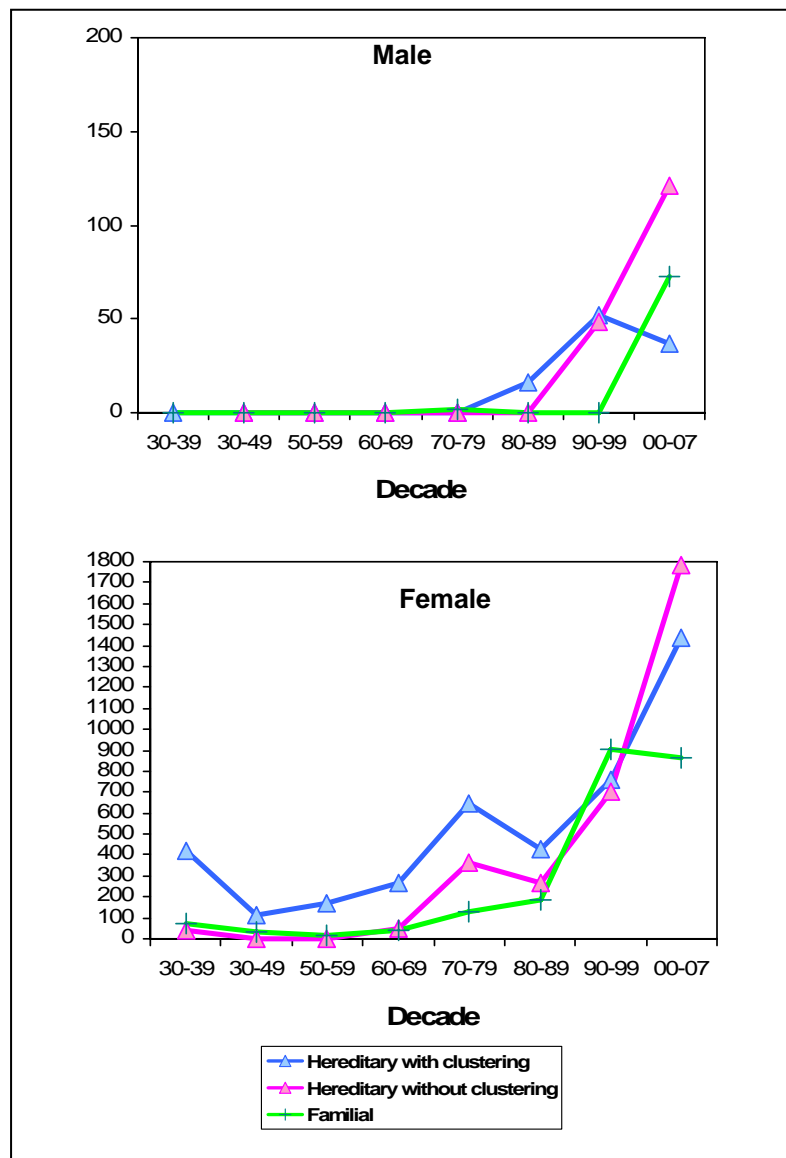
Figura 10. Standardized incidence rates (x 100,000 individuals) for breast cancer according with risk category (hereditary with clustering, hereditary without clustering and familial), sex and decades (1930-2007)

In hereditary risk with/without clustering groups a double percentage of breast cancer have been registered in male compared with the familial group.

The standardized incidence rates for breast cancer since 1930 to 2007 have shown a higher incidence of breast cancer in females than in males in the three categories for each decade, as *figure 10* shows. Male breast cancer have been registered in our series since 1980 in families belonging to the hereditary with clustering group and since 1990 in families belonging to hereditary without clustering group. In families belonging to familial group few cases have been registered in decades 1970-1979 and 2000-2007. Since 1970, 1980 and 1990 in the hereditary with clustering, hereditary without clustering and familial group respectively the frequency of breast cancer in male has been higher than the incidence reported in the Italian population that is one case every 100,000 individuals. Although at a first analysis male breast cancer seems to cluster higher in the hereditary groups than in familial group, the standardized incidence rates for breast cancer in male didn't gain differences of statistical value among risk categories (*figure 10*).
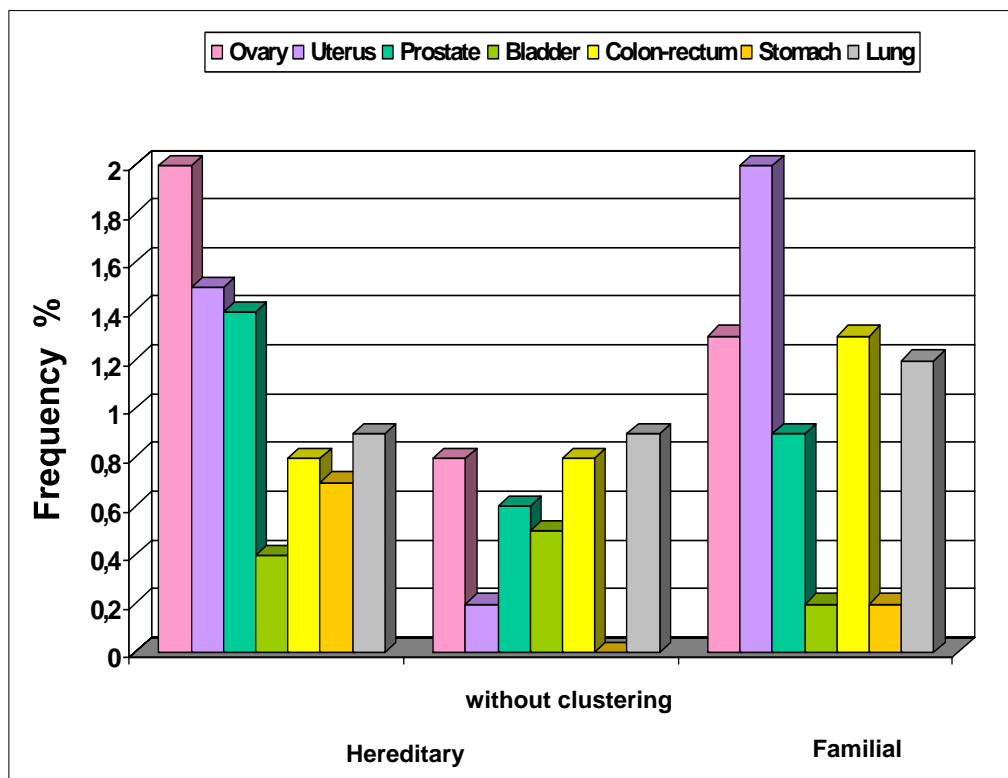
Figure 11. Cancer spectrum related to families with hereditary with/without clustering and familial breast cancers

In *figure 11* the frequency of tumors associated with hereditary with/without clustering and familial breast cancer has been reported for site and risk category. In the hereditary with clustering group a high frequency emerges for tumors of ovary (2%), uterus (1,4%), prostate (1,4%) and lung (0,9%). A

moderate frequency emerges for colon-rectum (0,8%) and stomach (0,7%) cancer. In the hereditary without clustering group a similar association has not been revealed except for colon-rectum cancer ((0,8%). In the familial group a high frequency has been registered for cancers of ovary (1,3%), uterus (2%) and colon-rectum (1,3%). A moderate frequency has been registered for prostate cancer (0,9%).

The standardized incidence rates for cancer at site different than breast cancer since 1930 to 2007 hasve not shown differences of statistical value among risk categories and sex. (*figure 12*). In our series the statistical analysis has not shown a cancer spectrum typical for hereditary and familial breast cancer. Our data don't support a previous European study (Bermejio et al. 2004) that confirmed the association of BRCA1/2 mutations with ovarian, pancreatic, prostate and stomach cancers at a population level.

The index case of the family allowed to acquiring pedigree by self-reported family and personal history and by giving data useful for our study for each family member. When possible, cancers have been confirmed by pathological report, clinical records or death certificate in order to maximize accuracy of tumor recording. In our series cancers have been confirmed in over 60% of cases. In evaluation of cancer spectrum we considered that the lack of tumor accuracy by pathological or other clinical reports could have introduced misclassification of the primary tumor site, especially for ovarian, uterus and colon cancers. Then the lack of cancer registries to verify events of tumors can be a possible source of underestimation/overestimation of cancer. Furthermore, in Campania the lack of a regional registry for cancers represents a limit for the present study because it didn't allow us to verify each diagnosis. In Italy also the lack of cancer registries is a factor that can introduce bias in such kind of studies because it didn't allow to conduct a population-based study. When using family history to assess risk, the accuracy and completeness of family history data must be taken into account. A self-reported family history may be erroneous, or a person may be unaware of relatives affected with cancer. In addition, small family sizes and premature deaths may limit the information obtained from a family history. A comparison of self-reported family history to data from the Utah Population Database indicates a sensitivity of 83% (95% CI, 66%-93%) for reported family history of breast cancer; a measure of overall agreement between the reported family history and the database was 0.63 (95% CI, 0.52-0.73), indicating moderate agreement. Family history was less accurate for most other cancers, e.g., the sensitivity of a family history of ovarian cancer was 60% (95% CI, 17%-93%). In a Canadian study, accuracy of a reported family history of breast cancer was assessed through review of the medical records of relatives reported as affected for a consecutive series of women with breast cancer and for a population-based sample of women without breast cancer. Among cases, 16% reported a first-degree relative with breast cancer; 91% of verifiable histories were confirmed. Among controls, 9% reported a first-degree relative with breast cancer; 97% of verifiable histories were confirmed (Kerber et al. 1997; Parent et al. 1997).
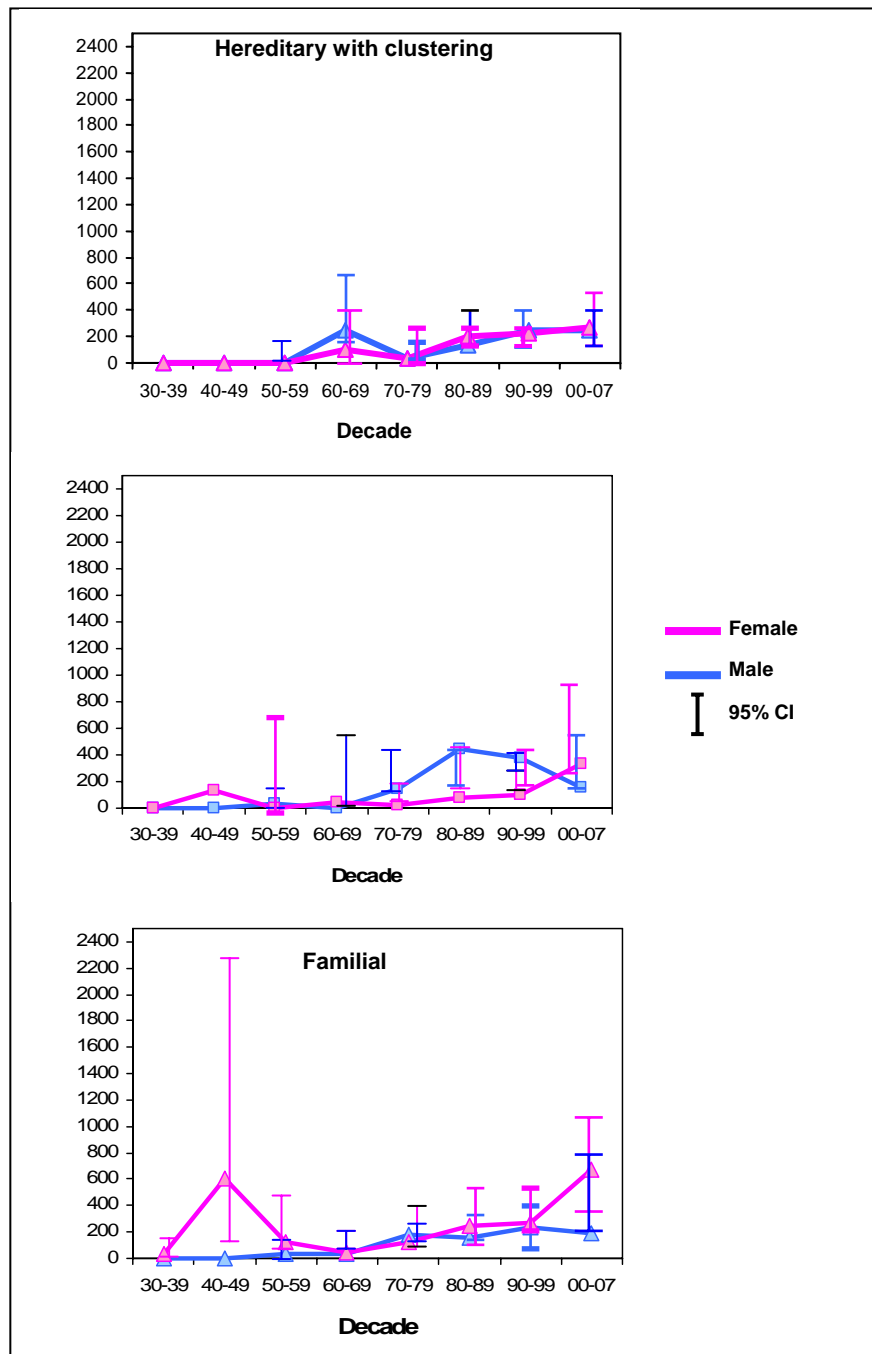
**Figura 12.** Standardized incidence rates (x 100,000 individuals) for cancer at site different than breast cancer according with risk category (hereditary with clustering, hereditary without clustering and familial), sex and decades (1930-2007)

At the evaluation of standardized incidence rates for other cancer, an earlier age of onset has not been registered for any cancer in different sites than

sporadic ones (data not shown). Childhood cancers, like one case of leukaemia at 6 years of age and one case of emangioblastoma in hereditary with clustering group, ganglioneuroblastoma at 3 years of age in hereditary without clustering group and retinoblastoma in familial group, have been reported respectively.

In *figure 13* the frequency of breast cancer in males and females has been reported in families divided in three groups according to genetic test results, such as BRCA1 positive group consisted of a cohort of 486 individuals (238 males; 248 females) distributed in 10 families, BRCA2 positive group consisted of a cohort of 185 individuals (77 males; 108 females) distributed in 6 families and BRCA1/2 negative group consisted of a cohort of 1155 individuals (568 males; 483 females) distributed in 32 families. The frequency of breast cancer in females is similar in the three groups, with an earlier age at diagnosis in BRCA1 group. In particular, in the group of BRCA1 positive the mean age at diagnosis of breast cancer has been 41 years (range 24-86), earlier than the age at diagnosis in the group of BRCA2 positive and in the group of BRCA1/2 negative, being of 43 and 49 years respectively. Male breast cancer has been clustered in families with BRCA1 genotype in 1/238 males (0.4%) of this cohort and in families with BRCA2 genotype in 1/77 males (1.2%) of this cohort compared to families without mutations in BRCA1/2 genes in which it accounts for 1/568 males (0.2%). Therefore, male breast cancer can be considered suggestive for a mutation in BRCA2 gene. This result confirms data of previous studies.
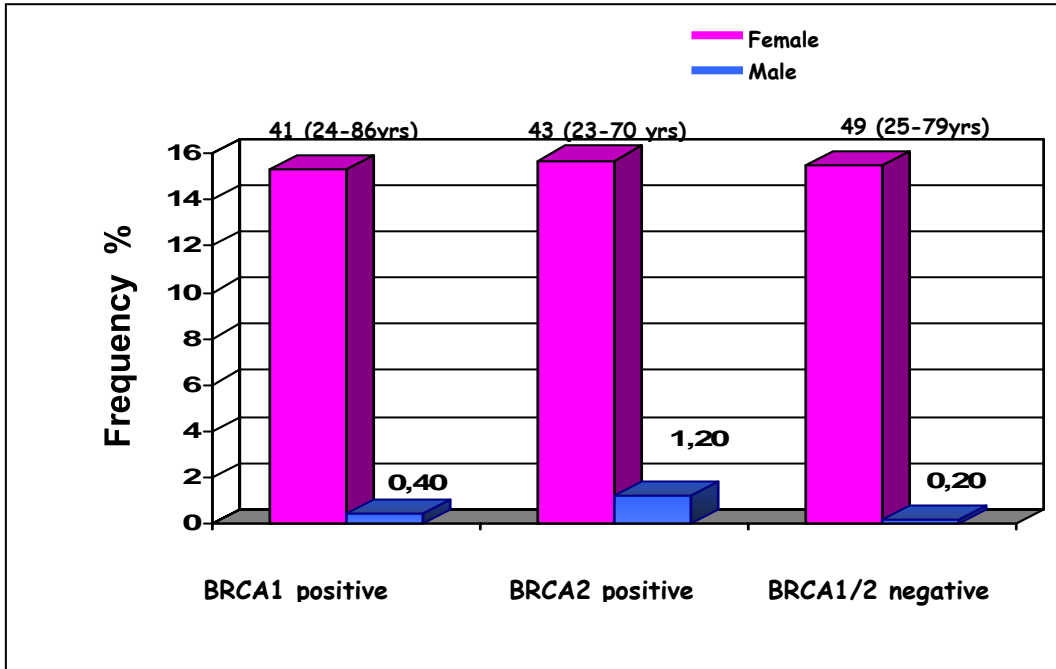
Figure 13. Frequency and age at diagnosis of breast cancer in the families positive and negative for mutations of BRCA1 and BRCA2 genes

In *figure 14* the frequency of tumors, associated in families that have been tested for mutations in BRCA1 and BRCA2 genes, has been reported for site and mutation status such as BRCA1 positive, BRCA2 positive and BRCA1/2 negative. In the 10 families with BRCA1 mutations, 76 events of cancers have been detected in a total of 486 individuals. It emerges mainly a clustering with ovarian cancer (4.9%), uterine cancer (1.2%) and bladder cancer (0.8%). In the 6 families with BRCA2 genotype, 33 events of cancers have been registered in a total of 185 individuals. It emerges a clustering with ovarian cancer (2.8%), uterine cancer (2,8%), colon-rectum cancer (1%), and prostate cancers (2,6%). In BRCA1 and BRCA2 mutation carriers tumors have not been diagnosed at an earlier age than sporadic ones. The low frequency of tumor clustering doesn't allow us to demonstrated definitively an increased risk of cancer for any specific site. In the 28 families negative for BRCA1/2 mutations, 151 events of cancers have been registered in a total of 1155 individuals. In this group the cancer spectrum have not revealed a peak for any site. The moderate frequency of uterine cancer (1,2%) and thyroid cancer seems to relate these tumors to this group. Perhaps, this group could include a plethora of hereditary syndromes unrelated to BRCA1/2 genotype. It can be assumed they are related rather to low penetrance genes.
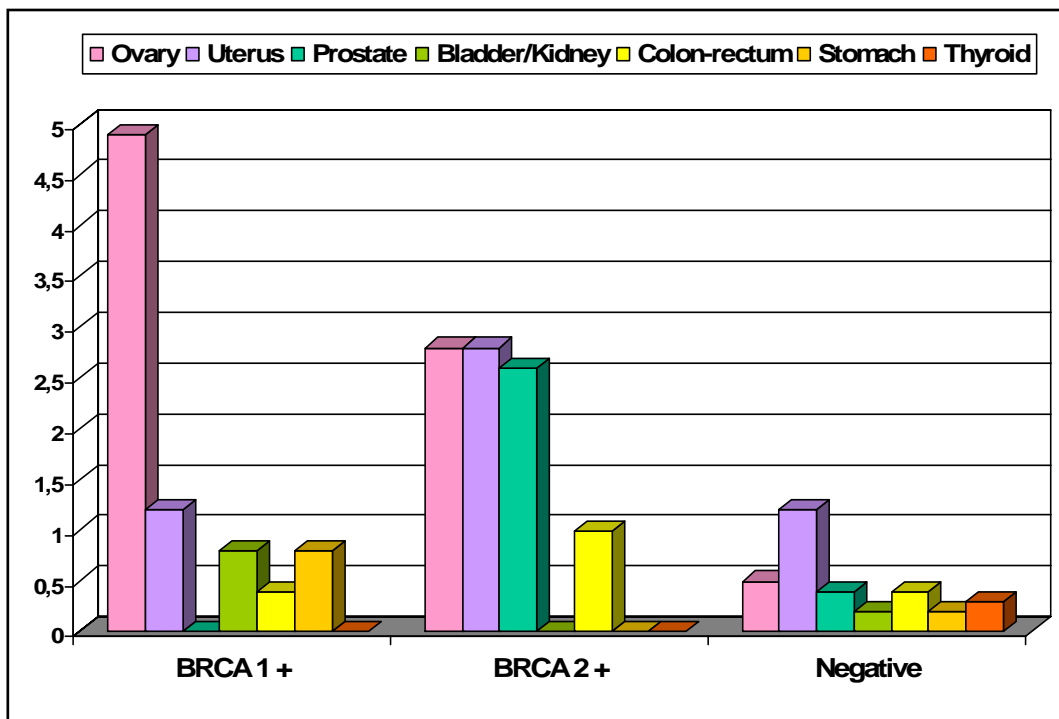
Figure 14. Cancer spectrum related to families with mutations of BRCA1 and BRCA2 genes

Ovarian cancer clustered in BRCA1 mutation carriers group with a frequency higher than that for other type of cancers in adults in this group. Ovarian cancer

seems to be predictive for BRCA1 mutations and less frequently for BRCA2 mutation. Prostate cancer seems to be predictive for mutations in BRCA2 gene.

Childhood cancers or early onset diagnosis of cancers in any other sites have not been reported. In one family in which the proband with an early onset breast cancer has been revealed with a mutation of BRCA1 gene, there was a diagnosis of ganglioneuroblastoma at 1 year of age (see family #5 in table 7 on page 33). Mutation status of this subject is unknown.

The evaluation of the standardized incidence rates of tumors at site different than breast has not been performed for the group of BRCA1 and BRCA2 mutation carriers because of the exiguousness of our sample in this setting.

5 CONCLUSIONS

In the hereditary with clustering group a high frequency emerges for cancer of ovary, prostate, uterus and lung. A moderate frequency emerges for colon-rectum and stomach cancer. In the hereditary without clustering group have not been revealed a similar association except for colon-rectum cancer. In the familial group a high frequency has been registered for cancers of the uterus, ovary and colon-rectum. A moderate frequency has been registered for prostate cancer. In these setting, an earlier age of onset for cancers in different sites than sporadic ones has not been registered for any cancer. In the families with BRCA1 mutations, it emerges mainly a clustering with ovarian cancer, uterine cancer and bladder cancer. In the families with BRCA2 genotype, it emerges a clustering with cancers of ovary, uterus, colon-rectum and prostate. In BRCA1 and BRCA2 mutation carriers tumors have not been diagnosed at an earlier age than sporadic ones. Childhood cancers have not been reported in any sites.

Standardized incidence rate of cancers have been computed by the appropriate age-, sex-, period-, site-, and population-specific incidence rates. The incidence rates of tumors have been estimated for decades starting from 1930 to 2007. Although at the first analysis a typical cancer spectrum has emerged for each category of risk and for mutation status, at least the statistical analysis for the evaluation of the standardized incidence rates of tumors in sites other than breast has not shown a high frequency of clustering cancer for any site in our sample.

In conclusion, our data suggest that apart from breast and ovarian cancer, the incidence of cancers at other sites does not appear to be statistically increased in families supposed to be at hereditary and familial risk. The exiguousness of our sample for BRCA1 and BRCA2 mutation carriers didn't allow us to evaluate the standardized incidence rates of tumors at site other than breast in this setting. These findings suggest to be careful as possible in considering a specific clinical surveillance on the basis of risk categories and mutation status, until data derived from population-based studies will be available.

6 ACKNOWLEDGEMENTS

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Reconstructing the genealogy of a BRCA1 founder mutation by phylogenetic analysis

Reconstructing the genealogy of a *BRCA1* founder mutation by phylogenetic analysis

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ABSTRACT

Estimating the age of founder mutations may contribute to improve our knowledge of population genetics and evolutionary history of diseases. Previous haplotype analysis suggested that the *BRCA1**1499insA mutation was a founder allele, probably originated in Tuscany (Italy). Here, we collected additional pedigrees carrying this mutation, and applied a phylogenetic method for estimating mutation age. A chromosome segment of about 25 cM, including 37 short tandem repeats (STRs) around the *BRCA1* gene (DeCode map), was typed in 50 subjects (28 mutation carriers) from 14 unrelated families. The time to the most recent common ancestor (MRCA) of the mutation carriers was estimated by the length of the shared haplotype between all possible pairs of individuals. A function relating the length of the shared haplotype to the time to the MRCA was obtained by a computer simulation. This approach gives results comparable with those of other existing mutation-dating methods, but does not depend on population-specific parameters such as allele frequencies, provides narrower confidence intervals (CI), and allows to build an extended genealogical tree of all mutation carriers. The 1499insA mutation shared by the investigated subjects was present in an individual living about 30 generations ago (95% CL 22-56), or 750 years (95% CL 550-1,400).

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INTRODUCTION

A considerable proportion of germline mutations of *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) are identical by descent (IBD) in unrelated individuals. In populations derived from a small number of founders, a few mutations may be responsible for a large proportion of all hereditary breast and ovarian cancers. For example, three different alleles (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*) have reached a cumulative frequency of about 1/40 in the Ashkenazi Jewish population (Struewing et al. 1997), explaining some 20% of all breast cancers (Satagopan et al. 2001), whereas the *BRCA2**999del5 allele has a population frequency of 0.5% in Iceland, and accounts for 40% of the familial risk of breast cancer (Mikaelsdottir et al. 2004; Tulinius et al. 2002). In genetically more diverse ethnic groups, like the Italian populations, the mutation spectra of both genes is broader, though some recurring mutations in apparently unrelated families have been reported (Aretini et al. 2003). Many of them are probably identical by descent, and in some instances this has been confirmed by haplotype analysis (Baudi et al. 2001; Rudkin et al. 2006). The *BRCA1**1499insA mutation was initially detected in three unrelated families from Tuscany (Caligo et al. 1996), a region from central Italy, and it was later reported in other Italian regions. Preliminary haplotype analysis was carried out with four closely linked markers in nine families, and the presence of a common compatible haplotype was determined. We have now extended the

collection of independent pedigrees carrying this mutation to 14 families (7 ascertained in Tuscany, 6 in Northern Italy, and 1 in Southern Italy).

Determining the IBD status of identical mutations may be useful to design population specific, efficient mutational screening, and estimating the age of founder mutations may improve our understanding of the population genetics of hereditary breast cancer. Several methods for dating mutations have been developed (Bergman et al. 2001; Lander and Botstein 1986; Risch et al. 1995; Serre et al. 1990; Sham 1998), which are all based on the presence of linkage disequilibrium (LD) between the disease allele and linked markers. Some of them are easily applicable, but use one marker at a time, whereas others can be extended to more than one marker, but with limitations (Risch et al. 1995); in addition, they usually depend on the knowledge of the ancestral haplotype. A maximum likelihood approach allowing for multiple markers (Neuhausen et al. 1996), was shown to give results similar to those obtained by LD-based methods (Ciotti et al. 2000). Here, we apply a phylogenetic method for estimating the time to the most recent common ancestor (MRCA) of the available mutation carriers; it allows us to take into account an arbitrary number of markers and is free from assumptions about the unknown ancestral haplotype. We also compare our results with those obtained by applying other methods to our data and investigate the robustness of our approach with respect to possible errors in haplotype reconstruction or genetic map definition.

MATERIALS AND METHODS

Families and genotyping

Establishing a common database of *BRCA1* and *BRCA2* mutations between collaborating centers in Italy (Aretini et al. 2003) has greatly facilitated the collection of all known families carrying the *BRCA1**1499insA mutation. Fifteen probands, 6 recruited in Pisa – Center Italy (PI), 6 in Milan – North (MI), 2 in Padova – North-East (PD), and 1 in Naples – South (NA), were independently ascertained by four centers. Probands of families MI-E and PI-17 resulted to be second cousins upon comparing their pedigrees, and their families were merged in a single large pedigree (PI-17). We thus refer to 14 apparently unrelated families as the final dataset of the present study. We estimate that these 1499insA mutations represent about 3% of the total number of newly detected *BRCA1* mutations in Italy, and about 15% of those detected in Tuscany.

DNA was obtained from 50 subjects, 28 of whom carried the 1499insA mutation. The carrier status of the family members was determined by the contributing centers, which also collected appropriate informed consent. Thirty-seven STR markers spanning 24.6 cM around *BRCA1* (mean intermarker distance 0.7 cM), 15 upstream (14.4 cM) and 22 downstream (10.2 cM) were chosen from the DeCode map for genotyping. Genotyping was performed by DeCode Genetics. The number of successfully typed genotypes was 1,770 (95.7%).

Haplotype reconstruction

The phase of the markers in pedigrees was determined by MERLIN 0.9 (Abecasis et al. 2002), and double checked with GENEHUNTER 2.1 (Kruglyak et al. 1996). MERLIN has the advantage that it allows for slightly larger pedigrees and let the user know when the inference on haplotype reconstruction is uncertain. We assigned the phase only when it was unequivocal. To determine the phase in probands without relatives and in subjects for whom family-based reconstruction was uncertain, we used the program PHASE 2.0.2 (Stephens et al. 2001). This program infer haplotypes from population-based genotype data, and has been shown to be accurate even in case of low LD (Marroni et al. 2005). When assigning haplotypes, this software also provides a probability that the phase of each marker is correct; we considered as unequivocally reconstructed only the haplotypes for which this probability was 1. After such reconstruction, the median number of markers per individual for which phase was unambiguously reconstructed was 36, ranging 22 to 37.

Estimating mutation age by LD-based methods

We followed the approach of Bergman et al. (Bergman et al. 2001), which was derived from a previous work by Sham (Sham 1998) and produces results very similar to other published formulas (Lander and Botstein 1986; Risch et al. 1995; Serre et al. 1990). The number of generations (t) since the appearance of the mutation can be estimated by

$$\hat{t} = \frac{\ln\left(\dfrac{\hat{p}_{d1} - \hat{p}_{n1}}{1 - \hat{p}_{n1}}\right)}{\ln(1-r)} \qquad (1)$$

where the caret denotes estimated values, r is the recombination fraction between a marker and the disease allele, p_{d1} is the frequency of the founder marker allele in mutation-carrying haplotypes and p_{n1} is the corresponding frequency in non-mutated haplotypes. All LD-based methods rely on the knowledge of the ancestral haplotype, which is difficult to determine. We therefore assumed, in accordance to (Bergman et al. 2001), that the most frequent alleles among the present-day mutation-carrying haplotypes define the founder haplotype.

A limitation of LD-based methods is that the calculation is not feasible or meaningful for all available markers. At least two situations exist in which such a situation can occur (Bergman et al. 2001): 1) all alleles of the conserved haplotype, for which $p_{d1} = 1$, and thus $\hat{t} = \frac{\ln(1)}{\ln(1-r)} = 0$. This is obviously incorrect, since the time separating any two individuals is at least one generation; for this reason, previous studies excluded the ancestral haplotype from calculation; 2) the alleles for which $p_{d1} < p_{n1}$, i.e. for which the ancestral allele is more frequent in the non-carrying chromosomes than in the mutation-carrying chromosomes (this leads to the logarithm of a negative number). A further limitation of the LD-based methods is that t can usually be calculated only one marker at a time. In calculating the age of the 1499insA mutation based on LD, we adopted the usual way of calculating

t separately for each marker (equation above), and then of averaging results over all markers.

Inferring the genealogy of a founder mutation by phylogenetic analysis

The method used to infer the time to the MRCA of the 1499insA mutation carriers and to obtain a dendrogram of the carrying haplotypes (their extended genealogical tree) consisted in the following three steps, which are further detailed below: 1) building a matrix of haplotype sharing between the mutation carriers (a similarity matrix); 2) converting the similarity matrix into an evolutionary distance matrix: a function relating the length of a shared haplotype between any two individuals to the number of generations elapsed from the common ancestor was obtained by computer simulations; 3) obtaining a dendrogram (a tree with a specified branching order along a time scale) from the distance matrix.

Building the pair-wise haplotype-sharing matrix. The mutation carriers of the last generation in each family were selected, and their mutation carrying haplotypes were paired to all others; the length of the shared haplotypes expressed in cM was arranged in a triangular matrix.

Obtaining a function relating the length of shared haplotypes to the number of generations. A virtual chromosome segment consisting of the 37 typed markers was generated using the allele frequencies estimated from the sample of non-carrying founder haplotypes present in our families, and it was replicated along two parallel lines for 100 generations. The entire process was replicated 5,000 times in duplicate, using two independent algorithms, implemented in R

(R_Development_Core_Team 2005) and in Excel, respectively. Simulations in R started with a fixed haplotype, whereas a random haplotype was generated at each new simulation in Excel; this difference was purposely introduced to assess whether results depended on the starting haplotype. At each step (generation), recombination was simulated in the two parallel chromosomes using the known intermarker distances, and the length of the shared segment carrying the mutated *BRCA1* allele remaining after recombination was determined. In the absence of interference, the probability of observing a recombination event between any two markers is, by definition, equal to the distance between them expressed in Morgan (Sham 1998). When a recombination occurred in a chromosome, the alleles of the markers distal to *BRCA1* were randomly chosen from the population pool. The average length of the shared haplotype among the 5,000 replicates and their 95% confidence intervals (obtained empirically from the observed distributions) were determined at each generation. The average length of the shared haplotype as a function of the number of generations was interpolated by a hyperbola, and the obtained equation was used to convert the similarity matrix (the length of shared haplotypes) into a distance matrix (the number of generations elapsed from the common ancestor).

Building the genealogical tree of the mutation carriers. To build a dendrogram of the carrying haplotypes, the distance matrix was submitted to the program KITSCH of the package PHYLIP (Felsenstein 2003). This program builds a phylogenetic tree of a number of "species" using the

Fitch-Margoliash and Minimum Evolution methods (Kidd and Sgaramella-Zonta 1971; Rzhetsky and Nei 1993), assuming that all tip species are contemporaneous. This means that branches of the tree are constrained so that the total length from the root of the tree to all species is the same (we can reasonably assume that individuals from different families are separated from the MRCA by approximately the same number of generations). To prepare the data for analysis, five subjects who had a genotyped descendant, thus explicitly violating the method's assumption, were removed. The standard error of the time to MRCA was obtained by jackknifing (Weir 1996); in this method, the estimate of MRCA is repeated n times (n being the number of subjects included in the analysis), removing a different subject each time.

Robustness of the model to mispecified haplotypes

In order to check the robustness of our method to possible errors in haplotype reconstruction or genetic map definition, which could lead to biases in estimating the length of shared haplotypes, we repeated the analyses introducing systematic errors, *i.e.* systematically modifying the length of the shared haplotype in the similarity matrix. We devised different scenarios, in which all of the shared haplotype segments were under- or over-estimated by one, two or three markers. As the average inter-marker distance was 0.7 cM, we added or subtracted from the shared segments the corresponding mean inter-marker distances, 0.7, 1.4, and 2.1 cM respectively, and re-estimated the age of the founder mutation. In addition, we designed a worst-case scenario, in which we supposed that

all the alleles attributed to haplotypes with probability <1 were mistaken. In other words, we built a similarity matrix in which the shared haplotype between any two subjects was disrupted every time the phase of one of the two haplotypes was not unambiguously reconstructed.

RESULTS

Genotypes and haplotypes

The final dataset of the present study included 14 independent families with the 1499insA mutation, including 50 subjects in total (28 mutation carriers), genotyped for 37 STRs spanning 24.6 cM around *BRCA1*. A total of 10 recombinations were observed in 35 informative meioses (recombination fraction = 0.286), in good accordance with the expected value of 0.242. Four "families" consisted of the proband only, for whom unambiguous phase reconstruction was not possible, whereas one (the PI-17 family) included 17 typed subjects (seven carriers); the others nine families included two to six typed subjects. Among these, three did not carry information for haplotyping. Thus, haplotype reconstruction from pedigrees (MERLIN) was possible in 21 mutation carriers of seven different families. For these subjects, the median number of markers for which phase was reconstructed unambiguously was 26.5 (range 8 to 38, 38 being the number of markers of the whole haplotype including *BRCA1*). Haplotype analysis was integrated using population-based methods (PHASE). As shown in Table 1, the phase of some

markers was still not unambiguous; the median number of markers for which phase could be unambiguously assigned was 37 (range 23 to 38). The mutation carriers shared a common haplotype spanning 2.69 cM (2.83 Mb) around the *BRCA1* gene; this haplotype was not observed in any of the non-carrier chromosomes. The length of the shared haplotype among different families was higher for Northern families (range 4.4-15.6 cM), and shorter for the Southern family paired to all others (range 2.7-8.5 cM). From the table of haplotypes, arranging the length of the shared haplotypes among all pair-wise mutation-carrying haplotypes in a triangular matrix was straightforward (not shown).

Table 1

Converting the length of a shared haplotype into the number of generations since the MRCA

The model of a founder mutation implies that a particular chromosome carrying the 1499insA mutation replicated at a certain time, giving origin to two independent lines of descent (which later originated other branches). The segment of identical haplotype was gradually shortened by random recombination events on both sides of the gene, leaving in present-day descendants a shared haplotype whose expected length is a function of the number of generations elapsed since the original duplication. We estimated this function by recourse to computer simulations of the process of recombination occurring in the particular chromosome segment investigated in the present study. The results of our two independent algorithms were in excellent agreement to one another (Pearson's correlation coefficient > 0.99), thus meaning that the

haplotype used for starting the simulation does not affect the results. We then interpolated the obtained curve (see Fig. 1) with a hyperbola of the form (a*b)/(x + a), where *a* and *b* were the parameters to be estimated, and *x* was the time expressed in generations. The estimated parameter values were a = 5.46 and b = 27.0. This function was used to convert the matrix of similarities (length of shared haplotypes) into a matrix of distances (number of generations elapsed from the common ancestor). Figure 1 also shows (black squares) the length of the shared haplotype and the time to the MRCA for all pairs for which this information was available from pedigrees. Most data points are included in the 95% confidence limits of the expectation, though several outliers are visible; in particular, PI17-56 and PI17-37, being five generation apart, share only 5.79 cM, and PI17-52 and PI17-56 are six generations apart and share only 6.6 cM.

Figure 1

Drawing the extended genealogical tree of the mutation carriers

The obtained distance matrix was submitted to the program KITSCH, which produced the dendrogram, or the "extended genealogical tree", of all mutation carriers that best fitted the data (Fig. 2). As expected, the subjects of the same families cluster together, and their inferred pedigree resembles those already known. Interestingly, we can also infer how much the different families are related to each other, even though they are unaware of any relationship. It appears that the region of ascertainment of the probands (in particular considering the several families from Lombardy, Northern Italy, and from Tuscany, Central Italy) does not

Figure 2

obviously discriminate different lines of descent, as different sub-pedigrees include probands from both regions.

The time to the MRCA

The point estimate of the coalescence time of all mutation-carrying haplotypes was 30 generations, or 750 years assuming a generation interval of 25 years. The jackknife estimates of the 95% upper and lower confidence limits were 56 and 22 generations (1400 and 550 years), respectively.

We compared the above estimate with that obtained by LD analysis (equation 1). The last column of Table 1 shows the value of *(t)* computed for each of the 37 markers. Five markers showed the same allele on all mutation-carrying chromosomes (p_{n1} =1), leading to $t = 0$, and had to be excluded from the analysis. In addition, two markers (D17S788 and D17S1799) showed $p_{n1} > p_{d1}$, meaning that the supposed founder allele was more common in normal chromosomes that in mutation-carrying chromosomes. In the remaining 30 markers, estimated *(t)* ranged from 5.1 (D17S1788) to 94.9 generations (D17S1818). Average was 25 generations ± 21 (95% CL 0-67).

Checking for the robustness of the model

The phylogenetic analysis was repeated after introducing systematic errors in the estimates of the length of the shared haplotype between individuals. We first assumed that in all the 253 possible pair-wise comparisons the shared haplotype was one marker longer or one marker

shorter than our estimates; we therefore added and subtracted to each cell of the similarity matrix the value of 0.7 cM, and repeated the estimation of the time to MRCA in both scenarios. We also considered the cases in which the error was two-fold and three-fold this quantity. As shown in Table 2, adding one marker to the shared haplotype of each pair shortened the time to MRCA by 5 generations (from 30 to 25), and adding two markers shortened it by 8 generations, thus coinciding with the lower CL of the original estimate (22 generations). The time to MRCA fell outside the confidence interval only introducing an error equivalent to increasing the length of shared haplotype by three markers. Shortening the shared segment (i.e., assuming that the estimated length of the shared haplotype was biased in excess) had similar effects in the opposite direction.

As a further analysis of the robustness of the phylogenetic approach, we investigated the situation in which the length of the shared haplotype between all pairs of individuals was limited to the markers for which phase probability was unambiguous. This scenario led to an estimate of time to MRCA of 39 generations (975 years).

Table 2

DISCUSSION

We applied a phylogenetic method for estimating the time to MRCA of a *BRCA1* founder mutation. This approach is conceptually easy, as it depends on the length of the shared haplotype between individuals (for this reason we will refer to it as a haplotype sharing method), which in turn is a function of the number of generation since the MRCA. Once the

matrix of the length of shared haplotypes between all pairs of mutation carriers is converted into a distance matrix, a phylogenetic tree, or the extended genealogical tree of the carriers, can plainly be obtained using available software. Determining the phase of the typed alleles could be a problem, as it is not always possible to infer it unambiguously. In our data, only 8% of all genotypes showed phase uncertainties, but this could introduce a bias in estimating the length of the shared haplotypes in some cases. We therefore examined to what extent wrong assumptions about haplotypes could affect the results. In the extreme situation in which all markers with uncertain phase generated a disruption of the shared haplotype, the time to the MRCA shifted from 30 to 39 generations; we then concluded that this represents probably a minor problem in our analysis. In addition, we examined the effect of changing the length of shared haplotypes by one, two and even three markers, and only in the last case the estimate of the time to the MRCA fell outside the confidence interval of the initial estimation.

A major advantage of haplotype sharing methods is that the length of the shared haplotype depends solely on the accuracy of the genetic map of the investigated markers, whereas methods based on LD depend both on genetic map accuracy and on level of LD, which is strongly influenced by population histories. This can lead to inconsistencies in the estimates of mutation age between different populations. For example, Bergman and colleagues estimated an age of approximately 50 generations for a 3.7 cM haplotype, whereas we estimated an age of 25 generations for a 2.7 cM haplotype using the same LD-based method. A shorter shared

haplotype should result in a longer time to MRCA independent of the markers used, while the opposite happens. Another advantage of haplotype sharing methods is that they do not depend on the correctness of the inferred ancestral haplotype. It follows that all typed markers contribute information, whereas in LD-based methods the marker alleles common to all mutation carriers and the alleles of the ancestral haplotype whose frequency is higher in the non-affected haplotypes, must be disregarded. The ancestral haplotype itself has also to be inferred, which adds a further level of uncertainty for LD-based methods. All that means that age estimates obtained by haplotype sharing methods possess intrinsically narrower confidence intervals. In our analysis, the CL obtained by the haplotype sharing method were 22-56, compared to 0-67 obtained by the LD based method.

A critical aspect of haplotype sharing methods is the conversion of the similarity matrix into a distance matrix. We adopted a computer simulation approach because it can take into account a bias potentially affecting the estimate of the length of the shared haplotype; we considered as being IBD any two identical chromosome segments, whereas some of the distal markers could in fact be shared IBS. This happens when two recombinant chromosomes carry the same array of alleles in the region of the crossing over, and causes the true length of the shared segment appearing longer than it is in reality. By picking up alleles at random from the population pool beyond a crossing over, we obtained at least a partial solution to this problem. However, other approaches may

probably be proposed. Once the distance matrix is obtained, a phylogenetic analysis follows straightforwardly.

Drawing of a dendrogram of all mutation carriers is another major advantage of haplotype sharing methods. In this way it is possible to estimate the time to MRCA of any two individuals, and not only of the MRCA common to all investigated subjects. In other words, it is the entire evolutionary history of a particular founder mutation that can be examined. This can be of interest for checking the consistency between genealogical and genetic data, for example in large families like PI-17. In addition, drawing the genealogy of mutation carriers makes it possible to compare the geographic distribution of the families with the inferred tree. For example, family MI-B (ascertained in Milan) appears to be closely related to families PI-223 and PI-227 (ascertained in Tuscany), which would imply the recent migration to Northern Italy by an ancestor of MI-B; upon examination, however, it turned out that family MI-B was in fact resident of Tuscany. Family MI-F also reported to be originated from this region. Thus, it seems likely that the common ancestor of all mutation carriers lived in Tuscany. Our best estimate is that this mutations was already present in the population in late Medieval times.

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Legends to figures and tables

Table 1. Inferred haplotypes carring the BRCA1*1499insA mutation. cM: distance in cM from BRCA1. Het: Heterozygosity. Bold type: alleles assigned by pedigree analysis; normal font: alleles assigned with probability = 1.0 by PHASE; when probability of assignment is < 1.0 both alleles are reported; "?": genotype not available. The last two columns report data relevant for age estimate based on the LD method (the inferred ancestral allele and the corresponding calculated mutation age, respectively)

Table 2: Variation of estimated time to MRCA when introducing systematic errors.

Figure 1. Decay of the length of shared haplotype as a function of time: simulation results (thin line) and interpolated function (thick line). Dotted lines: confidence limits of the simulated process. Black squares: length of the shared haplotype for the pairs with known time to MRCA.

Figure 2. Extended genealogic tree inferred for the 14 independently ascertained families carrying the BRCA1*1499insA mutation
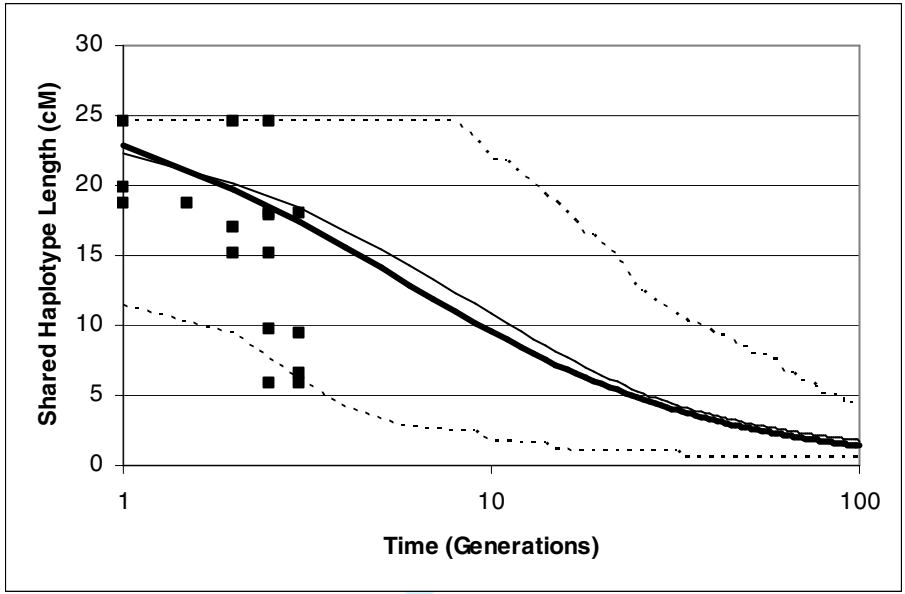
cM	Marker	Het	III-A-4	III-B-3	III-D-10	III-D-7	III-D-9	III-F-3	III-F-1	IIIA-93-3	IPD-113-3	IPD-113-5	IP-124-3	IP-207-6	IP-207-9	IP-223-4	IP-233-3	IP-223-4	IP-313	IP-316	IP-318	IP-6-1	IP-6-5	IP-17-37	IP-17-40	IP-17-47	IP-17-52	IP-17-56	IP-17-61	IP-17-62	Ancestral	(t)
2.75	D17S2194	0.76	3,4	4	4	4	4	5,4	3	3	2	2	4,3	3	2	4	4	4	6	3	5	3	5	4,3	2,5	4	4	4	4	4	4	6.9
1.47	D17S1293	0.86	9,6	10	5	5	5	9,6	10	2	11	4	6	3	11,4	5	3	3	6	6	9	3	5	3	3	1,6	2	3	3	3	3	17.4
0.51	D17S1842	0.48	4	?	5	4	4	4	4	4	4	3	4,5	3	5,4	5	3	4	4	4	5	5	5	4	4	4	5	4	5	3	3	22.5
0.17	D17S933	0.78	2	3	3	3	3	2,4	?	2	4	2,3	2	4	8	3	3	2	3	2	3	3	4	4	3,4	4	4	4	4	2	2	18.3
0.67	D17S1846	0.55	2,5	?	3	3	3	3	?	3	5	5,6	3,5	4	5,3	3	3	3	3	5	4	3	2	2	3	3	2	2	2	2	2	54.5
2.2	D17S1833	0.52	2	3	2	2	2	2	3	5	2	5,6	3,1	6	2	2	2	3	3	3	3	3	2	2	2	3	3	2	2	2	2	59.8
0.88	D17S1867	0.62	3	3	3	3	3	1,2	3	2	2	2	2	2	4	2	2	2	2	3	2	2	2	2	3	3	3	3	3	3	3	18.9
0.92	D17S1788	0.63	1	4	4,3	4	4,3	3,1	4	4	4	4	2,4	2,4	4	2	2	4	2	4	4	4	4	4	2,4	1	4	3	3	3	3	5.1
0.58	D17S1836	0.56	2,3	2	3	3	3	3	2,3	3	2	2	2	2	3	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	34.7
0	D17S2262	0.79	5,3	3	5	5	5	5	2,3	5	3	3	3	3	2	3	2	3	3	4	2	2	2	2	3	7	7	2	2	3	3	46.2
1	D17S1818	0.82	12,2	10	6	6	6	6	10,4	6	10	10	10	10	4	10,11	7	7	7	7	4	4	4	4	4	4	3	4	4	4	4	94.9
0.81	D17S1814	0.73	6	6	5	5	5	5	6	5	6	6	6,1	6,1	6	6	6	1	1	6	6	6	6	6	7,1	1	6	6	6	6	6	20.3
0.94	D17S1299	0.49	3	3	3	3	3	3	4,3	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	4,3	3	3	3	3	3	3	0.0
0.74	D17S1563	0.61	1,2	1,2	2	2	2	2	2	2	2	2,7	2,7	2	2	2,3	2,3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.0
0.49	D17S1801	0.51	2	2	2	2	2	2	2	2	2,7	2,7	2	2	2,7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.0
0.52	BRCA1	N/A	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
0.60	D17S951	0.73	6	6	6	6	6	6	6	6	6	6	6	6	6	3,6	3,6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0.0
0.88	D17S1860	0.78	1	1	1	1	1	1	1	6,4	1	1	6,1	1	1	5	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10.8
0.11	D17S1861	0.79	6	6	6	6	6	6	6	2,6	6	6	7	5	6	6	6	6	6	7	7	6	6	6	6	6	6	6	6	6	6	15.5
0.08	D17S1920	0.67	2	2	2	2	2	2,1	2	2,1	2	2	3	2	2	2	1	1	1	1	1	2	2	1	1	1	1	2	2	2	2	14.3
0.24	D17S693	0.28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.0
0.94	D17S931	0.70	3	3	3	3	3	6,3	3	6	3	3	3	3	3	3	3	6	6	5	5	3	3	3	3	3	3	3	3	3	3	18.8
0	D17S1859	0.68	2	2	2	2	2	5	2	4	2	2	2	2	2	2	2	2	2	6	4	4	2	2	3	2	2	2	2	2	2	8.4
0	D17S1785	0.78	1	1	1	1	1	6	1	3	1	1	1	1	1	1	1	3	3	3	5	5	1	1	1	1	1	1	1	1	1	11.4
0.22	D17S958	0.67	3	3	3	3	3	1	3	1	3	3	3	3	3	3	3	3	6	6	3	3	3	3	3	3	3	3	3	3	3	14.4
0.63	D17S2180	0.71	4	4	4	3,4	4	2,3	4	4	4	4	4	4	4	4	4	1	1	1	4	4	4	4	4	4	4	4	4	4	4	9.2
0.37	D17S1868	0.72	5	5	5	5	5	3	5	5	5	5	5	5	5	5	5	5	5	3	6	6	5	5	5	5	5	5	5	2	2	13.8
2.28	D17S797	0.57	2	2	2	2	2	1,2	1	1	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	16.1
1.04	D17S1795	0.71	6	3	3	3	3	2,4	6	3	3	3	3	3	3	6	3	3	7	7	2	2	2	2	3	5	5	3	3	3	3	17.4
0.16	D17S1815	0.57	4	3	4	4	4	4,3	4	3	2	2	3	3	2	3	4	3	3	4	4	3	3	3	3	3	3	3	3	3	3	80.3
2.07	D17S1877	0.71	1	4,1	1	1	1	3,1	1	1	3	3	1,3	3	4	1	1	1,4	3	3	3	1	1	1	1	1	1	1	1	1	1	18.3
0.28	D17S956	0.70	2	1	2	2	2	2,3	1	3	5	5	3,2	?	1,3	1	3	3	1	1	3	3	1	1	4	5	1	3	3	1	1	39.7
1.16	D17S788	0.72	5	5	3	3	3	5	5	1	2,6	1	5	1	1	3	1	3	6	5	2	2	2	3	5	6	1	3	5	5	5	N/A
0.00	D17S1865	0.65	2	7	5	5	5	5	7	5	7	5	1	5	5	5	5	6	6	2	2	2	5	3	1	2	5	5	5	5	2	28.1
0.89	D17S752	0.74	3	5	2	2	2	2,1	1	2	3	3	1	3	6,5	4	1	1	1	1	1	1	1	2	2,1	1	1	1	1	1	1	9.9
0.72	D17S790	0.72	7	7	4	4	4	9,3	7	1	6	2	4	4	3,5	4	3	8	5	5,1	4	4	4	5	3	4	7	3	4	6,4	4	15.6
0.25	D17S787	0.77	2	3	3	3	3	2	1	6	2	2	5,4	2	5,2	3	3	3	1	1	2	2	2	2	5	3	2	2	2	2	2	12.5
	D17S1799	0.54	3	1	3	3	3	1	3	1	1	1	1	1	1	1	1	3	3	3	1	1	1	3	3	1	3	3	3	1	1	N/A

Variation (cM)	Generations	Years
-2.1	63	1575
-1.4	48	1200
-0.7	37	925
0	30	750
0.7	25	625
1.4	22	550
2.1	19	475

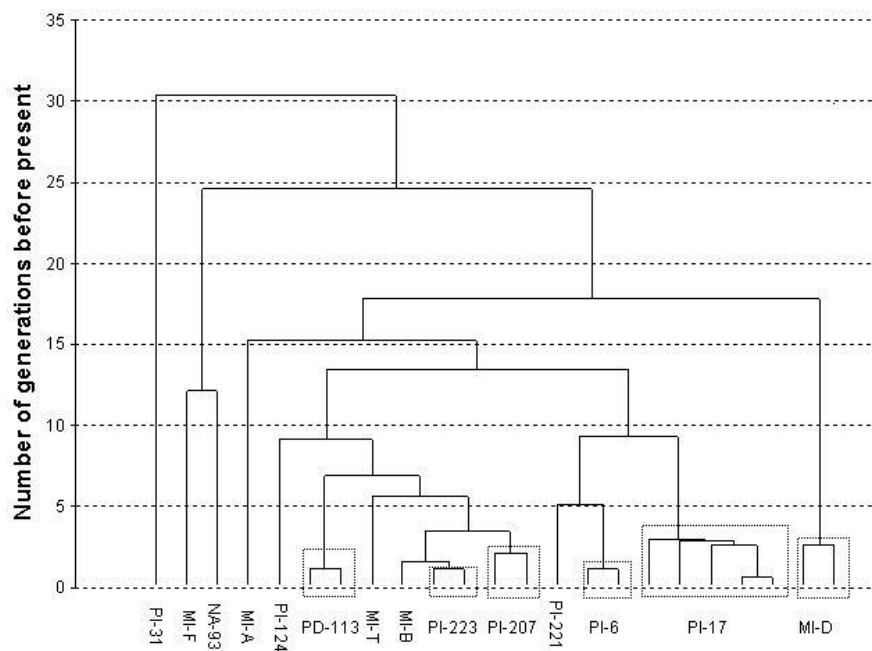**Figure 2. Extended genealogic tree inferred for the 14 independently ascertained families carrying the BRCA1*1499insA mutation**

Capitolo 3

TUMORI EREDO-FAMILIARI

A. Contegiacomo, M. Pensabene, C. Condello, I. Capuano, I. Spagnoletti, E. De Maio

INTRODUZIONE

È attualmente noto che i tumori sono rappresentati da forme sporadiche e da forme a carattere familiare ed ereditario. Le forme familiari sono contraddistinte dallo sviluppo del tumore in più membri della famiglia appartenenti tutti alla stessa generazione. Le forme ereditarie, invece, si presentano con caratteristiche cliniche peculiari, contraddistinte dallo sviluppo del tumore in più membri della famiglia appartenenti a generazioni successive, in età più precoce dell'età tipica di sviluppo, in forma bilaterale per tumori che originano da organi pari e in più organi nello stesso soggetto. Nella pratica clinica oncologica appare dunque evidente la necessità di riconoscere queste diverse forme tumorali.

I tumori familiari rappresentano il 20% di tutti i tumori e per queste forme si ipotizza la condivisione a livello familiare di fattori ereditari, quali geni a bassa penetranza, e fattori ambientali.

I tumori ereditari costituiscono il 5-10% di tutti i tumori e si sviluppano in soggetti che hanno ereditato una mutazione genetica che conferisce loro una *predisposizione* allo sviluppo di patologie neoplastiche. Molti geni di predisposizione sono stati identificati e clonati e, per alcuni di essi, è anche possibile effettuare un test genetico. La tabella 3.1 fornisce un elenco delle sindromi tumorali con predisposizione su base ereditaria attualmente note.

I progressi scientifici nell'ambito dei tumori su base eredo-familiare hanno aperto nuovi scenari nel campo della prevenzione, della diagnosi e della gestione di queste forme tumorali. Nella pratica clinica, ciò si è tradotto nella necessità di *servizi clinici specifici* (*cancer family clinic*), di *oncologi esperti nella gestione* dei tumori su base ereditaria e familiare e della presenza dello *psiconcologo* nelle équipe curanti. Inoltre, considerando gli aspetti clinici, psicologici ed etici della problematica dei tumori eredo-familiari, si è resa necessaria l'introduzione di un counseling genetico specifico per il *setting oncologico* (*counseling oncogenetico*). Il counseling oncogenetico sta acquisendo anche in Italia una precisa identità scientifica e metodologica, definendo precisamente le finalità d'intervento, i modelli organizzativi e le metodiche da utilizzare nella pratica clinica.

In questo capitolo verranno trattati il counseling oncogenetico, per gli aspetti sia medici sia psicologici, e alcune tra le sindromi tumorali eredo-familiari, relativamente agli aspetti clinici, genetici e di management, che più frequentemente richiedono un approccio oncologico specifico (tab. 3.1).

COUNSELING ONCOGENETICO

Il concetto di counseling oncogenetico rappresenta un'evoluzione in ambito oncologico della definizione di counseling proposta dall'*American Society of Human Genetics*, quale "processo di comunicazione tra professionisti esperti nel settore dei tumori eredo-familiari e una o più persone di una famiglia che si ritengono a rischio di tumore". Sulla base di questo concetto il counseling oncogenetico si configura come una tecnica di intervento che consente di individuare in modo appropriato il rischio eredo-familiare, di definirlo e di gestirlo. In particolare, il counseling oncogenetico viene offerto a:
- soggetti affetti con anamnesi personale e/o familiare oncologica suggestiva per forme di tumore eredo-familiare;
- soggetti sani con anamnesi familiare oncologica suggestiva per forme di tumore eredo-familiare.

Tabella 3.1 Sindromi tumorali eredo-familiari che più frequentemente richiedono un approccio oncologico

Sindrome	Spettro tumorale	Trasmissione	Geni
Mammella/ovaio (HBOC)	Mammella; ovaio; endometrio; cervice; prostata; stomaco; colon; pancreas; vie biliari; melanoma	Autosomica dominante	BRCA1 BRCA2
Li-Fraumeni	Tessuti molli (sarcomi); mammella; Osso (osteosarcoma); leucemia; encefalo; surrene	Autosomica dominante	p53
Cowden	Mammella; tiroide; endometrio	Autosomica dominante	PTEN
Poliposi familiare del colon (FAP)	Colon	Autosomica dominante	APC
Tumore del colon-retto ereditario non poliposico (HNPCC)	Colon; endometrio; ovaio; vescica; pelvi renale; uretere; pancreas; stomaco; piccolo intestino; vie biliari	Autosomica dominante	MLH1 MSH2 MSH6
Tumore gastrico diffuso ereditario (HDGC)	Stomaco	Autosomica dominante	CDH1
Melanoma ereditario	Cute (melanoma); pancreas	Autosomica dominante	CDKN2A CDK4
Prostata ereditaria	Prostata	Autosomica dominante	HPC1 HPC2 PCAP PCBC PRCA
		X-linked	HPCX
MEN1	Paratiroidi; pancreas; ipofisi; tiroide	Autosomica dominante	MEN
MEN2	Tiroide (midollare); surrene (feocromocitoma); paratiroidi	Autosomica dominante	RET

Scopi peculiari del counseling oncogenetico sono:
- effettuare la stima del rischio di sviluppare tumori su base ereditaria e familiare;
- offrire la possibilità del test genetico nei soggetti con rischio ereditario, laddove disponibile;
- programmare adeguate misure di gestione del rischio, secondo le linee guida nazionali e internazionali o programmi locali di ricerca specificamente formalizzati e approvati;
- promuovere il processo educazionale e di consenso consapevole attraverso una partecipazione attiva del soggetto con particolare riguardo alle fasi decisionali, quali la scelta di sottoporsi al test genetico e/o a specifiche misure di gestione del proprio rischio;
- effettuare un *assessment* psicologico e fornire uno spazio di elaborazione psichica e di contenimento emotivo.

È necessario identificare un *setting* adeguato agli scopi dichiarati di counseling, con particolare attenzione al luogo e alle modalità di esecuzione. È molto importante che il counseling sia svolto utilizzando un *linguaggio* semplice e chiaro, adeguato e adattato alla persona a cui è rivolto.

Un aspetto peculiare del counseling è la *multidisciplinarietà*. È necessario l'intervento di diverse competenze, dal momento che nell'ambito del counseling si impiegano tecnologie diagnostiche complesse, procedure medico-legali specifiche, misure di prevenzione medica e chirurgica specialistiche. È per questo motivo che frequentemente entrano in gioco diverse figure professionali quali, per esempio, l'oncologo, il genetista, lo psicologo, il biologo molecolare, il medico legale, il ginecologo, il chirurgo. Considerando la necessità di una specifica competenza nel management dei tumori eredo-familiari e la necessità di fornire informazioni aggiornate rispetto ai metodi diagnostici e alle opzioni di prevenzione e di trattamento disponibili, l'oncologo sembra avere un ruolo chiave nel processo di counseling,

dall'identificazione alla gestione del rischio. Per la complessità della problematica dei tumori eredo-familiari sul piano oncologico e psicosociale, particolarmente importante è il lavoro in équipe integrata, che contempla il ruolo preminente dell'oncologo affiancato dallo specialista dell'area psicologica.

A partire dal 1999, nel contesto nazionale italiano si è costituito un Network, supportato dal Ministero dell'Università e della Ricerca (MIUR), per lo "studio dei tumori eredo-familiari della mammella e/o dell'ovaio" nell'ambito del quale è stato validato un ***modello di counseling a multistep***. Tale modello è strutturato in più ***fasi***, ciascuna con diversi e specifici momenti di intervento dell'oncologo. L'intervallo tra due fasi successive è volto a promuovere nel consultante la rielaborazione dei contenuti di ciascun intervento della consulenza, consentendogli altresì di acquisire maggiore consapevolezza e autodeterminazione delle proprie scelte. In questo modo il consultante può elaborare le informazioni ricevute e adattarsi ai contenuti, esprimendo un consenso non solo "informato" ma anche "consapevole" (fig. 3.1). Tale modello contempla un approccio globale al soggetto affetto o al soggetto sano a rischio
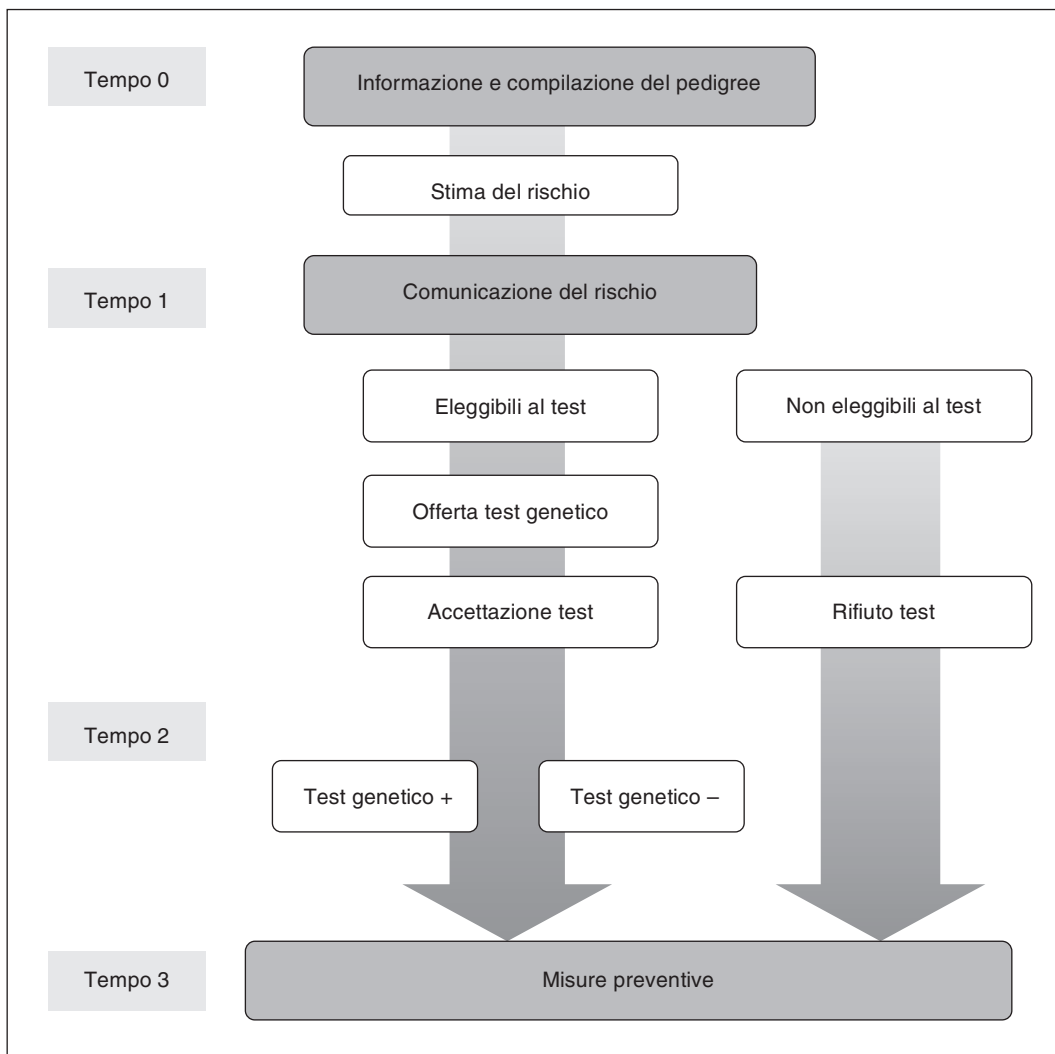
Figura 3.1 Modello di counseling oncogenetico a multistep (adattato da Contegiacomo et al. *Annals of Oncology*, 2004).
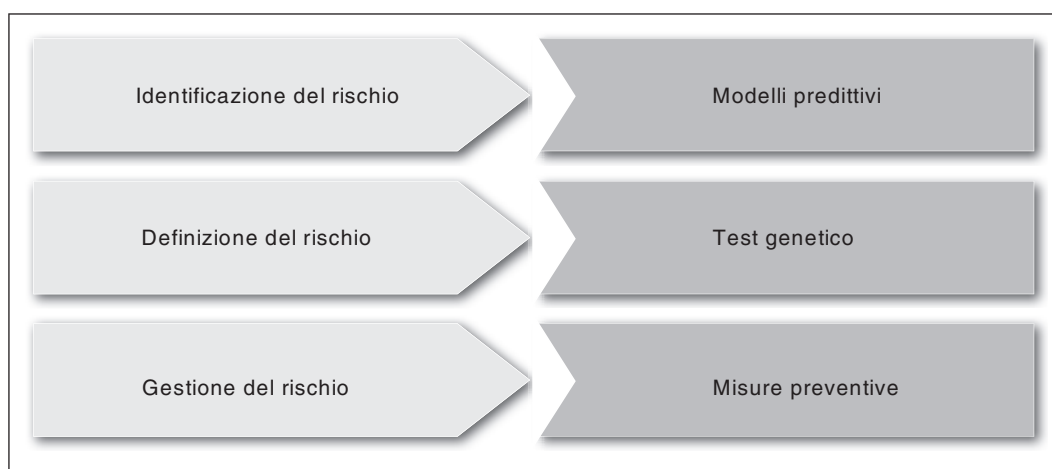
Figura 3.2 Finalità del counseling oncogenetico.

per tumore eredo-familiare, attraverso l'identificazione, la definizione e la gestione del rischio con l'obiettivo principale di promuovere la diagnosi precoce di tumori eredo-familiari (fig. 3.2).

La prima fase d'intervento, *Tempo 0*, consiste nell'informare il soggetto circa lo stato delle conoscenze scientifiche sui tumori ereditari e familiari, sui modelli e sulle procedure disponibili per stimare il rischio di predisposizione ereditaria al cancro, sui vantaggi e sui limiti del test genetico, sulle implicazioni del risultato del test genetico, nonché sulle misure disponibili per la prevenzione secondaria e le modifiche dello stile di vita. Si procede, previa acquisizione del consenso informato, alla raccolta dell'anamnesi personale e familiare oncologica attraverso la costruzione dell'albero genealogico per almeno tre generazioni, considerando sia la linea materna sia quella paterna. Per ciascun caso di tumore è necessaria la verifica attraverso l'acquisizione dell'esame istologico o di documentazione clinica inerente alla diagnosi. L'anamnesi personale e l'analisi del pedigree consentono di definire, in alcuni casi, il *profilo di rischio* del consultante a tre livelli – ereditario, familiare e personale – attraverso l'impiego dei modelli predittivi di rischio disponibili.

A oggi, sono disponibili modelli di stima del rischio su base clinica, epidemiologica e statistica, specifici per alcune delle sindromi tumorali eredo-familiari; il loro utilizzo integrato consente di attribuire i soggetti a diverse categorie di rischio. Nei soggetti a rischio ereditario si considerano l'eleggibilità all'analisi mutazionale e, a prescindere dall'esecuzione del test genetico, le misure di gestione del rischio. Nei soggetti a rischio familiare, non eleggibili all'analisi mutazionale, si considerano misure di sorveglianza adeguate alla categoria di rischio.

Nella fase successiva, *Tempo 1*, al soggetto viene data la comunicazione del rischio, incoraggiando e sollecitando eventuali richieste di chiarimento. In tale contesto, vengono discusse con il consultante le implicazioni che la stima del rischio ha sia per se stesso sia per i familiari. In presenza di un rischio ereditario, si comunica al soggetto la possibilità di eseguire il test genetico per l'analisi mutazionale dei geni a oggi noti, in accordo con le linee guida dell'American Society of Clinical Oncology (ASCO) (tab. 3.2). Sono discusse con il soggetto le problematiche relative ai vantaggi e ai limiti del test genetico (tab. 3.3). L'analisi mutazionale viene, in genere, eseguita sul soggetto affetto della famiglia che ha sviluppato il tumore in età più precoce in accordo con le linee guida nazionali di biosicurezza che regolamentano l'esecuzione del test genetico in Italia. L'analisi mutazionale viene eseguita presso laboratori di biologia molecolare che soddisfino gli standard di accuratezza previsti. Al *Tempo 2* viene comunicato il risultato del test genetico e, successivamente, al *Tempo 3,* vengono riconsiderate le misure di gestione del rischio, quali le modifiche dello stile di vita, la sorveglianza cli-

Tabella 3.2 Linee guida ASCO sui test per l'analisi dei geni di suscettibilità

Indicazioni per il test genetico	Offerto quando il soggetto ha un'anamnesi personale o familiare suggestiva di una condizione di predisposizione ereditaria allo sviluppo del tumore
	Il test può essere interpretato adeguatamente
	Il risultato del test guida la diagnosi o influenza la gestione clinica e/o chirurgica del soggetto e/o dei membri della famiglia a rischio ereditario per lo sviluppo del tumore
	Offerto soltanto nel contesto di un counseling pre e post-test
Counseling per la gestione clinica dopo il test	L'oncologo nel counseling pre e post-test discute gli eventuali rischi e/o benefici delle diverse misure di prevenzione
Regolamento del test genetico	Regolamento dei laboratori che forniscono i test genetici per la verifica della predisposizione ereditaria allo sviluppo del tumore: reagenti utilizzati, verifiche crociate tra laboratori di riferimento, standardizzazione del referto del test genetico
Protezione dalle discriminazioni assicurative e lavorative (USA)	Leggi federali proibiscono la discriminazione da parte di assicurazioni e/o datori di lavoro sulla base di una suscettibilità individuale allo sviluppo di un tumore
Copertura dei servizi	Tutti gli individui a rischio significativamente aumentato per lo sviluppo di un tumore ereditario devono avere accesso al counseling, al test, allo screening, alla sorveglianza e a tutti gli interventi medici e chirurgici correlati

Tabella 3.3 Vantaggi e limiti del test genetico per sindromi tumorali ereditarie

Vantaggi	Ridurre il distress in caso di test negativo
	Evitare la sorveglianza clinico-strumentale intensiva e altre strategie di prevenzione in caso di test negativo
	Prendere decisioni cliniche e di stile di vita sulla base del risultato del test
	Prendere decisioni riguardanti misure di chirurgia profilattica in caso di test positivo
	Coinvolgere altri membri della famiglia in caso di test positivo
	Eliminare l'incertezza circa la suscettibilità ereditaria in caso di test positivo
Limiti	Avere la percezione di assoluta assenza di rischio di sviluppare tumori nel corso della propria vita in caso di test negativo
	Difficoltà a comunicare il risultato del proprio test ai familiari in caso di risultato positivo
	Senso di colpa sulla possibile trasmissione di rischio genetico alla prole in caso di test positivo
	Eventuale discriminazione sociale in caso di test positivo
	Eventuale aumento del distress in caso di test positivo

nico-strumentale, la chirurgia profilattica e la chemioprevenzione, evidenziando i pro e i contro di ciascuna di esse (tab. 3.4). In caso di test genetico positivo, viene offerta al soggetto la possibilità di estendere il counseling oncogenetico agli altri membri sani e affetti della famiglia. Laddove è stimato un rischio di tipo familiare, pur non essendoci indicazione all'esecuzione del test genetico, individuando comunque un rischio di sviluppare tumori superiore a quello della popolazione generale, vengono discusse con il soggetto le modalità di gestione del rischio disponibili a oggi e la necessità di aderire a programmi di sorveglianza modellati sul livello di rischio.

ASPETTI PSICOLOGICI

Impatto psicologico della problematica eredo-familiare

La patologia oncologica eredo-familiare è considerata, a ragione, una *relational disease* per le numerose implicazioni biologiche, intrapsichiche e interpersonali e i peculiari risvolti sul piano personale, familiare e sociale. Il "carattere familiare" dei tumori ereditari implica necessariamente il coin-

Tabella 3.4 Vantaggi e limiti delle misure di gestione del rischio

Misura	Vantaggi	Limiti
Sorveglianza	Preservare l'organo	Mancanza di linee guida
	Preservare la capacità riproduttiva	Mancanza di dati di efficacia
	Conservare l'immagine corporea	Cancro intervallo
	Disponibilità di metodiche di diagnostica per immagini più sensibili	Possibile distress psicologico legato ai controlli frequenti
Chirurgia	Riduzione del rischio	Rischio residuo
		Morbilità e mortalità legate alla procedura chirurgica
		Possibile distress psicologico
Chemioprevenzione	Prevenzione primaria	Disponibili solo *trials*
		Effetti collaterali del farmaco

volgimento reale o fantasmatico della famiglia. L'ereditarietà, infatti, pone sempre l'individuo in rapporto con i familiari, siano essi collocati sullo stesso piano generazionale, o piuttosto in linea ascendente o discendente. In molti casi la valutazione del rischio ereditario, così come la sua definizione attraverso l'analisi del genoma, consente di prefigurare possibili scenari di rischio non soltanto per il consultante ma anche per i consanguinei, con la necessità di un adeguato management sia sul piano oncologico che psicologico.

Particolare valenza sul piano psicologico riveste la ***percezione del rischio***. Infatti, un'alta prevalenza in famiglia di patologie neoplastiche o il risultato positivo al test genetico, benché non rappresentino una diagnosi di cancro, possono avere un significativo impatto sul piano psicologico ed emozionale del soggetto. A tale riguardo, numerosi studi attestano che la percezione del rischio è spesso irrealistica e associata ad alti livelli di *"cancer distress"*, che possono interferire con l'equilibrio psicologico del consultante, così come con la partnership e la compliance. La percezione del rischio, infatti, essendo di per sé un'astrazione, risulta modulata non soltanto dal dato empirico, ma anche dalle caratteristiche di personalità e dal contesto familiare e sociale a cui il soggetto si riferisce. Individui che tendono ad attribuire a se stessi la responsabilità di ciò che accade loro (*locus of control interno*) sembrano disporre di un maggiore senso di autoefficacia e sembrano aderire più facilmente ai programmi di prevenzione, rispetto a coloro che danno grande peso a entità o a situazioni esterne non controllabili, come, per esempio, al destino (*locus of control esterno*). Anche la tipologia dei legami familiari può condizionare negativamente la percezione del rischio, nel senso che individui con vincoli identificatori più forti nel contesto del proprio sistema familiare tenderebbero a vedere una comunanza di destino anche rispetto alla malattia.

Un altro aspetto dibattuto in seno alla comunità scientifica riguarda l'***impatto*** del risultato del ***test genetico*** in popolazioni a rischio. Sebbene i risultati degli studi siano per molti versi contrastanti, è stato dimostrato, per la sindrome ereditaria della mammella e dell'ovaio (HBOC), un impatto psicologico positivo del test genetico nella popolazione di donne sane a rischio non portatrici di mutazione genetica e non sono stati altresì registrati effetti psicologici abnormi tra le portatrici di mutazione. Sembra che il test genetico per BRCA1/2 alteri i livelli di distress psicologico soltanto temporaneamente, mentre altre caratteristiche, esterne al test, legate a fattori personologici, familiari e sociali e al supporto emozionale possano incidere significativamente sull'intensità del distress nel lungo periodo. A tale riguardo, studi recenti hanno rilevato che il supporto sociale ed emozionale fornito dal partner e/o dai familiari può essere considerato un'importante risorsa di coping in relazione alla problematica eredo-familiare, con un impatto positivo sui livelli di distress. Inoltre, reazioni abnormi al *dépistage* genetico sembrano essere poco frequenti quando il test genetico è proposto in un percorso di counseling che contempli chiare informazioni circa la problematica oncologica eredo-familiare e il supporto emozionale soprattutto in fase pre e post test.

Anche la ***gestione del rischio***, attraverso i programmi di prevenzione oggi disponibili, può avere un impatto sulla sfera psicologica. In linea generale, sembra che la sorveglianza clinico-strumentale non alteri significativamente l'equilibrio psicologico del consultante nel lungo periodo, mentre la chirurgia profilattica, in taluni casi, può influire negativamente sui livelli di distress, sul funzionamento sessuale e sull'immagine corporea. Diversi studi, tuttavia, attestano che i livelli di distress associati alla chirurgia profilattica tendono a normalizzarsi in fase postchirurgica e la maggior parte dei soggetti sembra soddisfatta della scelta compiuta. Inoltre, la chirurgia ricostruttiva, quando richiesta, così come la disponibilità di un adeguato supporto psicologico sembrano esitare in un migliore adattamento alla problematica, con un positivo impatto sulla qualità di vita dei soggetti che vi si sottopongono.

Per questa complessa serie di fattori, il counseling oncogenetico, che contempla una presa in carico globale della persona, sembra fornire un adeguato supporto emozionale durante tutto il percorso della consulenza, dall'identificazione alla gestione del rischio. A tale riguardo, i risultati di due metanalisi hanno mostrato che il counseling, attraverso il lavoro in equipe integrata, riduce i livelli di distress, promuove l'accuratezza della percezione del rischio e favorisce la scelta di programmi di prevenzione *ad hoc*.

Aspetti psicologici del counseling oncogenetico

Il counseling viene considerato un *processo relazionale di tipo professionale*, ispirato ai principi di onestà, di empatia e di rispetto e finalizzato a interventi informativi, supportivi, educazionali e di problem-solving, che nel contesto dei tumori eredo-familiari è rivolto sia al consultante sia ai familiari**. Tra i modelli operativi utilizzati in ambito oncologico, il counseling integrato multidiscplinare assume un ruolo di primo piano vuoi per la ricerca, vuoi per gli aspetti assistenziali. La ricerca scientifica in ambito psico-oncologico, infatti, pone in risalto l'importanza della stretta relazione tra aspetti oncologici e psicologici, al fine di organizzare le strategie di counseling in linea con le risorse, il sistema valoriale e le capacità di autodeterminazione del consultante e della sua famiglia.

Il ***ruolo dello psiconcologo*** nell'équipe multidisciplinare, durante tutto il percorso di counseling, è teso a promuovere l'autodeterminazione consapevole, il miglioramento del senso di autoefficacia e il contenimento dell'impatto intrapsichico e interpersonale della problematica oncologica eredo-familiare.

Gli aspetti cardine dell'intervento psiconcologico contemplano:
- il ***colloquio clinico***, considerato quale strumento di promozione del processo di conoscenza e di cambiamento, che privilegia e valorizza la soggettività dell'utente. Particolare attenzione viene data all'"analisi della domanda", focalizzata su motivazioni, aspettative e fantasie rispetto al counseling oncogenetico, al fine di orientare la pianificazione del progetto di cura dell'équipe con il coinvolgimento attivo e proficuo del consultante e della sua famiglia;
- le ***strategie di counseling*** finalizzate alla gestione della problematica eredo-familiare, agli aspetti relazionali e familiari, ai disagi legati alla malattia e alle eventuali angosce di morte che a essa spesso si accompagnano. Viene, quindi, dato uno spazio all'individuazione e alla pianificazione di strategie di gestione di problematiche esistenziali e alla decisione consapevole rispetto al test genetico, favorendo la riorganizzazione delle risorse esistenti, nel rispetto delle capacità di autodeterminazione dell'individuo;
- l'utilizzo dei ***test psicologici*** per una valutazione comparativa, incentrata su parametri psicologici, in riferimento a un sistema nosografico condiviso dalla comunità scientifica. L'utilizzo dei test è giustificato dalla necessità di completare un percorso di assessment e per monitorare alcuni parametri anche a scopo di ricerca.

La valutazione dell'assetto psicologico, la conoscenza delle variabili psicosociali dell'utente e la disponibilità del supporto emotivo sono parte integrante del counseling oncogenetico, mirato ai bisogni e rispettoso delle capacità e della condizione psicologica peculiare del consultante.

CONSENSO INFORMATO E PRIVACY

Il consenso informato, così come la privacy, è un elemento di fondamentale importanza nelle procedure di counseling oncogenetico.

In particolare, per quanto concerne il consenso informato, le linee guida ASCO suggeriscono di includere i seguenti aspetti:

- elicitazione e discussione delle motivazioni, delle aspettative e degli obiettivi del consultante;
- informazioni circa i fattori genetici che possono ripercuotersi sulla suscettibilità ai tumori;
- informazioni relative alla stima del rischio;
- discussione dei potenziali benefici, rischi e limiti del test genetico;
- informazione circa i laboratori deputati a eseguire il test;
- implicazioni relative ai possibili risultati del test;
- opzioni preventive disponibili e loro efficacia;
- discussione sulle implicazioni psicologiche, familiari e sociali;
- possibili ripercussioni del risultato del test sulle misure preventive e sullo stile di vita;
- possibilità di declinare la scelta e la comunicazione del risultato del test.

Nel rispetto delle norme sulla privacy, un ulteriore aspetto di rilievo è quello di assicurare la riservatezza dei dati relativi al counseling, ivi compresi i risultati del test genetico. L'ottemperanza delle norme prevede che i dati relativi al profilo di rischio e al risultato del test genetico non debbano essere riportati routinariamente all'interno della documentazione clinica.

SINDROMI EREDITARIE DELLA MAMMELLA

Sindrome ereditaria della mammella e/o dell'ovaio (HBOC)

CARATTERISTICHE CLINICHE

Il 5-10% dei tumori della mammella è considerato ereditario. L'84% è attribuibile a mutazioni a carico dei geni BRCA1 e BRCA2 responsabili della sindrome ereditaria della mammella e dell'ovaio (HBOC). Le *caratteristiche cliniche* che fanno sospettare un tumore della mammella ereditario sono l'alta frequenza con cui il tumore mammario e/o dell'ovaio si presenta nella famiglia, la distribuzione in due generazioni diverse secondo un pattern di trasmissione autosomica dominante. Inoltre, è necessario che vi siano altre caratteristiche di presentazione quali almeno tre membri della famiglia affetti da tumore della mammella e/o dell'ovaio, l'età di insorgenza precoce (minore di 40 anni), la bilateralità del tumore. Altre caratteristiche cliniche suggestive di una forma di tumore mammario ereditario sono l'insorgenza nel maschio, la diagnosi nella stessa donna di tumore sia della mammella sia dell'ovaio, l'etnia. Infatti, l'etnia degli Ebrei Ashekenazi ha un'alta prevalenza di tumori ereditari sostenuti da mutazioni a carico dei geni BRCA1 e BRCA2 (2,3% *versus* 0,1% della popolazione generale).

Il Breast Cancer Linkage Consortium ha riportato un *rischio cumulativo di tumore della mammella* per i portatori di mutazioni a carico di BRCA1 e BRCA2 dell'80-85% nel corso della vita e un rischio cumulativo di tumore dell'ovaio pari al 30-60%. Inoltre, la sindrome del tumore della mammella e/o dell'ovaio ereditario si caratterizza per l'alta aggregazione di altri tumori in famiglia. Infatti, è riportato un rischio cumulativo di sviluppare tumori della mammella controlaterale, tumori della prostata e tumori del colon-retto. Inoltre, è riportato un rischio relativo alto di sviluppare tumori dell'endometrio, della cervice e del pancreas per i portatori di mutazioni nel gene BRCA1, mentre per i portatori di mutazioni nel gene BRCA2 è riportato un rischio relativo alto per i tumori della prostata, del pancreas, delle vie biliari, dello stomaco e per il melanoma.

I *tumori a genotipo BRCA1* sono caratterizzati da una più alta incidenza dell'istotipo midollare tipico e atipico, dall'alto grado, dall'alta frazione di crescita e dalla negatività per i recettori per gli estrogeni (ER), per il progesterone (PgR) e per HER2/neu. Inoltre, sono frequentemente positivi per le citocheratine basali 5 e 6, overesprimono la ciclica E e p53, hanno bassa espressione di p27, caratteristiche tipiche dei tumori basal-like. I *tumori a genotipo BRCA2* non hanno un fenotipo parti-

colarmente diverso dalle forme sporadiche, eccetto che per l'alta espressione dei recettori per estrogeni (ER) e progesterone (PgR). L'istotipo dei tumori dell'ovaio a genotipo BRCA è di solito il siero-papillare, sebbene siano stati osservati tumori endometrioidi e a cellule chiare.

I modelli di Frank e il BRCAPRO sono tra i modelli probabilistici più utilizzati per la stima della probabilità a priori di mutazioni nei geni di predisposizione BRCA1 e BRCA2. Il *modello di Frank* è indirizzato a stimare la probabilità a priori di mutazioni a carico di BRCA1 e BRCA2 in donne che hanno sviluppato un tumore della mammella prima dei 50 anni o un tumore dell'ovaio a qualsiasi età e che hanno almeno un parente di primo o secondo grado con tumore della mammella insorto prima dei 50 anni. Esso considera, inoltre, la bilateralità o l'età di 40 anni, quali altri criteri per la stima del rischio. Il ***BRCAPRO*** è un programma computerizzato che applica un'analisi bayesiana per stimare la probabilità di mutazione per un dato individuo sulla base di vari fattori, quali la penetranza delle mutazioni BRCA1 e BRCA2 nella popolazione di riferimento, l'etnia, il numero di soggetti affetti e non affetti in famiglia, l'età media di insorgenza del tumore mammario e ovarico in famiglia, l'età dei soggetti viventi e deceduti non affetti, la bilateralità e il sesso.

GENETICA

Questa sindrome si trasmette secondo un pattern di tipo autosomico dominante a penetranza incompleta**.** Qualora venga sospettato un rischio ereditario sulla base dei modelli di stima del rischio o delle caratteristiche cliniche di presentazione del tumore della mammella, si può disporre nella pratica clinica del test genetico finalizzato alla identificazione di mutazioni a carico dei due geni di suscettibilità, BRCA1 (17q21) e/o BRCA2 (13q21-23). Le proteine BRCA sembrano partecipare al ***mantenimento della stabilità genomica***, attraverso il loro coinvolgimento nei processi di ricombinazione e di trascrizione associati al riparo dei Double Strand Break (DSB). Entrambe le proteine si associano, infatti, a RAD 51, responsabile della combinazione omologa e sono principalmente espresse a livello nucleare durante la transizione G1/S in tessuti altamente proliferanti. Sono note oltre 1200 mutazioni deleterie per ciascuno dei due geni, che portano alla sintesi di un prodotto proteico tronco non funzionante.

MANAGEMENT

La gestione del rischio eredo-familiare implica la disponibilità di tre tipologie di intervento, quali la sorveglianza clinico-strumentale, la chemioprevenzione e la chirurgia profilattica.

Sorveglianza clinico-strumentale

Attualmente, le principali raccomandazioni per la sorveglianza clinico-strumentale dei soggetti a rischio ereditario derivano da ***suggerimenti di esperti***, non essendo ancora disponibili linee guida internazionalmente accettate. Oltre alla chirurgia profilattica da prendere in considerazione per i soggetti portatori di mutazioni dei geni BRCA, ai carrier di mutazioni BRCA1 bisogna offrire misure atte a sorvegliare la mammella controlaterale, l'ovaio, la prostata e il colon. Per i carrier BRCA2 devono essere sorvegliati l'ovaio e la mammella, la prostata e la cute per l'eventualità di melanoma.

Ancora oggetto di discussione sono l'***età di inizio e di sospensione*** della sorveglianza, il rischio di esposizione alle ***radiazioni***, la ***cadenza*** con la quale eseguire gli esami strumentali. Gli esperti suggeriscono di iniziare a partire da 5-10 anni prima del caso con tumore della mammella insorto in età più precoce in famiglia.

Recenti studi indicano che lo screening mammografico iniziato in età precoce per i portatori di mutazioni in BRCA1 può essere efficace. Tuttavia, Brekelmans et al. hanno osservato un'alta prevalenza di cancri intervallo in donne tra i 25 e i 35 anni, suggerendo che la mammografia annuale può essere insufficiente in queste donne a rischio molto alto. Tali evidenze potrebbero essere attribuite agli elevati livelli di proliferazione dei tumori mammari a genotipo BRCA1 e alle caratteristiche del tessuto mammario in donne giovani che si presenta particolarmente denso, al punto da poter inficiare il risultato dello screening mammografico. Oltre alla diagnostica radiologica tradizionale, rappresentata dalla mammografia e dall'ecografia mammaria, studi recenti supportano il ruolo della ***risonanza magnetica nucleare*** (RMN) nella diagnosi precoce di tumori della mammella in donne a rischio elevato sulla

base della storia familiare o della condizione di carrier. Molti studi, infatti, hanno evidenziato che la RMN è più sensibile della mammografia e dell'ecografia nel diagnosticare tumori della mammella in donne ad alto rischio, in particolare donne giovani con tessuto mammario denso. Per la sorveglianza sull'ovaio sono indicate l'ecografia transvaginale e il CA125 ogni 6-12 mesi a partire dai 35 anni.

Chemioprevenzione

Il ruolo della chemioprevenzione nel tumore della mammella ereditario non è ben definito. Il **tamoxifene**, approvato dalla Food and Drug Administration (FDA) per la prevenzione del tumore mammario nelle donne a rischio, sembra ridurre l'incidenza di tumore della mammella con espressione dei recettori per estrogeni secondo lo studio NSABP-1. Considerando che nell'80% dei casi i tumori a genotipo BRCA1 non esprimono i recettori ormonali, mentre i tumori a genotipo BRCA2 esprimono tali recettori, sembra ragionevole ipotizzare un'azione chemiopreventiva del tamoxifene per i portatori di mutazioni a carico di BRCA2. Infatti, è riportato che il tamoxifene riduce il rischio di tumore della mammella in donne sane carrier BRCA2 del 62%, ma non riduce il rischio in donne portatrici di mutazioni in BRCA1. Attualmente, in Italia è in corso uno studio (studio APRES) finalizzato alla valutazione dell'effetto chemiopreventivo dell'**exemestane** in donne in postmenopausa portatrici di mutazioni in BRCA1 e/o BRCA2.

Chirurgia profilattica

La **mastectomia profilattica** fornisce un sostanziale miglioramento dell'aspettativa di vita (da 2,5 a 5,3 anni) per donne giovani portatrici di mutazioni a carico di BRCA1 e/o BRCA2 con una riduzione del 90% del rischio di sviluppare un tumore della mammella nel corso della propria vita. Il miglior approccio di chirurgia profilattica sembra essere la mastectomia totale, in quanto in seguito a mastectomia semplice sottocutanea sono riportati casi di tumore della mammella insorto su tessuto mammario ectopico presente a livello dell'ascella o della parete addominale. L'**ovariectomia profilattica** riduce del 50-85% il rischio di tumori ginecologici (tumori dell'ovaio, tumori delle tube di Fallopio e del peritoneo). Inoltre, due ampi studi hanno riportato un effetto protettivo anche per il tumore della mammella con una riduzione del 68%. Tuttavia, sono riportati casi di tumori ovarici o peritoneali dopo chirurgia profilattica. In questi casi, il tumore si sviluppa da foci occulti di tumore ovarico primitivo con successiva diffusione al peritoneo oppure insorge *de novo* a partire dal mesotelio del peritoneo che ha un'origine embrionale comune con l'epitelio del dotto di Muller. L'origine policlonale dei molteplici foci di tumore peritoneale avalla questa seconda ipotesi. L'ovariectomia profilattica dovrebbe essere praticata nei portatori di mutazioni in BRCA1 e BRCA2 dopo che queste abbiano portato a termine le gravidanze desiderate, a causa dell'età mediana di insorgenza del tumore dell'ovaio di 50,8 anni (*range* 30-73 anni).

Trattamento del tumore della mammella nei portatori di mutazioni

I pazienti con tumore della mammella a genotipo BRCA1 e/o BRCA2 hanno un rischio aumentato di sviluppare un secondo tumore sebbene non sia chiaro l'effetto sulla prognosi. Tale considerazione implica un trattamento diverso per questo subset di pazienti. Attualmente non si dispone di dati basati sull'evidenza sperimentale che confrontano l'attività di terapie sistemiche in donne con mutazioni BRCA. Comunque, il tamoxifene ha dimostrato, in studi caso-controllo, una riduzione del rischio di tumore della mammella controlaterale in portatori di mutazioni a carico di BRCA2 del 62%. Inoltre, da studi pre-clinici emerge un particolare livello di chemiosensibilità ai derivati del platino da parte di cellule a genotipo BRCA.

Stile di vita

Fattori non genetici potrebbero modificare il rischio di sviluppare il tumore della mammella, quali per esempio fattori riproduttivi, la terapia ormonale sostitutiva e/o contraccettiva, i grassi alimentari, l'incremento del peso corporeo, l'attività fisica, l'alcol e una dieta povera di vitamine antiossidanti. In particolare, l'uso prolungato di **contraccettivi orali** comporta un aumento del rischio di tumore della mammella nelle portatrici di mutazioni a carico di BRCA1 e BRCA2, mentre nei portatori di

mutazioni in BRCA1 sembra ridurre il rischio di tumore dell'ovaio del 50% circa. Studi osservazionali hanno suggerito che nelle donne in postmenopausa l'uso di terapia ormonale sostitutiva aumenta il rischio di tumore della mammella del 30-40%.

Sindrome di Li-Fraumeni

CARATTERISTICHE CLINICHE
La sindrome di Li-Fraumeni è caratterizzata dall'aggregazione di più membri della famiglia affetti da *sarcomi in età pediatrica* e da tumori in altre sedi diagnosticati in età precoce. Classicamente, i criteri che fanno sospettare questa sindrome sono dati dalla presenza in famiglia di tre parenti di primo grado con tumori diagnosticati prima dei 45 anni, quali tumori dei tessuti molli e osteosarcomi, tumori della mammella, tumori cerebrali, carcinomi del surrene e leucemia. *Altri tumori* associati con minore frequenza sono i tumori dello stomaco, del pancreas e tumori in età pediatrica.

GENETICA
Mutazioni germinali a carico del gene p53 sono state identificate nel 70% delle famiglie che soddisfano i criteri classici per la sindrome di Li-Fraumeni.

MANAGEMENT
I tumori che si sviluppano in pazienti in cui si riconosce la sindrome di Li-Fraumeni non differiscono per caratteristiche istopatologiche dai tumori sporadici. Tuttavia, in questi casi si dovrebbe evitare il *trattamento radiante*, poiché ci sono evidenze per un'aumentata incidenza di secondi tumori in seguito a radioterapia.

Sindrome di Cowden

CARATTERISTICHE CLINICHE
La sindrome di Cowden è un disordine genetico di tipo autosomico dominante caratterizzata dalla presenza di multipli *amartomi* con un alto rischio di sviluppare tumori sia benigni sia maligni della tiroide, della mammella e dell'endometrio. Criteri patognomonici suggestivi della sindrome sono: lesioni mucocutanee, trichilemmomi, cheratosi acrale, lesioni papillomatose, lesioni delle mucose.

GENETICA
Mutazioni germinali a carico del gene oncosoppressore PTEN sono riconosciute nell'80% dei pazienti con sindrome di Cowden.

MANAGEMENT
Ai soggetti carrier di mutazioni del gene PTEN dovrebbero essere offerti programmi di sorveglianza adeguati. In particolare, le donne dovrebbero eseguire esami senologici a partire dai 25 anni, aggiungendo la mammografia annuale dopo i 30 anni o a partire da 5 anni prima del caso di tumore della mammella più precoce in famiglia. Inoltre, per la prevenzione del tumore dell'endometrio, a partire dai 35 anni o da 5 anni prima del caso di tumore dell'endometrio più precoce in famiglia, le donne dovrebbero sottoporsi a una visita ginecologica. I maschi dovrebbero fare un'autopalpazione della mammella mensile. Inoltre, dai 18 anni potrebbero essere utili una visita dermatologica e un'ecografia della tiroide. Non ci sono evidenze di efficacia per la sorveglianza per lesioni gastriche.

SINDROMI EREDITARIE DEL TRATTO GASTROENTERICO

Poliposi familiare del colon (FAP)

CARATTERISTICHE CLINICHE
La poliposi familiare del colon (FAP) è una sindrome a trasmissione autosomica dominante caratterizzata dalla presenza di multipli (> 100) polipi adenomatosi del colon e del retto. I polipi adeno-

matosi iniziano a comparire nella prima decade di vita. All'età di 35 anni il 95% dei soggetti sviluppa polipi. Entro la quarta decade di vita la quasi totalità dei soggetti sviluppa un tumore del colon-retto. Caratteristiche cliniche aggiuntive sono rappresentate dalla presenza di polipi del tratto gastrointestinale alto (stomaco e piccolo intestino), da manifestazioni extraintestinali quali l'ipertrofia congenita dell'epitelio retinico, osteomi e cisti epidermoidi, denti soprannumerari, formazioni dermoidi e tumori, quali i tumori della tiroide, del piccolo intestino, epatoblastomi e tumori cerebrali.

L'associazione di poliposi del colon-retto con particolari caratteristiche cliniche definisce le seguenti sindromi:

1. *sindrome di Gardner (GS),* caratterizzata dall'associazione di polipi adenomatosi del colon con osteomi e tumori dei tessuti superficiali (cisti epidermoidi, fibromi, desmoidi). Interessa il 20% dei soggetti con FAP.
2. *sindrome di Turcot,* caratterizzata dall'associazione di polipi adenomatosi del colon e tumori del sistema nervoso centrale, prevalentemente il medulloblastoma.
3. *FAP attenuata (AFAP),* caratterizzata da un numero ridotto di polipi adenomatosi del colon rispetto alla FAP classica (< 100) sebbene persista l'elevato rischio di trasformazione neoplastica. I polipi tendono a localizzarsi nel colon prossimale. L'età media di sviluppo di tumore del colon, 50-55 anni, è meno precoce rispetto alla FAP classica.

GENETICA

La FAP deriva da mutazioni germinali a carico del gene APC (Adenomatous Polyposis Coli), localizzato sul cromosoma 5q2. Le caratteristiche cliniche sono di solito correlate con la localizzazione della mutazione nel gene e con il tipo di mutazione. In particolare, un'elevata densità di polipi adenomatosi è associata alle mutazioni localizzate tra i codoni 169 e 1393 del gene APC e il rischio di sviluppare tumori desmoidi è più alto per mutazioni localizzate tra i codoni 1445 e 1578. La forma attenuata di FAP è associata con mutazioni all'estremo 3' e 5' del gene. Una mutazione a bassa penetranza del gene APC, I1307, è stata identificata come mutazione fondatrice negli Ashekenazi. In circa il 5-30% dei casi in cui non si riesce a individuare alcuna mutazione a carico del gene APC, si è osservata di recente una mutazione a carico del gene MYH. La sindrome legata a mutazioni a carico di MYH (MAP) ha un pattern di trasmissione autosomico recessivo.

MANAGEMENT

La gestione del soggetto appartenente a famiglia con probabile diagnosi di FAP viene diversificata sulla base delle seguenti condizioni:

- **soggetti con storia personale suggestiva di FAP** (soggetti portatori di mutazione del gene APC o con evidenza di polipi adenomatosi del colon). Per questi soggetti la chirurgia profilattica rappresenta lo standard di trattamento. Le tre attuali opzioni chirurgiche sono rappresentate da: proctocolectomia totale con ileostomia permanente (TPC), colectomia totale con anastomosi ileorettale (IRA), proctocolectomia con anastomosi anale di pouch ileale (IPAA). La scelta dell'opzione chirurgica più adatta dipende da numerosi fattori, legati sia alle particolari caratteristiche della malattia, quali il rischio di sviluppare un tumore desmoide dopo chirurgia profilattica addominale, sia alle preferenze del soggetto. La sorveglianza postcolectomia viene praticata, per i pazienti che hanno conservato il retto, con una retto-sigmoidoscopia flessibile annuale con asportazione di eventuali polipi, una visita clinica annuale e una valutazione endoscopica basale del tratto gastrointestinale superiore all'età di 25-30 anni;
- **soggetti con storia familiare di FAP** e con mutazione identificata o non identificata in famiglia. Il test genetico andrebbe offerto all'età di 10-12 anni. Se il test risulta positivo, è indicata una retto-sigmoidoscopia flessibile o una colonscopia annuale a partire dall'età di 10-15 anni. La scelta del timing della chirurgia dipende da vari fattori, quali la comparsa di un considerevole numero di polipi intestinali, la presenza di grave displasia, di adenomi di grosse dimensioni e/o la comparsa di sintomi, quali il sanguinamento, la diarrea e/o l'anemizzazione.

Se il test risulta negativo, è raccomandato lo screening come per un soggetto a rischio moderato di sviluppare un tumore del colon-retto. Se il test non è praticato, è indicata una retto-sigmoidoscopia flessibile o una colonscopia annuale a partire dall'età di 10-15 anni. Se non compaiono polipi entro la seconda-terza decade di vita, è possibile allungare la frequenza dei controlli.

In termini di chemioprevenzione, l'utilizzo dei *farmaci antinfiammatori non steroidei* (FANS) ha dimostrato di ridurre l'incidenza e la recidiva di adenomi colorettali. Un follow-up a lungo termine è necessario per verificare tali risultati e definire il preciso ruolo di tali farmaci in questo ambito.

Tumore del colon-retto ereditario non poliposico (HNPCC)

CARATTERISTICHE CLINICHE

Il tumore del colon-retto ereditario non poliposico (HNPCC), conosciuto anche come *sindrome di Lynch*, è una sindrome caratterizzata da un pattern di trasmissione autosomica dominante ed è responsabile dello sviluppo di circa il 3-5% di tutti i tumori del colon e del retto. Le caratteristiche di presentazione sono: lo *sviluppo precoce di tumori del colon-retto*, situati nel 70% dei casi al colon destro e alla flessura epatica, un aumentato rischio di sviluppare tumori del colon *sincroni e metacroni* e uno spettro tipico di tumori benigni e maligni *in sede extracolonica*. Tale spettro include il tumore dell'endometrio, dello stomaco, dell'ovaio, del pancreas, del piccolo intestino, del fegato e delle vie biliari, dell'encefalo (tipicamente glioblastomi), della pelvi renale e delle vie urinarie. Una variante della sindrome HNPCC è rappresentata dalla *sindrome di Muir-Torre*, caratterizzata dallo sviluppo di neoplasie degli annessi cutanei (in particolare adenomi delle ghiandole sebacee, carcinomi sebacei e cheratoacantomi) e tumori del colon-retto e del tenue, dello stomaco, del rene e dell'ovaio. Il rischio di sviluppare un tumore del colon-retto nell'arco della vita nei portatori di mutazione è stimato intorno all'80%. L'età media di sviluppo dei tumori del colon-retto è di 44 anni, comparata con i 64 nel tumore del colon-retto sporadico. Soggetti con mutazione genetica sono anche ad aumentato rischio di sviluppare adenomi del colon a un'età più precoce rispetto alla popolazione generale.

La diagnosi clinica di sindrome HNPCC viene posta attraverso l'integrazioni di vari criteri (tab. 3.5).

GENETICA

La sindrome HNPCC è causata da mutazioni germinali a carico dei geni del riparo del disaccoppiamento del DNA ("mismatch repair genes", MMR). I geni più frequentemente coinvolti sono *hMSH2, hMLH1* e *hMSH6*. Mutazioni in altri due geni, hPMS1 e hPMS2 sono state ipotizzate come predisponenti alla sindrome. Conseguenza di mutazioni a carico di tali geni è un'instabilità genomica definita dal fenotipo RER ("replication error repair"). Tale fenotipo è evidenziato dalla presenza di *instabilità dei microsatelliti* (MSI) nel tessuto tumorale, cioè la presenza nelle diverse cellule di un diverso numero di ripetizioni di determinate sequenze di-trinucleotidiche, dovuta all'infedeltà nella duplicazione del DNA. Il fenotipo RER è presente in circa l'80% dei tumori del colon-retto correlati ad HNPCC e nel 15% nei tumori del colon-retto sporadici. L'instabilità dei microsatelliti viene quindi utilizzata nella pratica clinica quale marker di mutazione genetica. Un ulteriore marker è rappresentato dall'analisi immunoistochimica del tessuto tumorale per valutare l'assenza di espressione delle proteine hMSH2, hMLH1 e hMSH6.

I soggetti che soddisfano i criteri di Amsterdam e/o i criteri di Bethesda sono quindi indirizzati all'analisi dei microsatelliti sul tessuto tumorale. In presenza di instabilità dei microsatelliti viene offerta l'analisi mutazionale dei geni a oggi noti. In assenza di instabilità dei microsatelliti può essere considerata comunque l'offerta del test genetico se i criteri di Amsterdam sono soddisfatti.

MANAGEMENT

Le attuali *raccomandazioni* per i soggetti a rischio prevedono: 1) l'esecuzione della prima colonscopia all'età di 20-25 anni o 10 anni prima del caso di tumore del colon più giovane in famiglia, le suc-

Tabella 3.5 Criteri clinici per la diagnosi di tumore del colon-retto ereditario non poliposico (HNPCC)

Criteri di Amsterdam

Tre o più soggetti della famiglia affetti da tumore HNPCC correlato: tumore del colon-retto (CRC), endometrio, piccolo intestino, uretere o pelvi renale. Più tutti i seguenti criteri:
- un soggetto parente di primo grado degli altri due
- due o più generazioni successive coinvolte
- uno o più tumori diagnosticati prima dei 50 anni
- diagnosi di FAP esclusa
- tumori verificati istologicamente

Criteri di Amsterdam modificati

Uno dei seguenti criteri:
- in famiglie molto piccole che non possono essere ulteriormente estese, due parenti di primo grado con CRC, in almeno due generazioni diverse, almeno un caso di CRC diagnosticato prima dei 55 anni
- in famiglie con due parenti di primo grado affetti da CRC, la presenza di un terzo parente con un tumore inusuale in età precoce o un tumore dell'endometrio

Criteri di Bethesda

Uno dei seguenti criteri:
- famiglia che soddisfa i criteri di Amsterdam
- individui con due tumori HNPCC-correlati, inclusi i tumori del colon-retto sincroni e metacroni o tumori associati extracolonici (endometrio, ovaio, stomaco, vie biliari, piccolo intestino, pelvi renale, uretere)
- individui con CRC e un parente di primo grado con CRC o un tumore extracolonico associato o adenoma colorettale; uno dei tumori diagnosticato prima dei 45 anni o adenoma diagnosticato prima dei 40 anni
- CRC o tumore dell'endometrio diagnosticato prima dei 50 anni
- tumore del colon destro con pattern indifferenziato (solido/cribriforme) diagnosticato prima dei 50 anni
- individui con tumore del colon tipo signet-ring cell diagnosticato prima dei 50 anni
- Adenoma prima dei 40 anni

Criteri di Bethesda modificati

Uno dei seguenti criteri:
- CRC diagnosticato prima dei 50 anni
- CRC sincroni, metacroni o altri tumori HNPCC correlati (stomaco, vescica, uretere, pelvi renale, tratto biliare, glioblastoma, adenoma delle ghiandole sebacee, cheratoacantoma, piccolo intestino)
- CRC con istologia a elevata MSI (tumore con infiltrazione linfocitaria, reazione linfocitica Chron simile, istotipo mucinoso o a signet-ring cell o midollare) diagnosticato prima dei 60 anni
- CRC diagnosticato in uno o più parenti di primo grado con tumori HNPCC correlati, diagnosticati in almeno un caso prima dei 50 anni (incluso l'adenoma diagnosticato < 40 anni)
- CRC in due o più parenti di primo o secondo grado con tumori HNPCC correlati, indipendentemente dall'età

cessive andranno praticate con cadenza annuale o biannuale fino all'età di 40 anni, poi annualmente; 2) un'ecografia transvaginale annuale con biopsia endometriale e dosaggio sierico del Ca125 dall'età di 25-30 anni con cadenza annuale; 3) un'ecografia renale ed esame citologico urinario con cadenza annuale.

La *chirurgia profilattica* nei carrier di mutazione è ancora oggi una raccomandazione controversa. La colectomia profilattica può essere considerata nei soggetti in cui è tecnicamente impossibile o difficile effettuare un controllo endoscopico regolare o che rifiutino una sorveglianza regolare. L'isteroannessectomia può essere presa in considerazione quale misura profilattica nelle donne portatrici di mutazione dopo che abbiano portato a termine le gravidanze desiderate.

Sono in corso studi di *chemioprevenzione* che prevedono la somministrazione a lungo termine degli inibitori della cicloossigenasi-2.

Tumore gastrico diffuso ereditario (HDGC)

CARATTERISTICHE CLINICHE

Il tumore dello stomaco diffuso ereditario (HDGC) è una sindrome a carattere autosomico dominante caratterizzata dallo sviluppo di tumori dello stomaco diffusi, infiltranti la parete del viscere senza formare una precisa massa tumorale (linite plastica). Il rischio di sviluppare il tumore dello stomaco è del 60-80% nell'arco della vita, con un'età media di sviluppo di 38 anni (*range* 14-69). Soggetti di sesso femminile presentano anche un rischio del 39% di sviluppare un tumore lobulare della mammella.

I *criteri* clinici attualmente considerati per l'offerta del test genetico sono i seguenti:
- due o più casi di tumore dello stomaco nella famiglia, con almeno un caso di tumore dello stomaco diffuso diagnosticato prima dei 50 anni;
- tre o più casi di tumore gastrico nella famiglia, a ogni età, con almeno un caso documentato di tumore dello stomaco diffuso;
- un caso di tumore dello stomaco diffuso diagnosticato prima dei 45 anni;
- un caso di tumore multiplo dello stomaco diffuso e della mammella lobulare;
- un caso di tumore dello stomaco diffuso e un altro con tumore della mammella lobulare;
- un caso di tumore dello stomaco diffuso e un caso di tumore del colon signet-ring.

GENETICA

Mutazioni a carico del gene *CDH-1*, che codifica per una e-cadherina, sono responsabili di circa il 48% delle forme di tumore dello stomaco diffuso.

MANAGEMENT

La gastrectomia rappresenta l'unica misura possibile che riduce il rischio di sviluppare un tumore gastrico nei soggetti portatori di mutazione, visto che la sorveglianza clinico-strumentale periodica non ha dimostrato, a oggi, una sua reale efficacia.

MELANOMA EREDITARIO

CARATTERISTICHE CLINICHE

Il 10% di tutti i casi di melanoma è ascrivibile a mutazioni a carico di geni di suscettibilità, con trasmissione ereditaria di tipo autosomico dominante.

Una predisposizione ereditaria al melanoma deve essere sospettata in presenza di almeno uno dei seguenti criteri:
- soggetto con melanoma e con uno o più familiari affetti da melanoma;
- soggetto con melanomi multipli;
- soggetto con melanoma e tumore del pancreas;
- soggetto con multipli nevi atipici o displastici. L'associazione di melanoma e multipli nevi displastici è denominata FAMMM (*familial atypical multiple mole melanoma*) o sindrome dei nevi atipici;
- soggetto con melanoma diagnosticato in giovane età;
- soggetto con melanoma e storia familiare di tumore del pancreas.

GENETICA

Due geni sono implicati nella suscettibilità ereditaria al melanoma: l'oncosoppressore CDKN2A e il proto-oncogene CDK4.

CDKN2A, localizzato sul cromosoma 9p21, è risultato alterato in circa il 25% dei casi di melanoma ereditario. Il gene, attraverso un meccanismo di *splicing* alternativo, codifica per due distinte proteine. L'alfa trascritto codifica per la proteina p16^{INK4a}, che inibisce l'attività del complesso ciclica D1-CDK4, implicato nel controllo del ciclo cellulare. Il beta trascritto codifica per la proteina p14^{ARF}, che è implicata nel processo di apoptosi indotto da p53.

CDK4, localizzato sul cromosoma 12q13, è risultato alterato in poche famiglie. Il gene codifica per una proteina che funziona come partner di legame di p16^{INK4a}.

Sono stati scoperti anche geni di suscettibilità al melanoma a bassa penetranza, tra cui il gene MC1R (*melanocortin 1 receptor gene*) che codifica una proteina implicata nel processo di pigmentazione.

MANAGEMENT

La sorveglianza dei soggetti con melanoma ereditario e dei familiari di primo e secondo grado a rischio prevede l'autoesame della cute e la visita dermatologica ogni 6-12 mesi a partire dall'età di 12 anni.

TUMORE DELLA PROSTATA EREDITARIO

CARATTERISTICHE CLINICHE

Numerosi studi hanno dimostrato l'esistenza di una componente ereditaria nella suscettibilità al tumore della prostata.

A oggi non esiste una definizione standard di tumore ereditario della prostata, ma sono stati definiti alcuni criteri che consentono una diagnosi clinica. Si parla di tumore della prostata ereditario se in una famiglia è soddisfatto almeno uno dei seguenti criteri:
- presenza di tre o più parenti di primo grado affetti da tumore della prostata;
- presenza di due o più parenti di primo o secondo grado dello stesso ramo della famiglia affetti da tumore della prostata insorto prima dei 55 anni;
- presenza di parenti affetti da tumore della prostata in almeno tre generazioni successive del ramo paterno o materno.

GENETICA

Il tumore della prostata ereditario risconosce una trasmissione sia autosomica dominante sia X linked.

Nel 1996, un gene associato alla forma autosomica dominante di tumore della prostata è stato mappato sul braccio corto del cromosoma 1q24. Il gene oncosoppressore HPC-1 (*hereditary prostate cancer 1*) codifica l'enzima Ribonucleasi L (RNASEL). Mutazioni germinali di tale gene conferiscono una probabilità pari all'88% di sviluppare un tumore della prostata, con un'età media di diagnosi di 66 anni. Un secondo gene denominato HPCX (*hereditary prostate cancer on the X*) è stato trovato sul cromosoma X nella regione Xq27-28.

Successivamente sono stati individuati altri loci di suscettibilità sia ad alta penetranza sia a bassa penetranza.

MANAGEMENT

Raccomandazioni per lo ***screening dei soggetti a rischio*** sono basate su opinioni di esperti. Infatti, mancano studi randomizzati che indirizzino la sorveglianza dei soggetti a rischio e i dati di studi osservazionali appaiono contraddittori. Le raccomandazioni dell'American Cancer Society prevedono l'esplorazione rettale e il dosaggio del PSA annuale offerto agli uomini a partire dai 50 anni.

A oggi, non ci sono dati definitivi su strategie di ***prevenzione primaria*** per uomini a rischio. Un recente studio prospettico randomizzato su finasteride *versus* placebo, ha dimostrato una riduzione del 25% di tumore alla prostata tra i partecipanti allo studio. La riduzione del rischio era simile sia nei soggetti a rischio per storia familiare (19%) sia in quelli senza storia familiare (26%).

NEOPLASIE ENDOCRINE MULTIPLE

Le neoplasie endocrine multiple (*multiple endocrine neoplasia*, MEN) sono sindromi ereditarie caratterizzate dall'insorgenza di tumori benigni e/o maligni che interessano due o più ghiandole endocrine nello stesso soggetto. Nell'ambito delle MEN sono state identificate due sindromi: MEN 1 e

MEN 2. Ognuna di esse può essere ereditata come carattere autosomico dominante a elevata pene-tranza e con espressività variabile.

MEN 1

CARATTERISTICHE CLINICHE

La MEN 1 è una patologia rara con una prevalenza dello 0,2 ogni 1000 individui. La diagnosi viene effettuata comunemente tra la terza e la quinta decade di vita, ma l'età all'esordio può essere molto più precoce. Le ghiandole endocrine più frequentemente coinvolte sono le paratiroidi (90% dei casi), il pancreas (dal 50% al 70% dei casi) e l'ipofisi (dal 25% al 65% dei casi).

La manifestazione clinica più comune è l'iperparatiroidismo primario dovuto alla presenza di *adenomi multipli* o *iperplasia diffusa delle paratiroidi*. I tumori del *pancreas* endocrino, benigni o maligni, sono generalmente multicentrici e funzionanti. I più frequenti sono il gastrinoma, che può avere anche localizzazione duodenale ed è responsabile dell'insorgenza della sindrome di Zollin-ger-Ellison, e l'insulinoma. Più sporadicamente i tumori del pancreas endocrino possono causare ipersecrezione di glucagone, polipeptide intestinale vasoattivo, somatostatina, polipeptide pancrea-tico e secrezione ectopica di ACTH o somatotropina. I tumori non funzionanti vengono diagnosticati più tardivamente e hanno una maggiore probabilità di essere maligni. I tumori dell'*ipofisi* sono rap-presentati prevalentemente da microadenomi e possono essere multicentrici, secernenti o non secer-nenti. I più comuni sono i prolattinomi, che causano amenorrea e galattorrea nella donna, riduzione della libido e impotenza nell'uomo. Meno frequentemente i tumori dell'ipofisi producono un eccesso di GH, che causa acromegalia, o di ACTH, responsabile della sindrome di Cushing. Manifesta-zioni più rare della MEN 1 sono adenomi surrenalici, lipomi sottocutanei, collagenomi, angiofibro-mi, neoplasie tiroidee, carcinoidi insorti a livello bronchiale, intestinale, pancreatico o timico.

GENETICA

L'insorgenza di questa sindrome è legata a mutazioni germinali del gene oncosoppressore *MEN 1* localizzato sul cromosoma 11 (11q13). Questo gene codifica per la menina, una proteina nucleare coinvolta nella proliferazione cellulare.

MANAGEMENT

Non ci sono indicazioni univoche per la sorveglianza clinico-strumentale dei soggetti a rischio. I soggetti sani a rischio dovrebbero effettuare, periodicamente, almeno la determinazione del calcio sierico ionizzato e della prolattina a partire dai 5-10 anni di età. La terapia dei pazienti non si discosta da quella prevista per i singoli tumori. Il trattamento è quindi principalmente chirurgico, con atteg-giamento meno conservativo, considerando che l'interessamento è multighiandolare e spesso multi-centrico.

MEN 2

CARATTERISTICHE CLINICHE

La MEN2 viene suddivisa in MEN 2A (sindrome di Sipple), MEN 2B e FMTC (carcinoma midol-lare familiare della tiroide). Le sindromi si differenziano per lo spettro tumorale e la frequenza dei disordini endocrini che le caratterizzano. Quasi tutti i pazienti affetti da una delle sindromi MEN 2 sviluppano un *carcinoma midollare della tiroide* che produce elevati livelli di calcitonina, marker biochimico di questa malattia. Inoltre, circa la metà dei pazienti colpiti da MEN 2A o da MEN 2B può sviluppare un *feocromocitoma*, spesso bilaterale, che determina l'insorgenza di crisi ipertensi-ve, tachicardia, tremori, sudorazioni e cefalea. I pazienti con MEN 2A possono sviluppare anche adenomi delle *paratiroidi* (20% dei casi), mentre la maggior parte di quelli con MEN 2B presenta neurinomi mucosi, ganglioneuromi intestinali e habitus marfanoide. Il FMTC è caratterizzato dal solo sviluppo del carcinoma midollare della tiroide, tipicamente multifocale e preceduto da iperpla-sia delle cellule C.

GENETICA

Le MEN2 sono legate a mutazioni differenti dello stesso proto-oncogene *RET*. Esso è situato sul cromosoma 10 (10q11) e codifica per il recettore tirosina-chinasi. La maggior parte dei casi di MEN 2A è causata da mutazioni del dominio extracellulare del recettore che favoriscono la dimerizzazione costitutiva e quindi l'attivazione delle molecole recettoriali. Molti casi di FMTC sono dovuti a mutazioni simili. Le mutazioni responsabili della MEN 2B sono invece localizzate nel dominio intracellulare e influenzano in maniera positiva l'attività chinasica del recettore.

MANAGEMENT

Ai portatori di mutazioni del gene RET vengono proposte misure di profilassi come la tiroidectomia in età precoce (3-5 anni) e screening annuali per l'iperparatiroidismo e il feocromocitoma.

La terapia dei pazienti affetti da tumori nell'ambito della MEN2 non si discosta da quella attuata per i singoli tumori. Il trattamento è quindi principalmente chirurgico, con atteggiamento meno conservativo, considerando che l'interessamento è multighiandolare e spesso multicentrico.

TUMORI EREDO-FAMILIARI

Counseling oncogenetico

Il counseling oncogenetico rappresenta un processo di comunicazione tra professionisti esperti nel settore dei tumori eredo-familiari e una o più persone di una famiglia che si ritengono a rischio di tumore. Viene offerto a soggetti affetti e a soggetti sani con anamnesi familiare oncologica suggestiva per forme di tumore eredo-familiare. Gli scopi sono: effettuare la stima del rischio di sviluppare tumori su base ereditaria e familiare; offrire la possibilità del test genetico nei soggetti con rischio ereditario; programmare adeguate misure di gestione del rischio; promuovere il processo educazionale e di consenso consapevole; effettuare un assessment psicologico e fornire uno spazio di elaborazione psichica e di contenimento emotivo.

Un aspetto peculiare è la multidisciplinarietà attraverso l'integrazione di diverse figure professionali. Per la complessità della problematica dei tumori eredo-familiari, importante è il lavoro in équipe integrata con l'oncologo affiancato dallo specialista dell'area psicologica.

In Italia, è stato validato un modello di counseling strutturato in più fasi al fine di promuovere nel consultante la consapevolezza e l'autodeterminazione delle scelte. Il Tempo 0 è deputato all'informazione, all'acquisizione dell'albero genealogico e all'identificazione del rischio mediante l'uso di modelli predittivi. Al Tempo 1 viene data la comunicazione della stima del rischio e viene preso in considerazione il test genetico in caso di rischio ereditario. In ogni caso, per rischio ereditario e per rischio familiare, vengono suggerite misure preventive di gestione del rischio. Al Tempo 2 viene comunicato il risultato del test genetico e al Tempo 3, sulla base del risultato del test genetico, vengono riconsiderate le misure di gestione del rischio, quali lo stile di vita, la sorveglianza clinico-strumentale, la chirurgia profilattica e la chemioprevenzione. In caso di test genetico positivo, il counseling oncogenetico deve essere esteso agli altri membri, sani e affetti, della famiglia.

Aspetti psicologici

Impatto psicologico della problematica eredo-familiare. La patologia oncologica eredo-familiare è considerata una *relational disease* per i risvolti sul piano personale, familiare e sociale. La percezione del rischio è spesso irrealistica e associata ad alti livelli di *"cancer distress"*. La percezione del rischio risulta modulata dal dato empirico, così come dalle caratteristiche di personalità e dal contesto familiare e sociale del soggetto. Il test genetico, nel caso dei geni BRCA, sembra alterare i livelli di distress soltanto temporaneamente, mentre altre caratteristiche, legate a fattori psicosociali, sembrano incidere sull'intensità del distress nel lungo periodo. Per quanto concerne la gestione del rischio, in linea generale, sembra che la sorveglianza clinico-strumentale non alteri significativamente l'equilibrio psicologico del consultante nel lungo periodo, mentre la chirurgia profilattica, in taluni casi, può influire negativamente sui livelli di distress, sul funzionamento sessuale e sull'immagine corporea. Il counseling oncogenetico, attraverso il lavoro in équipe integrata e la disponibilità di un adeguato supporto emozionale, riduce i livelli di distress, promuove l'accuratezza della percezione del rischio e favorisce la scelta di programmi di prevenzione *ad hoc*.

(Segue)

(Continua)

Aspetti psicologici del counseling oncogenetico. Il ruolo dello psiconcologo nell'équipe multidisciplinare, durante tutto il percorso di counseling, è teso a promuovere l'autodeterminazione consapevole, il miglioramento del senso di autoefficacia e il contenimento dell'impatto intrapsichico e interpersonale della problematica oncologica eredo-familiare.

Gli aspetti cardine dell'intervento psiconcologico sono: a) il colloquio clinico, quale strumento di promozione del processo di conoscenza e di cambiamento, che privilegia e valorizza la soggettività dell'utente; b) le strategie di counseling, finalizzate alla gestione della problematica eredo-familiare; c) l'utilizzo dei test psicologici, al fine di completare un percorso di assessment e per scopi di ricerca.

Sindrome ereditaria della mammella e/o dell'ovaio (HBOC)

Caratteristiche cliniche. Caratteristiche suggestive per l'ereditarietà sono: alta frequenza del tumore mammario e/o ovarico in famiglia; coinvolgimento di più generazioni successive; età di insorgenza precoce; bilateralità; insorgenza nel maschio; tumori multipli nello stesso soggetto; etnia.

Per i carrier BRCA1 sono riportati un rischio cumulativo di sviluppare tumori della mammella controlaterale, della prostata e del colon-retto e un rischio relativo alto di sviluppare tumori dell'endometrio, della cervice e del pancreas. Per i carrier BRCA2 è riportato un rischio relativo alto per i tumori della prostata, del pancreas, delle vie biliari, dello stomaco e per il melanoma.

Genetica. Trasmissione autosomica dominante a penetranza incompleta**.** Mutazioni dei geni BRCA1 e/o BRCA2.

Management. Per i carrier BRCA1 bisogna sorvegliare la mammella controlaterale, l'ovaio, la prostata e il colon; mentre per i carrier BRCA2 l'ovaio, la mammella, la prostata e la cute.

La mastectomia profilattica riduce del 90% il rischio di sviluppare un tumore della mammella. L'ovariectomia profilattica riduce del 50-85% il rischio di tumori ginecologici e del 68% il rischio di tumore della mammella.

Sindrome di Li-Fraumeni

Caratteristiche cliniche. Criteri tipici sono rappresentati dalla presenza in famiglia di tre parenti di I grado con tumori diagnosticati prima dei 45 anni, quali i tumori dei tessuti molli e gli osteosarcomi, i tumori della mammella, i tumori cerebrali, i carcinomi del surrene e la leucemia.

Genetica. Trasmissione autosomica dominante. Mutazioni del gene p53.

Management. Evitare il trattamento radiante per un'aumentata incidenza di secondi tumori nelle sedi irradiate.

Sindrome di Cowden

Caratteristiche cliniche. È caratterizzata dalla presenza di multipli amartomi con un alto rischio di sviluppare tumori sia benigni sia maligni della tiroide, della mammella e dell'endometrio. Criteri patognomonici sono: lesioni mucocutanee, trichilemmomi, cheratosi acrale, lesioni papillomatose.

Genetica. Trasmissione autosomica dominante. Mutazioni del gene PTEN.

Management. Le donne dovrebbero sorvegliare la mammella, l'endometrio, la tiroide e la cute.

Poliposi familiare del colon (FAP)

Caratteristiche cliniche. È caratterizzata dalla presenza di multipli (> 100) polipi adenomatosi del colon e del retto, che compaiono a partire dalla prima decade di vita. Caratteristiche cliniche aggiuntive sono: polipi del tratto gastrointestinale alto, ipertrofia congenita dell'epitelio retinico, osteomi e cisti epidermoidi, denti soprannumerari, formazioni dermoidi e tumori della tiroide, del piccolo intestino, del SNC e del fegato.

Genetica. Trasmissione autosomica dominante. Mutazioni del gene APC.

Management. La gestione viene diversificata sulla base delle seguenti condizioni:

- nei soggetti con storia personale suggestiva di FAP, la chirurgia profilattica rappresenta lo standard di trattamento. Attualmente, le opzioni chirurgiche sono: proctocolectomia totale con ileostomia permanente (TPC), colectomia totale con anastomosi ileorettale (IRA), proctocolectomia con anastomosi anale di pouch ileale (IPAA);
- nei soggetti con storia familiare di FAP, con e senza mutazione identificata in famiglia, è indicata una retto-sigmoidoscopia flessibile o una colonscopia annuale a partire dall'età di 10-15 anni, seguite da chirurgia profilattica.

(Segue)

(Continua)

Tumore del colon-retto ereditario non poliposico (HNPCC)

Caratteristiche cliniche. Sviluppo precoce di tumori del colon-retto; di tumori del colon sincroni e meta-croni; di tumori associati in sede extracolonica. La diagnosi clinica di sindrome HNPCC viene posta attraverso l'integrazione dei criteri di Amsterdam e di Bethesda.

Genetica. Trasmissione autosomica dominante. Mutazioni dei geni del riparo (hMSH2, hMLH1 e hMSH6), con conseguente instabilità genomica definita dal fenotipo RER ("replication error repair").

Management. La gestione prevede:
- colonscopia a partire da 20-25 anni di età o 10 anni prima del caso di tumore del colon più giovane in famiglia;
- ecografia transvaginale annuale con biopsia endometriale e dosaggio sierico del CA125 dall'età di 25-30 anni;
- ecografia renale ed esame citologico urinario annuale.

In particolari condizioni possono essere proposte misure di chirurgia profilattica.

Tumore gastrico diffuso ereditario (HDGC)

Caratteristiche cliniche. È caratterizzato dallo sviluppo di tumori dello stomaco diffusi. Soggetti di sesso femminile presentano anche un rischio del 39% di sviluppare un tumore lobulare della mammella. I criteri clinici per l'offerta del test genetico sono: a) due o più casi di tumore dello stomaco nella famiglia, con almeno un caso di tumore prima dei 50 anni di età; b) tre o più casi di tumore in famiglia, a ogni età, con almeno un caso documentato di tumore dello stomaco diffuso; c) un caso di tumore dello stomaco diffuso diagnosticato prima dei 45 anni di età; d) un caso di tumore multiplo dello stomaco diffuso e della mammella lobulare; e) un caso di tumore dello stomaco diffuso e un altro con tumore della mammella lobulare; f) un caso di tumore dello stomaco diffuso e un caso di tumore del colon "signet ring".

Genetica. Trasmissione autosomica dominante. Mutazioni del gene CDH-1.

Management. La gastrectomia può rappresentare l'unica misura profilattica nei carrier.

Melanoma ereditario

Caratteristiche cliniche. Una predisposizione ereditaria deve essere sospettata in presenza di almeno uno dei seguenti criteri: a) soggetto con melanoma e con uno o più familiari affetti da melanoma; b) soggetto con melanomi multipli; c) soggetto con melanoma e tumore del pancreas; d) soggetto con multipli nevi atipici o displastici; e) soggetto con melanoma diagnosticato in giovane età; f) soggetto con melanoma e storia familiare di tumore del pancreas.

Genetica. Trasmissione autosomica dominante. Mutazioni dei geni CDKN2A e CDK4.

Management. I soggetti con melanoma e i familiari di I e II grado dovrebbero effettuare una visita dermatologica ogni 6-12 mesi a partire dall'età di 12 anni.

Tumore della prostata ereditario

Caratteristiche cliniche. Una predisposizione ereditaria deve essere sospettata in presenza di almeno uno dei seguenti criteri: a) presenza di tre o più parenti di I grado con tumore della prostata; b) presenza di due o più parenti di I e/o II grado con tumore della prostata in età inferiore ai 55 anni; c) presenza di parenti affetti da tumore della prostata in almeno tre generazioni successive.

Genetica. Trasmissione autosomica dominante e X-linked. Principali geni coinvolti: HPC-1 e HPCX.

Management. Esplorazione rettale e dosaggio del PSA annuale dai 50 anni di età.

Neoplasie endocrine multiple (MEN)
MEN 1

Caratteristiche cliniche. Le ghiandole endocrine più frequentemente coinvolte sono le paratiroidi, il pancreas e l'ipofisi, con una diagnosi tra la terza e la quinta decade di vita.

Genetica. Trasmissione autosomica dominante. Mutazioni del gene MEN 1.

Management. I soggetti sani a rischio dovrebbero effettuare periodicamente la determinazione del calcio sierico ionizzato e della prolattina a partire dai 5-10 anni di età.

(Segue)
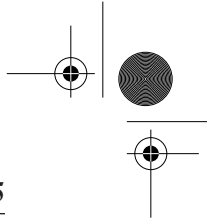
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MEN 2

Caratteristiche cliniche. È suddivisa in MEN 2A (sindrome di Sipple), MEN 2B e FMTC (carcinoma midollare familiare della tiroide). I tumori associati alla MEN 2 sono: il carcinoma midollare della tiroide, il feocromocitoma, spesso bilaterale. I pazienti con MEN 2A possono sviluppare anche adenomi delle paratiroidi, mentre la maggior parte di quelli con MEN 2B presenta neurinomi mucosi, ganglioneuromi intestinali e habitus marfanoide. Il FMTC è caratterizzato dal solo sviluppo del carcinoma midollare della tiroide.

Genetica. Trasmissione autosomica dominante. Mutazioni differenti del gene RET.

Management. Ai carrier vengono proposte la tiroidectomia profilattica in età precoce e screening annuali per l'iperparatiroidismo e il feocromocitoma.
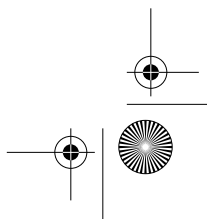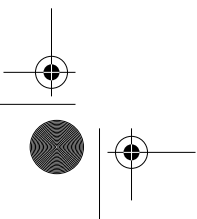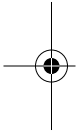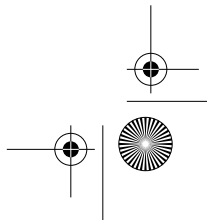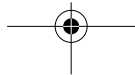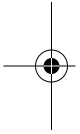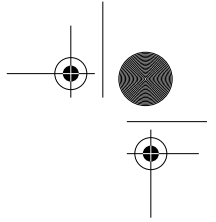
Journal of Genetic Counseling

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ORIGINAL RESEARCH

Distress and Family Functioning in Oncogenetic Counselling for Hereditary and Familial Breast and/or Ovarian Cancers

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I. Spagnoletti · I. Capuano · C. Baldi ·
F. Carle · A. Contegiacomo

Abstract We conducted a psychological assessment during oncogenetic counseling for hereditary breast/ovarian cancer. Anxiety and depression were assessed with the HAD scale, and family functioning and satisfaction with FACES III. HAD was administered at baseline (t_1), at risk communication (t_2), at genetic test result communication, or at first surveillance in not tested subjects (t_3); FACES III was administered at baseline only. We analysed a total of 185 questionnaires administered to the 37 subjects studied. Although not pathological, distress was significantly higher at t_2 and t_3 ($p=0.027$ and $p=0.039$, respectively). Health and marital status were significantly associated with distress. In a disease-free condition, anxiety was higher ($p=0.027$) at t_2, and for single status, depression increased from t_1 to t_2 ($p=0.026$). Families were perceived to be well functioning, and subjects were satisfied with their families. The data collected in this analysis could help to improve the quality of oncogenetic counselling in clinical practice.

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Introduction

Hereditary breast/ovarian cancers are genetic diseases. Germline alterations of the *BRCA1/2* genes are associated with a 45–65% lifetime (to age 70) risk of breast cancer and with an 11–39% lifetime (to age 70) risk of ovarian cancer (Antoniou et al. 2003). It is widely agreed that there is a need for specific genetic testing and surveillance measures for subjects at risk of hereditary and familial cancers (Burke et al. 1997; Vasen et al. 1998).

Given the complexity of hereditary and familial cancer, anxiety and depression variables are aspects of the psychological impact linked to the risk and susceptibility for genetic tumours. In fact, the threat of cancer could negatively affect the psychological sphere and result in anxiety and depression (Claes et al. 2005; Bleiker et al. 2003; DudokdeWit et al. 1998; Kash et al. 1992, 2000). Non-pathological distress levels could be considered a result of cognitive and behavioural strategies used to cope with the disease threat (Lazarus and Folkman 1984).

Assessment of anxiety and depression can advance our understanding of the emotional burden of hereditary and familial cancer in the oncogenetic counselling setting. In fact, high levels of distress could interfere with the counselee–counsellor partnership and undermine medical and psychological interventions (Andersen and Tewfik 1985; Stark et al. 2002).

Several studies have demonstrated a positive association between test uptake and breast cancer anxiety. The test uptake for BRCA1/2 is associated with increased personal risk perception for breast/ovarian cancer (Andrews et al. 2004; Foster et al. 2004; Meiser 2005; McInerney-Leo et al. 2006), although few subjects who choose genetic testing have an accurate perception of their risk (Evans et al. 1994). It is not surprising that subjects with a family history

of cancer generally perceive themselves to be at a high risk of cancer (Evans *et al.* 1994; Kash *et al.* 1992; Mouchawar *et al.* 1999). Empirical findings have shown a significant association between high risk perception and psychological distress (Drossaert *et al.* 1996; Brain *et al.* 1999).

It has been suggested that subjects seek genetic testing particularly to obtain relief from cancer-related anxiety and to feel reassured (Meiser 2005). Considering DNA-testing as a potential tool of risk reduction, and anxiety as a healthy response to cancer-related threat, genetic testing could be in line with the search for strategies to manage the risk of cancer (Geirdal *et al.* 2005). In this context, another debated issue concerns the psychological impact of genetic testing. In a systematic review, Meiser (2005) showed that the psychological impact of genetic testing is related more to pre-test psychological distress than to the test result itself. In addition, it seems that high pre-test anxiety levels were significantly related to high anxiety levels after genetic testing (Lodder *et al.* 2001). Marteau and Croyle (1998) reported that the psychological impact of genetic testing depends more on pre-test expectations, mood and social support than on the test result itself. It seems that the pre-test mood is positively correlated with the post-test mood. Furthermore, the impact of genetic testing is higher in subjects who had an unexpected test result and do not have social support. Therefore, oncogenetic counselling, which involves sessions with the psychologist, favours psychological assessment and emotional support from risk identification to genetic testing (Butow *et al.* 2003).

We previously described an oncogenetic counselling model specifically designed for the oncological setting and aimed at promoting the early diagnosis of invasive and pre-invasive hereditary and familial tumours and prevention. This model is led by an oncologist because of the highly technical expertise required for cancer management, and the need to provide updated information about diagnostic methods and treatment options. It differs from genetic counselling led by the geneticist as occurs in many other countries including Italy. It is not meant to replace classical genetic counselling, but rather to provide an alternative. Indeed, the oncologist is able to play a comprehensive role in assessing familial cancer risk and in the counselling process starting from risk identification to risk management of the user as a healthy or affected subject. Considering the multidisciplinary nature of cancer genetic counselling, our model also foresees close links with the psychologist, geneticist and other professional figures required in the management of at-risk subjects (Contegiacomo *et al.* 2004, 2005).

The model consists of distinct steps, namely, information-giving, pedigree construction and risk estimation, genetic test result communication, and surveillance for affected and disease-free subjects. This model is based on a global approach that includes the bio-psychosocial perspec-

tive in order to promote awareness and adaptive coping strategies. The model foresees structured sessions with a psycho-oncologist for psychological assessment, psychological counselling interventions and emotional support when requested (Contegiacomo *et al.* 2005; WHOQOL Group 1994). An evaluation of the "consent" of 311 subjects who underwent oncogenetic counselling with this model revealed a bi-modal profile, namely, a high level of consent that decreased at the crucial points of counselling (Contegiacomo *et al.* 2004). The crucial points of counselling occurred when the question of genetic testing became a reality and when the user had to decide whether or not to involve relatives. At these times "consent", expressed as adhesion to counselling, decreased. This result demonstrates that the users felt completely free to reconsider their decision at any counselling step, which is in agreement with the educational aim and the promotion of awareness of our counselling model.

In an attempt to improve the counselling modalities, we carried out a psychological assessment to monitor several psychological variables during the oncogenetic counselling process. Sessions with the psycho-oncologist and the psychological assessment took place at baseline and at the critical points of counselling, namely, risk and genetic test result communication or the first surveillance in not tested subjects.

Given the role played by the family and socio-cultural characteristics in hereditary and familial cancers (DudokdeWit *et al.* 1997; Meiser 2005), in this study we evaluated distress levels related to health status, education and marital status. Moreover, we assessed the subject's perception of family functioning and satisfaction. The level of satisfaction that family members have with their family functioning is based on the gap between the real and the ideal perception of the family situation.

The model of family assessment we used in this study is Olson's Circumplex Model of Marital and Family System. This model was designed for research, clinical assessment, treatment planning and marital and family therapy. It is related to a family perspective in which the family is conceived as a dynamic system in which the structure and functioning changes over time.

Here we report the distress assessment data obtained during oncogenetic counselling, together with the counselee's perception of family functioning and his/her family satisfaction. These preliminary data will help us to improve the quality of oncogenetic counselling in clinical practice.

Materials and Methods

The study sample consisted of subjects consecutively recruited from subjects referring to the Screening and Follow-up for Hereditary and Familial Tumours Unit

(University "Federico II," Naples) during the calendar years 2001 and 2003. Inclusion criteria were: affected and disease-free probands, affected and disease-free relatives; absence of major mental illness and an ability to give informed consent as detected by a baseline psychological interview. Subjects underwent counselling with the onco-genetic counselling model used in our clinic and reported elsewhere (Contegiacomo et al. 2004, 2005).

Our institutional Ethics Committee approved the counselling procedures. Each subject provided informed consent to the study, and data were handled in accordance with the Italian privacy law.

Psychological Assessment

Anxiety and depression levels, perception of family functioning and family satisfaction were assessed from replies to self-report questionnaires administered by the psycho-oncologist. Anxiety and depression levels were evaluated with respect to health status (presence or absence of breast and/or ovarian cancer), education level (low level: 8 years of schooling including primary school and secondary school; high level refers to >8 years including junior high school and university) and marital status (single or not single).

Psychological assessment was performed during counselling according to the steps of the oncogenetic counselling model used. Each counselling session devoted to psychological assessment occurred a week after each oncological session. After information-giving and pedigree construction, subjects are assessed by the psycho-oncologist to determine the presence or not of distress. After risk communication, subjects have another assessment session with the psycho-oncologist. After communication of the genetic test results or at the first surveillance session in not tested subjects, subjects undergo the last psychological assessment.

The Hospital Anxiety and Depression Scale (HAD Scale) (Costantini et al. 1999; Zigmond and Snaith 1983) was used to assess anxiety and depression and the Family Adaptability and Cohesion Scale (FACES III) to determine the subject's perception of family functioning and the level of family satisfaction (Olson 1985). We analysed a total of 185 questionnaires administered to the 37 subjects studied. The HAD Scale was compiled in each of the three steps of counselling for a total of 111 administrations. The FACES questionnaire was compiled in the real and ideal forms only at the baseline step of counselling for a total of 74 administrations.

a) Hospital Anxiety and Depression Scale—HAD Scale

The HAD Scale is a self-rating screening instrument for anxiety and depression in patients with physical and mental problems. This questionnaire covers the following aspects of anxiety and depression: tension, worry, panic, restless-

ness and autonomic overactivity. The HAD Scale was administered at baseline, namely at the information-giving and pedigree construction step (first administration=t_1), at risk communication (second administration=t_2) and at genetic test result communication or first surveillance in not tested subjects (third administration=t_3). It consists of 14 items and two subscales, with maximum scores of 21 for anxiety and depression, rated on a four point-scale. On either subscale, scores 0–7 are considered "normal", scores 8–9 represent "borderline," and scores of 10 or more represent clinical morbidity, namely "pathological" (Costantini et al. 1999; Hopwood et al. 1991; Payne et al. 1999; Söllner et al. 2004; Zigmond and Snaith 1983).

b) Family Adaptability and Cohesion Scale—FACES III

FACES III is a questionnaire developed to assess the perception of family functioning on the basis of adaptability and cohesion, according to Olson's circumplex model (Olson 2000) (Figure 1).

As shown in Figure 1, there are four levels of family cohesion (disengaged, separated, connected and enmeshed), and four levels of adaptability (rigid, structured, flexible and chaotic).

FACES III includes 16 characteristic family types and three or more general family types (balanced, mid-range and extreme) (Table I). It consists of two scales: one measures what the subject perceives to be his/her own real familial situation, and the other measures what the subject would like to be the ideal situation. Assessment of family satisfaction is based on the difference between the score of the ideal version and the score of the real version of the questionnaire (Olson 1985; 2000).

Statistical Analysis

We used Fisher's exact test to evaluate the association between anxiety and depression levels considered singly at each of the three administrations of the questionnaire, and to evaluate the association among the levels of anxiety and depression and health status, education and marital status. The analysis of variance for repeated measures was used to evaluate the association of anxiety and depression levels with the above-mentioned variables. We used two distinct multiple linear regression models to quantify the effect of these variables on anxiety levels and depression levels, respectively. Dependent variables were analysed at the third measurement only. When anxiety levels were evaluated, the analysis was adjusted both for anxiety levels detected at the first two measurements and for the three depression levels. The same approach was used for depression levels. A level of probability equal to 5% was used to assess statistical significance. The statistical package SAS System version 8.2 was used for all analyses.

Figure 1 Olson's Circumplex Model—Family Map (Adapted from Olson, 2000)
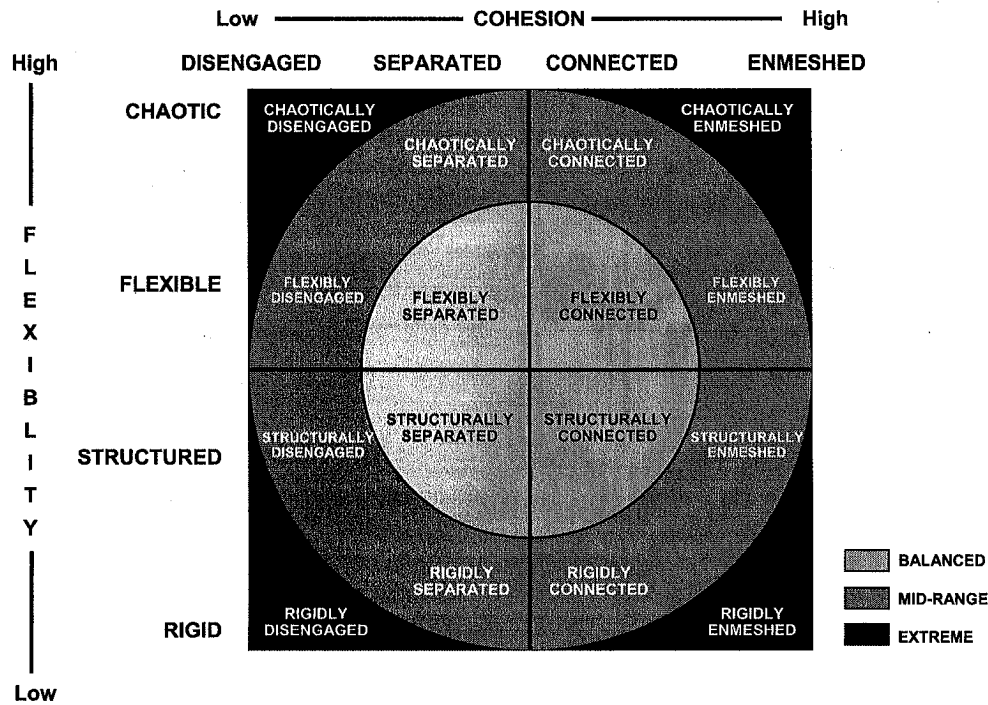
Results

We assessed distress and family functioning during the steps of oncogenetic counselling in 37 subjects. The characteristics of the sample are reported in Table II. The age at counselling ranged between 25 and 71 years and the mean age was 47.43 years. Twenty-five subjects were affected by breast cancer in 23 cases, ovarian cancer in one case and breast plus ovarian cancer in the remaining case. The majority of affected subjects were in follow-up, whereas only four subjects were undergoing chemotherapy. No major mental illness was detected from the psychological interview.

The analysis of the 185 self-report questionnaires revealed a significant association between anxiety and depression. As shown in Table III, high anxiety levels corresponded to high depression levels at the t_2 and t_3 assessments ($p=0.027$ and $p=0.039$, respectively).

Table IV shows the analysis of the association of health status, education and marital status with anxiety and depression identified at the three measurements and considered singularly. There was no significant association

Table I Family Types According to Olson's Circumplex Model

Family types	Description	
Extreme	Chaotically disengaged	Low level of cohesion and high level of adaptability
	Rigidly disengaged	Low level of cohesion and low level of adaptability
	Chaotically enmeshed	High level of cohesion and high level of adaptability
	Rigidly enmeshed	High level of cohesion and low level of adaptability
Mid-range	Chaotically separated	Moderately low level of cohesion and high level of adaptability
	Chaotically connected	Moderately high level of cohesion and high level of adaptability
	Flexibly enmeshed	High level of cohesion and moderately high level of adaptability
	Structurally enmeshed	High level of cohesion and moderately low level of adaptability
	Rigidly connected	Moderately high level of cohesion and low level of adaptability
	Rigidly separated	Moderately low level of cohesion and low level of adaptability
	Structurally disengaged	Low level of cohesion and moderately low level of adaptability
	Flexible disengaged	Low level of cohesion and moderately high level of adaptability
Balanced	Flexibly separated	Moderately high level of adaptability and moderately low level of cohesion
	Flexibly connected	Moderately high level of adaptability and cohesion
	Structurally separated	Moderately low level of adaptability and cohesion
	Structurally connected	Moderately low level of adaptability and moderately high level of cohesion

Table II Characteristics of 37 Subjects who Underwent Psychological Assessment

Characteristics	N (%)
Race	
Caucasian	37 (100)
Gender	
Female	34 (92)
Male	3 (8)
Education	
Low level	14 (38)
High level	23 (62)
Marital status	
Not single	30 (81)
Single	7 (19)
Health status	
Healthy	12 (32)
Affected	25 (68)
Cancer management	
Follow-up	21 (84)
Chemotherapy	4 (16)
Genetic testing	
Tested	29 (78)
Not tested	8 (22)

among the variables considered and anxiety and depression levels.

Table V shows the results of the analysis of variance for repeated measurements expressed as adjusted means and standard errors (SE). The mean levels of anxiety were borderline (i.e., between 8 and 9; see "Materials and Methods" Section) at almost all measurements (Table V, anxiety panel). The only exception was a greater mean anxiety score of disease-free subjects versus affected subjects ($p=0.027$) at the second measurement. The three variables evaluated did not significantly affect anxiety levels. In contrast, time affected anxiety ($p=0.047$). In particular, anxiety scores decreased significantly between the first and third measurements ($p=0.029$).

The mean depression level of the subjects enrolled in the study were normal at each measurement (Table V, depression panel). Two of the three variables studied, namely health status and education, did not affect depression level in any instance. Instead, the mean depression level of singles increased from t_1 to t_2 ($p=0.026$), whereas the mean depression level of non-singles decreased. We found that time exerted an independent effect ($p=0.009$). In fact, depression scores decreased between the first and third measurements ($p=0.002$), while they increased between the second and third measurements ($p=0.045$).

The multiple linear regression analysis confirmed the above-reported observations and allowed us to quantify the associations identified. In fact, this analysis showed that the increase of one unit of depression score implies a significant increase of 0.67 points in the anxiety score at t_3 (95% CI=0.36–0.98). Similarly, variation of the anxiety score determines a significant increase of 0.61 points on the depression score at t_3 (95% CI=0.33–0.90). Moreover, the analysis showed that depression at the third measurement was related to the basal depression level (95% CI=0.13–0.84). The results of linear regression analysis did not show any significant change in anxiety and depression scores at t_3 versus t_1 and t_2 (data not shown).

In the FACES III questionnaire, the perception of family functioning is evaluated based on cohesion and adaptability. As shown in Figure 2, 21 subjects (56%) perceived themselves as belonging to a "flexibly connected" family, 1 (2.7%) to a "flexibly separated" family, 7 (18.92%) to a "structurally connected" family and 8 cases (21.62%) to a

Table III Association Between Anxiety and Depression Levels

	Normal n (%)	Anxiety Borderline n (%)	Pathological n (%)	p
t_1				
Depression T_1				
Normal	13 (46.4)	0 (0)	1 (20)	
Borderline	6 (21.4)	2 (50)	0 (0)	$p=0.108$
Pathological	9 (32.1)	2 (50)	4 (80)	
t_2				
Depression t_2				
Normal	14 (48.3)	0 (0)	1 (12.5)	
Borderline	6 (20.7)	0 (0)	0 (0)	$p=0.027$
Pathological	9 (31)	0 (0)	7 (87.5)	
t_3				
Depression t_3				
Normal	17 (89.5)	1 (50)	1 (14.3)	
Borderline	1 (5.3)	0 (0)	0 (0)	$p=0.039$
Pathological	1 (5.3)	1 (50)	6 (85.7)	

Table IV Association Among Anxiety and Depression Levels with Health Status, Education, and Marital Status

	Anxiety				Depression			
	Normal n (%)	Borderline n (%)	Pathological n (%)		Normal n (%)	Borderline n (%)	Pathological n (%)	p
Information-giving and pedigree construction (t_1)								
Health status								
Healthy	4 (28.6)	3 (37.5)	5 (33.3)	p=1	8 (28.6)	2 (50)	2 (40)	p=0.597
Affected	10 (71.4)	5 (62.5)	10 (66.7)		20 (71.4)	2 (50)	3 (60)	
Education								
Low	5 (35.7)	1 (12.5)	8 (53.3)	p=0.160	11 (39.3)	1 (25)	2 (40)	p=1
High	9 (64.3)	7 (87.5)	7 (46.7)		17 (60.7)	3 (75)	3 (60)	
Marital status								
Not single	11 (78.6)	7 (87.5)	12 (80)	p=1	22 (78.6)	4 (100)	4 (80)	p=0.809
Single	3 (21.4)	1 (12.5)	3 (20)		6 (21.4)	0 (0)	1 (20)	
Risk communication (t_2)								
Health status								
Healthy	2 (13.3)	4 (66.7)	6 (37.5)	p=0.053	10 (34.5)	0	2 (25)	p=1
Affected	13 (86.7)	2 (33.3)	10 (62.5)		19 (65.5)	0	6 (75)	
Education								
Low	4 (26.7)	9 (75)	7 (43.8)	p=0.542	11 (37.9)	0	3 (37.5)	p=1
High	11 (73.3)	3 (25)	9 (56.3)		18 (62.1)	0	5 (62.5)	
Marital status								
Not single	11 (73.3)	5 (83.3)	14 (87.5)	p=0.740	25 (86.2)	0	5 (62.5)	p=0.156
Single	4 (26.7)	1 (16.7)	2 (12.5)		4 (13.8)	0	3 (37.5)	
Genetic test result communication/First surveillance in not tested subjects (t_3)								
Health status								
Healthy	4 (21.1)	1 (25)	7 (50)	p=0.239	10 (35.7)	1 (50)	1 (14.3)	p=0.447
Affected	15 (78.9)	3 (75)	7 (50)		18 (64.3)	1 (50)	6 (85.7)	
Education								
Low	9 (47.4)	1 (25)	4 (28.6)	p=0.487	10 (35.7)	1 (50)	3 (42.9)	p=1
High	10 (52.6)	3 (75)	10 (71.4)		18 (64.3)	1 (50)	4 (57.1)	
Marital status								
Not single	15 (78.9)	4 (100)	11 (78.6)	p=1	23 (82.1)	2 (100)	5 (71.4)	p=0.744
Single	4 (21.1)	0 (0)	3 (21.4)		5 (17.9)	0 (0)	2 (28.6)	

"structurally separated" family. Moreover, all 37 cases were found to belong to the general family type of "balanced" family. In the family satisfaction assessment, 31 subjects (83.7%) considered themselves "satisfied," and 6 (16.22%) "rather satisfied."

Discussion

Hereditary and familial breast and ovarian cancers involve a complex array of medical and psychological aspects that impact on affected individuals and their families. Oncogenetic counselling, in which the psychological effect is related to both the genetic test result and the diverse aspects of these cancers, seems to be the most suitable approach to these cases.

Despite a wealth of data about psychological assessments before and after BRCA1/2 testing (DiCastro *et al.*

2002; Lodder *et al.* 2001; Watson *et al.* 2004) and about the impact of genetic testing in relation to distress assessment (DiCastro *et al.* 2002; Lodder *et al.* 2001; Meiser 2005), several aspects of the long-term effects of oncogenetic counselling and testing on psychosocial parameters is far from clear (DiCastro *et al.* 2002).

We have carried out a general distress assessment during oncogenetic counselling for hereditary and familial breast/ ovarian cancers using our model, which involves a psycho-oncologist (Contegiacomo *et al.* 2004, 2005). Specifically, we evaluated the interaction among several socio-demo-graphic variables and anxiety and depression levels, and the subject's perception of family functioning and satisfaction.

We found a significant association between anxiety and depression at the time of risk communication and at communication of the genetic test result, or first surveillance for not tested subjects. The link between the two variables emerged also from a simultaneous change in

Table V Mean Levels of Anxiety and Depression with Respect to Health Status, Education and Marital Status

Anxiety

	N	t_1 Mean	SE	t_2 Mean	SE	t_3 Mean	SE	p^a	p^c	p^d
Health status										
Healthy	12	9.83	1.16	10.56	1.10	8.81	0.81	0.075	0.257	
Affected	25	8.59	0.89	7.77	0.85	7.71	0.62			
p^b		0.340		0.027		0.227				
Education										
Low	14	10.23	1.19	9.92	1.13	7.99	0.83	0.279	0.055	
High	23	8.19	0.83	8.40	0.79	8.54	0.58			
p^b		0.113		0.208		0.534				
Marital status										
Not single	30	8.97	0.70	9.77	0.67	8.39	0.49	0.770	0.055	
Single	7	9.46	1.42	8.56	1.34	8.13	0.99			
p^b		0.752		0.409		0.808				
Total	37	8.59*	0.68	8.86	0.76	8.22*	0.67			0.047

Depression

	No.	t_1 Mean	SE	t_2 Mean	SE	t_3 Mean	SE	p^a	p^c	p^d
Health status										
Healthy	12	5.01	0.97	5.60	1.06	4.61	0.80	0.411	0.767	
Affected	25	5.52	0.74	6.02	0.82	5.76	0.62			
p^b		0.634		0.723		0.200				
Education										
Low	14	5.75	1.00	6.50	1.10	5.41	0.83	0.263	0.687	
High	23	4.78	0.69	5.12	0.76	4.96	0.57			
p^b		0.359		0.240		0.611				
Marital status										
Not single	30	5.62	0.59	4.40	0.65	5.09	0.49	0.449	0.026	
Single	7	4.91	1.18	7.22	1.30	5.28	0.98			
p^b		0.581		0.054		0.856				
Total	37	5.46*	0.53	4.8**	0.65	5.27*,**	0.67			0.009

Anxiety: *t_1 vs t_3 p=0.029. Depression: *t_1 vs t_3 p=0.002; **t_2 vs t_3 p=0.045. [a] significance of variable (health status, education, marital status); [b] significance of variable (health status, education, marital status) at each time point; [c] significance of interaction between time and health status, education and marital status; [d] significance of time effect.

scores, although anxiety and depression levels were generally below pathological limits. Another association between depression levels was identified at baseline (information giving and pedigree construction) versus the third assessment (communication of genetic test result or first surveillance in not tested subjects). We found that the psychological assessment at the t_3 counselling step seems to be linked to baseline anxiety and depression levels rather than to the test result itself. This observation is in accordance with the finding that genetic counselling and BRCA testing are not associated with major adverse psychological effects (DiCastro et al. 2002; Meiser 2005).

In our study, health status, education level and marital status did not significantly affect distress levels during oncogenetic counselling. However, at the risk communication step, the anxiety levels of affected subjects were normal, whereas those of disease-free subjects were pathological. This difference could be due to the motivations implicit in the request for oncogenetic counselling. Lower distress levels of affected subjects at the time of risk communication could be due to a better psychological adjustment to cancer and motivation to help family members. Disease-free members of an at-risk family could experience greater distress when they learn about their own risk (DudokdeWit et al. 1997; Meiser 2005). In fact, a family history of cancer is known to impact negatively on distress levels among healthy subjects (Kim et al. 2005).

We also found a significant relationship between marital status and depression levels at the time of risk communication: depression levels increased in singles and decreased in non-singles. This interaction could reflect a lower level of emotional support in singles versus partnered subjects.

Figure 2 Family Types According to FACES III in 37 Subjects
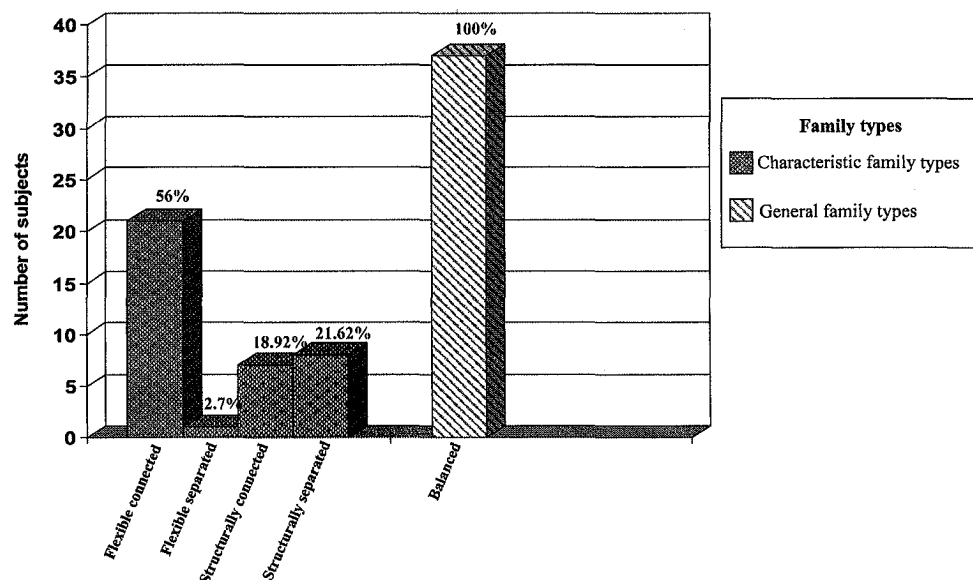
This coincides with the results of breast cancer studies in which married or partnered women had a natural support system not found in subjects who are not in stable relationships. The social and emotional support provided by partners and relatives facilitates adjustment to genetic testing (Bloom *et al.* 2001; Wong-Kim and Bloom 2005). Moreover, the perception of emotional support is considered a psychological resource used by the subject to cope with oncological stressful events and to improve health outcomes (Cohen and Wills 1985; Lutgendorf *et al.* 2005).

We also identified a significant interaction between time and distress during oncogenetic counselling. Specifically, anxiety levels were higher at baseline than at communication of the genetic test result or first surveillance in not tested subjects, whereas depression levels were lower upon risk communication than upon communication of the genetic test result or first surveillance in not tested subjects. Since distress is frequently a response to worry, a moderate increase in anxiety and depression levels at a critical time of counselling indicates a perception of risk for one's own health and can be seen as a natural psychological response (Geirdal *et al.* 2005).

Considering the role played by the family in the context of hereditary and familial cancers (DudokdeWit *et al.* 1997; Kim *et al.* 2005; Meiser 2005; Wong-Kim and Bloom 2005), we evaluated the counselee's perception of family functioning and satisfaction with his/her family. Evaluation of this perception was based on family cohesion and adaptability, and on the degree of discrepancy perception between the actual family and the ideal family. The cohesion is defined as the "emotional bonding that family members have toward one another," while the adaptability dimension is defined as the "ability of the marital or family system to change in its power structure, role relationships,

and relationships rules in response to situational and developmental stress" (Olson 2000). In our study, most families were positioned in the two central levels of the adaptability and the cohesion dimensions (flexible, structured and connected, separated, respectively). Moreover, all families were of the "balanced" type, namely, an open system in which family members have balanced level of autonomy and relationship with each other. Almost all our subjects perceived their families to be well functioning, and they were satisfied with their families. Consequently, family types falling within this category were generally considered well-functioning families. These conditions could encourage subjects to adhere to the counselling process.

In this context, Minuchin (1976) and Olson (1985, 2000) stressed that the main task of the family is to favour a sense of identify of its members as well as a sense of belonging and self-identity. Friedman *et al.* (2006) found that greater family cohesion and a satisfactory marriage were related to better adjustment to breast cancer. The greatest cohesion levels seem to constitute a positive resource of emotional support to cope with stressful life events (Bowles Biesecker *et al.* 2000). Moreover, Olson and Gorall (2003) argue that a balanced family type copes more functionally with distress-producing events than an extreme type. In particular, a balanced family modifies itself in relation to stressful life events, whereas an extreme family does not change probably because of limited behavioural repertoires. Counselling for hereditary and familial cancers can be a stressful health care process that impacts on the emotional and relational (interpersonal) spheres, and the perception of good family functioning could favour more adaptive coping strategies. The wide homogeneity of family types in our sample did not allow us to evaluate the association between FACES III and HAD scores, although this assessment could

provide interesting insights into family functioning in relation to distress levels.

Study Limitations and Future Directions

This is a preliminary study based on the evaluation of 37 subjects and the results should also be considered preliminary. The study sample was ascertained from the initial activities of the Screening and Follow-up for Hereditary and Familial Cancers Unit at the University of Naples "Federico II." Identification and management programs for at-risk subjects have recently been introduced into clinical practice in Italy. Oncogenetic counseling involves complex aspects that require studies on larger populations. Because of the small sample size, we can consider only a few variables related to the counselling program. We are extending the sample study to include evaluations on current cancer treatment, tested versus not tested patients, and the results of genetic testing. Similarly, further psychological investigations are required to determine the relationship among anxiety, depression and family functioning, which are lacking in the present study because of the small number of subjects studied and the wide homogeneity of the family types. A larger number of subjects would consent longitudinal studies to explore better the relationship between distress and family functioning. Moreover, it could be interesting to understand how family types impact on oncogenetic counseling.

Conclusions

Hereditary and familial cancers can be considered a "relational disease" as regards both biological and psychological aspects (Koehly et al. 2003). The characteristics of neoplasias, and the psychological and interpersonal factors have a complex emotional impact on affected subjects and on the family as a whole. There is general consensus that the issue of hereditary and familial cancer requires a multidimensional approach. A multidisciplinary team including the psycho-oncologist is needed to offer counselees psychological counselling.

Our study confirms a previous report (Butow et al. 2003; Meiser 2005) that oncogenetic counselling is a successful modality in the management of at-risk subjects and is not associated with major adverse psychological effects. Moreover, our study suggests that the steps of risk communication and genetic test result communication or first surveillance for non-tested subjects are associated with an increase in distress levels although they do not general reach pathological limits.

Our preliminary data on family functioning and satisfaction seem to indicate good adjustment to adverse illness events, such as hereditary and familial cancer. The study could improve the quality of oncogenetic counselling in clinical practice based on medical and psychological personalized interventions.

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Comment on 'Cancer genetic counselling' by P. Mandich et al. (Ann Oncol 2005; 16: 171)

With the advent of genetic tests, genetic counselling is attracting increasing attention, as also shown by the recent letter by Mandich et al. [1], which addressed some aspects of our oncologist-based multistep model of cancer genetic counselling [2]. Perhaps the features of our model can be appreciated if we explain the rationale that prompted it. The philosophy and practice of the model emerged from a clinical oncological setting [2]. It was specifically designed to meet the user's needs of physical, mental and social well-being as recommended by the WHO [3], and is in keeping with the Italian National Health Plan in force when the model was designed, in that it empowers users to make an informed, fully aware choice among the various preventive, diagnostic and therapeutic options available [4]. The model, which employs an interdisciplinary team, identifies and manages at-risk subjects, and promotes the early diagnosis of invasive and preinvasive hereditary and familial tumours.

Pedigree construction and genetic testing (T1) occur only when the user is fully empowered to decide whether he/she wishes to know their cancer risk. Decisional empowerment derives from extensive information-giving about all aspects of familial or hereditary cancer (T0). At this step, the counsellor also obtains all the information necessary, including clinical-pathological files, to construct the pedigree and to estimate risk, thereby avoiding piecemeal data collection that would delay risk estimation. Communication modalities are geared to the user's educational/cultural level and their motivations and expectations in requesting counselling. The oncologist defines the user's risk profile (hereditary, familial or personal) and

informs them of the possibility, limits and implications, also for their family, of risk estimation, and of prevention options so that the user can decide whether to proceed or not with counselling. At crucial steps of counselling, the psycho-oncologist evaluates also the user's coping style, which is an indicator of psychological well being [5]. A grave cognitive deficit and a severe psychopathologic condition preclude continuation of counselling because fully aware consent (i.e. 'empowerment') and not just informed consent is required to proceed from step to step of the model. The counsellor verifies acquisition of information by questioning the user. The counsellor–user relationship is considered a partnership in which a dynamic feedback of information from and to the user is established. Gene testing is not appropriate for everyone [6]. Not all users have a genetic risk.

Given the high psychological impact of cancer, global counselling is particularly important and requires the specific professional figures in the field of hereditary and familial cancer. It is conceivable that, given their training and daily exposure to patients, oncologists are able to estimate personal risk, to propose diagnostic/therapeutic strategies and to explain these to the user considering their healthy or disease status.

The multistep counselling model, endorsed by the Italian National Health Service for application in patient care, is being used in some centers of the Network for Hereditary Breast and Ovarian Cancer. Information provided by the media or on educational websites, even when 'officially' sanctioned, needs to be 'interpreted' by the health professional according to each user's needs.

In conclusion, our multistep model is not intended to replace classical genetic counselling, but rather to provide an alternative that fosters the oncologist–user partnership in order to promote early diagnosis and prevention.

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Does the concurrent use of anthracycline and granulocyte colony-stimulating factor influence the risk of secondary leukaemia in breast cancer women?

Topoisomerase II inhibitors and alkylating agents induce secondary acute leukaemia (sAL) differently. The risk of this complication peaks 5–10 years after the start of chemotherapy in patients receiving alkylating agents. These patients frequently present with myelodysplasia (MDS), which may then progress to overt acute myeloid leukaemia (AML). Unlike the sAL associated with alkylating agents, that induced by anthracylines is monocytic, involves a specific cytogenetic abnormality (11q23) and develops within a few years (generally 2–3 years) after treatment, without prior MDS in some cases [1].

Although granulocyte colony-stimulating factor (G-CSF) induced the growth of primary acute myeloid leukaemic blasts *in vitro* in about 50% of cases, it was not leukaemogenic and even had an antileukaemic effect in some preclinical models [2]. In early breast cancer, Crump *et al.* [3] found no cases of sAL among patients given epirubicin-based adjuvant chemotherapy plus G-CSF, and Citron *et al.* [4] reported no correlation between the use of G-CSF and the incidence of sAL among 2005 patients randomized to standard or dose-dense chemotherapy. Conversely, in the cross-protocol analysis on six complete NSABP trials with different regimens of anthracycline and cyclophosphamide, Smith *et al.* found a positive association between the use and the dose of G-CSF and the risk of sAL in patients receiving standard anthracycline and dose-intensified cyclophosphamide [5]; the estimated risk of AML/MDS was 3.58 for patients given more than the median dose of G-CSF (242 µg/kg).

A total of 497 evaluable stage I–II breast cancer patients were randomly assigned to receive epirubicin 120 mg/m^2 and cyclophosphamide 600 mg/m^2 i.v. (hEC) on day 1 every 21 days for four cycles with or without lonidamine and with or without prophylactic G-CSF according to a factorial 2×2 design [6]. Among these patients we encountered, at median follow-up of 55 months, a 58-year-old woman who developed AML (monocytic, M5) 19 months after completion of chemotherapy. She had received filgrastim (480 µg/day s.c) every other day on days 8, 10, 12 and 14 of each hEC course and chest-wall irradiation (50 Gy plus a boost of 10 Gy) after completion of chemotherapy. She died 10 days after diagnosis of sAL. Although the cumulative epirubicin dose (480 mg/m^2) was less than that reported by Crump et al. [3], we found no other cases of sAL among the 243 evaluable patients in our series receiving hEC without G-CSF. Thus the crude incidence of sAL after adjuvant hEC with G-CSF support was 0.41%.

The case presented here and the recent update on the incidence of sAL after adjuvant chemotherapy for early breast cancer deserve some consideration. Several studies have demonstrated the possibility of achieving a modest to moderate increase in dose intensity using growth factors as an adjunct to higher-dose or dose-dense chemotherapy regimens, which were able to improve the clinical outcome. However, since the dose intensity of anticancer therapy has increased in parallel with the introduction of G-CSF in current clinical practice, distinguishing the contribution of intensified therapy versus G-CSF is often difficult. Above all, the leukaemogenic hazards of cancer treatment should always be weighed against its therapeutic benefits. Considering the recent development of indications even for subgroups of patients at moderate risk of relapse, it is crucial to balance the absolute survival benefit against the risk of severe complications caused by chemotherapy itself, particularly secondary acute leukaemia. In conclusion, this single case cannot prove the role of G-CSF in the development of sAL, but does point out the importance of being prudent when prescribing high-dose chemotherapy with growth factor support.

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Original article

An oncologist-based model of cancer genetic counselling for hereditary breast and ovarian cancer

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On behalf of the Italian Network on Hereditary Breast Cancer

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Background: We describe a multistep model of cancer genetic counselling designed to promote awareness, and disease surveillance and preventive measures for hereditary and familial breast and ovarian cancer.

Patients and methods: Step T0 of the model entails information giving; this is followed by pedigree analysis and risk estimation (T1), risk communication and genetic testing (T2), and genetic test result communication (T3). User consent was required to proceed from one step to the next. Surveillance and preventive measures are proposed to at-risk users. Of the 311 subjects who requested cancer genetic counselling, consent data to each counselling step were available for 295: 93 were disease-free, 187 had breast cancer, 12 had ovarian cancer and three had breast plus ovarian cancer.

Results: Consent was high at T0 (98.39%), T1 (96.40%) and T2 (99.65%). Consent decreased at the crucial points of counselling: T2 (87.71%) and T3 [genetic test result communication (85.08%), and extension of counselling to and testing of relatives (65.36%)].

Conclusions: The model fosters the user's knowledge about cancer and favours identification of at-risk subjects. Furthermore, by promoting awareness about genetic testing and surveillance measures, the algorithm enables users to make a fully informed choice of action in case of predisposing or familial cancer risk.

Key words: breast cancer, genetic counselling, ovarian cancer

Introduction

It has been estimated that ~70% of all primary breast cancers are sporadic forms, between 15% and 20% are familial forms and the remaining 5–10% are hereditary [1–4]. In this context, identification of the *BRCA1* and *BRCA2* susceptibility genes [5, 6] provided a molecular basis for genetic testing. This, together with increased breast cancer awareness in the general population, has increased the demand for identification of the hereditary risk, mainly as regards identification of the susceptibility gene. Moreover, the identification of familial risk favours the use of surveillance measures also in relatives at moderate risk of cancer. Consequently, when one of these forms of hereditary or familial breast and/or ovarian cancer is suspected in clinical practice, the general practitioner should address the patient to an oncological centre specialising in cancer genetic counselling for risk identification, definition and management [7–11].

Genetic counselling, defined by the American Society of Human Genetics as 'a *communication process* which deals with the *human problems* associated with the occurrence or risk of occurrence of a genetic disorder in a family' (our italics), involves one or more appropriately trained persons to help the affected individual or family [9, 10, 12, 13]. Genetic counselling in the oncological setting (cancer genetic counselling) should also provide sufficient information to enable the user to make a fully informed choice of action, particularly as regards prevention, in case of identification of a mutation or of a familial cancer risk [11].

In Italy, where health care is mainly a public service, cancer genetic counselling is a relatively new concept and is almost invariably offered within the framework of research projects [14]. The onset of cancer genetic counselling, which at first focused on genetic testing, coincided with a change in the physician/patient relationship as the Italian public became more aware of improvements in cancer treatment, in palliative care and in prevention. In recognition of this new scenario, the Ministry of Research funded a research project entitled the Development of a National Network for the Study of Hereditary Breast Cancer [15]. Five clinically oriented centres of this network (representing northern, central and southern areas of the country) are implementing a multistep

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model of cancer genetic counselling based on the experience initiated and promoted by the Naples Unit.

Given the highly technical expertise required for cancer management, and the need to provide updated information about diagnostic methods and treatment options, the oncologist seems to be the most appropriate professional figure for the role of counsellor. In fact, the oncologist is able to play a comprehensive role in assessing familial cancer risks and in the counselling process starting from risk identification to risk management [16]. Considering the multidisciplinary nature of cancer genetic counselling, our model also foresees close links with the psychologist, geneticist, radiologist, gynaecologist and surgeon during the patient's educational process and as required in the various counselling steps.

The defining features of the model described herein are: (a) it is an educational model; (b) it aims at promoting awareness; and (c) it aims at promoting prevention and surveillance measures in subjects who have been identified as being at hereditary or familial risk. Here we describe this model and report the 'consent' to each counselling step obtained in 311 subjects.

Patients and methods

Subjects who requested counselling were referred by their physician or came spontaneously to the Screening and Follow-up for Hereditary and Familial Tumours Unit (Azienda Ospedaliera Universitaria 'Federico II', Naples), the Centre for the Study of Familial Breast and Ovarian Tumours (Modena Polyclinic), the Medical Oncology Division (University of L'Aquila), and the Regional Reference Centre for Genetic Counselling and High Technology

Therapies in Medical Oncology ('Mater Domini' Polyclinic, Catanzaro), between 1999 and 2001. The Ethics Committees of the participating units approved the counselling procedures. Each participating centre adhered to the counselling model proposed by the Naples unit.

Counselling was addressed to: (a) cancer-affected subjects with a personal history suggesting genetic risk (e.g. early onset breast cancer, male breast cancer, breast and ovarian cancer in the same subject and multiple cancers besides breast or ovarian cancer in the same subject), or with a family history of cancer; and (b) disease-free subjects belonging to families with cancer clustering.
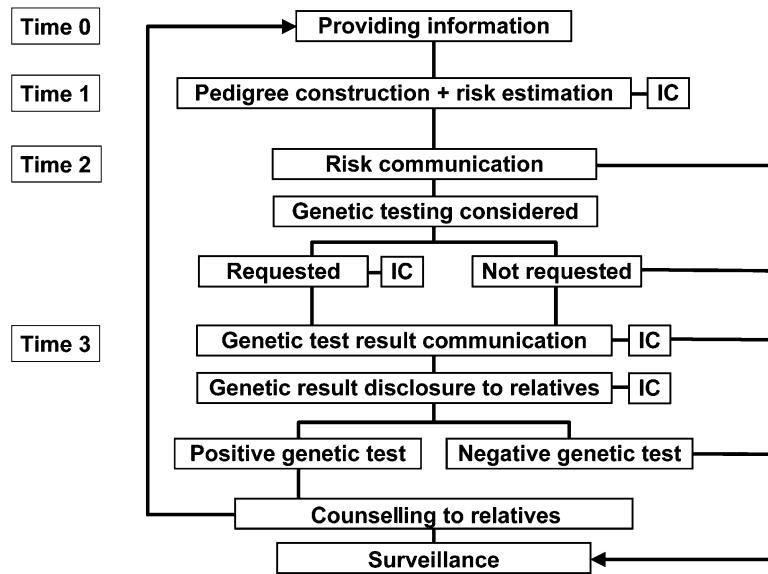
The multistep counselling model

The counselling teams included an oncologist/counsellor, psychologist, geneticist, radiologist, gynaecologist and surgeon, except in the Catanzaro unit where there was a psychiatrist instead of a psychologist. The model was designed to promote awareness using a multistep approach in order to allow users to assimilate fully the information given, to adapt to the new reality and to become fully aware of their condition and all its implications. Sessions with a psychologist are structured within the model, and subjects may request a session with the psychologist whenever they want information or need support. Adequate time is set aside for each counselling step, and each subject decides when he/she is ready for the next step. Every effort is made to protect the user's privacy. Easy-to-understand language adapted to each subject is used. The communicative modalities are modelled according to the affected or disease-free condition of the proband and to his/her cultural profile. Interaction between users and the oncologist is informal and respects the communication process typical of the clinical setting.

The steps of the model are shown in Figure 1 and in Table 1. At step T0, the aims and organisation of cancer genetic counselling are explained by the

Table 1. Methodological scheme at the various steps of the model and the professionals involved in each step

Step		Description	Professionals involved
T0	Providing information	Information/education about sporadic, familial and hereditary breast cancers.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
		Information about risk assessment procedures.	
		Information/education about preventive strategies, lifestyle implications and health-promoting behaviour.	
		Collection of personal history, histological report.	
T1	Pedigree construction	Pedigree construction for at least three generations.	Oncologist counsellor
	Risk estimation	Analysis of pedigree acquired. The risk profile is defined as individual, familial and inherited (Claus, Modena and Frank models).	Oncologist counsellor; geneticist (when requested)
T2	Risk communication	Communication about individual and/or familial and/or inherited risk.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
		Communication about the implication of the risk estimation for the user and for the user's relatives.	
	Genetic testing considered	Genetic test offered in case of suspected inherited risk.	
		Discussion about advantages and limits of genetic testing.	
T3	Genetic test result communication	Communication of the results and discussion about implications.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
	Genetic results disclosure to relatives	The proband informs his/her relatives about genetic test results and informs them about counselling.	
	Counselling for relatives	Relatives interested in counselling contact the unit for an appointment.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
	Surveillance	Surveillance measures modelled on different levels of risk. Discussion of preventive measures available, including chemoprevention and/or prophylactic surgery.	Oncologist; surgeon; gynaecologist; radiologist

Figure 1. The multistep cancer genetic counselling model. IC, informed consent.

oncologist counsellor, and the user's motivations and expectations with regard to counselling are elicited. In this information-giving process the subject learns about the hereditary, familial and sporadic forms of breast cancer, and about the methods available to identify the risk of developing these forms of cancer (i.e. pedigree construction and analysis, personal history collection and susceptibility gene testing) [17–21]. The counsellor then discusses the implications of cancer risk in general terms, and the strategies available for risk management [20], i.e. surveillance [22–24] and prevention [25–29, 30]. During counselling, the user is repeatedly encouraged to ask questions and seek explanations; the user's responses also allow the counsellor to verify the user's understanding. Lastly, users are instructed to collect information about their family in preparation for step T1 (pedigree construction and risk estimation). After information-giving by the counsellor, subjects have a session with a psychologist to define, by way of a semi-structured interview, their cognitive level and the presence or not of psychological distress, evaluated by self-administered questionnaires. A low cognitive level and psychological distress preclude continuation of counselling. The psychologist becomes familiar with the subject's medical condition, and explores his/her socio-cultural background, relationship with the medical team, family members and others, personality profile and ability to adapt to changing situations.

At T1 (Figure 1 and Table 1), the proband is required to give written informed consent (IC) to allow the counsellor to acquire information about the family and, eventually, to disclose the results of pedigree analysis to other family members should they request counselling in the future. Information about the subject's ethnic background is recorded. The proband's family history going back at least three generations (maternal and paternal), is collected. The diagnosis is verified from the histological notes of the affected proband and his/her family. Each pedigree is assigned a code, which is used throughout the counselling process to guarantee privacy [31].

Risk is established according to Frank et al. [18], whereby the *a priori* risk of being a carrier of the susceptibility genes *BRCA1* and/or *BRCA2* is calculated. Risk estimation is also determined according to the criteria of Modena University [17] considering breast or ovarian cancer clustering within the family and within generations, the degree of relationship (first and second degree), vertical transmission, skipping a generation in case of interposition of a male due to incomplete BRCA1 and/or BRCA2 penetrance, mono or bilateral tumour and onset age of cancer, breast and ovarian cancer in the same subject, and multiple cancers other than breast or ovarian in the same subject.

Pedigree analysis was performed by the oncologist and discussed with the geneticist in the more complex cases, in uncertain cases and when the pedigree did not contain a known cancer syndrome. At T2, the counsellor informs the user about the presence or not of a hereditary or familial risk, or, in the case of a disease-free proband, about an individual risk exceeding that found in the general population. If a hereditary risk is identified, the advantages and limits of genetic testing in defining the risk are illustrated and discussed. The user is encouraged to ask questions to ensure that the information given has been fully understood. The genetic test notwithstanding, the counsellor explains surveillance and prevention measures for the proband and disease-free relatives who could be at an increased risk with respect to the general population. Subjects who are contemplating a genetic test have another session with the psychologist in order to clarify further the psychological aspects related to genetic testing. Users then return to counselling and give written informed consent to blood withdrawal for *BRCA1/BRCA2* gene analysis. Gene analysis was performed at each unit by molecular biologists with whom the counsellor discussed the test results. The network laboratories use a standardised procedure and periodically verify testing proficiency. The costs of genetic testing are covered, at present, by an MIUR grant. Only the affected proband or, in the case of a disease-free proband, the youngest affected family member, has access to gene testing. As required by Italian guidelines, unaffected probands from families with no living affected relatives were not offered genetic testing [32].

When the test result becomes available, the user returns to counselling and is again required to give written consent to test result disclosure, and eventually to disclosure to relatives. Thus, if a relative requests counselling, the counsellor is free to use the pedigree information previously obtained for this family. At this time (T3), the counsellor explains the test results (positive or negative), taking care that the user understands all aspects and implications of the result with respect to relatives and progeny. The counsellor also explains the advantages of a positive test (i.e. preventive measures can be scheduled) and disadvantages of a negative result in cases of suspected hereditary risk (i.e. possible involvement of an unknown susceptibility gene) [33]. In the case of a positive test, the counsellor discusses with the gene carrier the possibility that first-degree disease-free relatives undergo the genetic test. Importantly, the user is instructed to vehicle the suggestions concerning surveillance to relatives at an increased risk with respect to the general population. The user also receives a written report that includes the test results, the procedure used for

the test, and an explanation of the significance of the test result, together with a copy of the pedigree. The risk information collected in the onco-genetic clinic is integrated into the medical management of the patient by the multi-disciplinary counselling team. The user is also advised to contact a clinical oncological outpatients unit of the Network or a local oncological unit [15]. If a patient requests in writing, a report is sent to the general practitioner who referred the subject to counselling. The results of genetic testing are recorded on a separate chart that is kept in the Family Cancer Genetics Office.

The oncologist consults the gynaecologist, surgeon and radiologist as required to clarify aspects related to counselling. The proband decides whether or not to inform relatives that they belong to an at-risk family or to a *BRCA1/BRCA2*-carrying family, about taking surveillance measures and the possibility of genetic testing. The relatives so informed can request counselling and start the counselling cycle from T0. In such cases the contents of each counselling step are adapted to the user's level of information and to his/her expectations.

At the end of each step, the proband is given ample opportunity to discuss any questions or problems at length in order to clarify all aspects of their condition. In this regard, the inter-step interval must be sufficient so as to allow users to elaborate the contents of the previous step so that they can express a truly 'aware' consent, and not just 'informed' consent, to the various steps and actions selected during counselling. Consequently, the proband decides when he/she is ready for the subsequent counselling session based on appointments offered after 1 week, 2 weeks or longer. Each time informed consent is required, users are reminded that they have the right to rescind their decision at any time.

Results

Cancer genetic counselling was requested by 311 subjects, 21 (6.7%) of whom were referred by their physician, 243 (78.2%) were recruited from the clinical service of the participating departments, and the remaining 47 (15.1%) requested counselling spontaneously.

Of the 311 subjects who requested cancer genetic counselling in the five participating centres of the National Network for the Study of Hereditary Breast Cancer, 306 underwent step T0 (Figure 1). After information-giving by the oncologist, these subjects underwent an interview with a psychologist. Eleven subjects did not return to counselling or refused the psychologist interview or showed low motivation for counselling after referral by their physicians. The remaining 295 subjects (all Caucasian) returned to counselling and gave their informed consent for pedigree construction and risk estimation (step T1). Of these, 93 subjects were disease-free, 187 had primary breast cancer, 12 primary ovarian cancer and three primary breast cancer (BC) and ovarian cancer (OC). Patients were evaluated by counsellors at different stages of their oncological history: 146 (72.2%) during follow-up and 56 (27.8%) during advanced disease. Disease-free subjects were referred to counselling for various reasons: 54 (58.2%) for high familial clustering (at least three cases of BC and/or OC), 22 (23.6%) had at least one first-degree relative affected by BC and/or OC, and 17 (18.2%) with early onset BC or male breast cancer in the family. The age at diagnosis of breast cancer was <35 years in 28 subjects and ≥35 years in the remaining 159 breast cancer subjects (overall age range 27–80 years; median age 47 years). The age range of ovarian cancer patients was 29–63 years (median age 40 years). Sixty-six disease-free subjects were premenopausal

and 23 postmenopausal, and four of the disease-free subjects were male.

Based on pedigree analysis, and personal history data in the case of disease-free individuals, we used the criteria of Modena University [17] and the Frank model [18] to divide the families into risk categories based on the hereditary and familial risk in all subjects.

A total of 292 (99.65%) subjects attended the T2 counselling session (risk communication). Of the three subjects who did not attend this session, one died and two decided not to proceed. At T2, the oncologist communicates the results of the pedigree analysis. In case of hereditary or familial risk or when a disease-free subject had a risk greater than that of the general population, information about surveillance and prevention measures was given to the subjects undergoing counselling and to their relatives if requested. Of these 292 subjects, 140 belonged to genetic at-risk families and were given details about identifying the risk by genetic testing.

At a subsequent appointment, 122 (87.71%) subjects from at-risk families gave written informed consent to blood withdrawal for genetic testing. Of these, 106 subjects were probands with primary breast cancer, eight had primary ovarian cancer, four had primary breast plus ovarian cancer, and four were relatives of disease-free probands who had participated in counselling from T0. Eighteen subjects decided not to undergo genetic testing. These subjects were encouraged to take disease surveillance and prevention measures. Of the 114 subjects informed that their test result was ready, 97 decided to learn the result (T3). As with the 18 subjects who did not take the genetic test, the 17 subjects who preferred not to know the result of their genetic test were informed of the importance of taking surveillance and prevention measures, and advised to contact a clinical oncological outpatients unit of the Network or their local oncological unit. They were also advised that they could request their test result at any time in the future should they change their mind.

Fifty-nine disease-free subjects, who were relatives of probands with a positive test, were informed by the proband that they belonged to an at-risk family. Thirty-four of these relatives requested counselling and underwent counselling starting at T0 (Figure 1); in these cases the contents of each step were modified depending on the user's level of information and on his/her expectations. Twenty-five of the 59 disease-free subjects did not undergo counselling, even though they had been informed by the proband that they belonged to an at-risk family.

Consent to the counselling model differed among the various steps of the model (Figure 2). The interstep interval was usually around 1–2 weeks. At T0, T1 and T2 (as regards risk communication), the percentage of consent was very high, with only a few cases of non-adhesion due to missed appointments (T0), a change of mind about pedigree construction and risk estimation (T1), and a change of mind about risk communication (T2). In contrast, the per cent of consent decreased in steps T2 (genetic testing) and T3. The drop-outs were: (a) subjects who, although they belonged to a family at genetic risk, did not undergo genetic testing at T2 (subjects who died and subjects who changed their mind about genetic testing); (b) subjects who underwent blood sampling for genetic
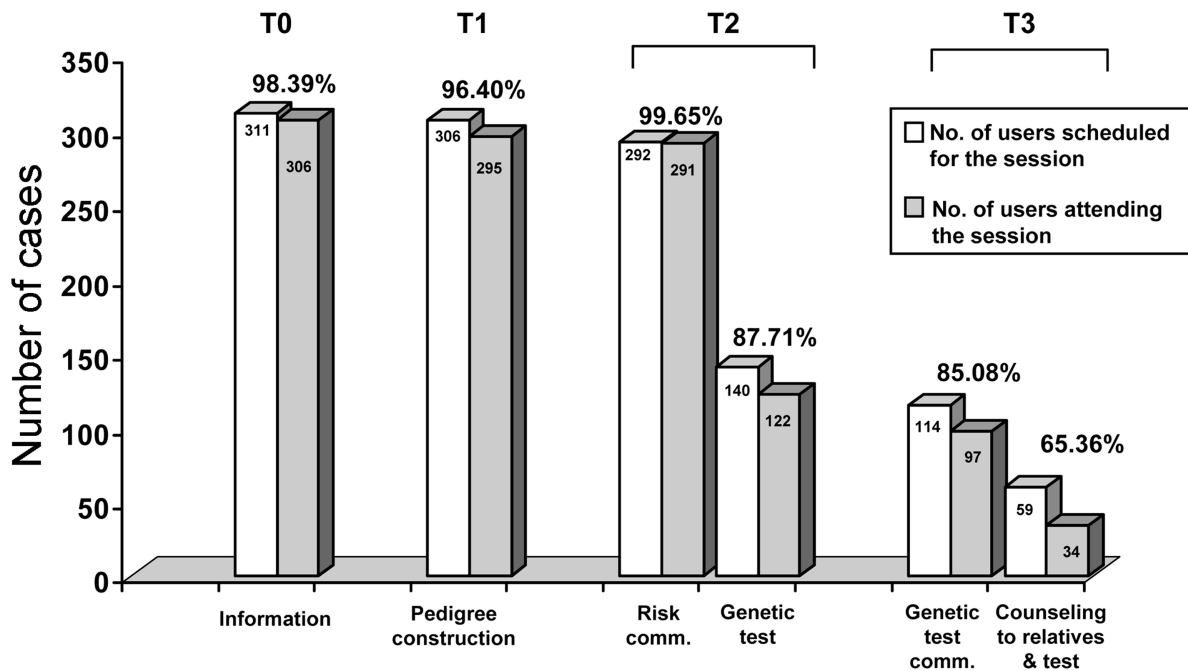
Figure 2. Consent to the multistep cancer genetic counselling model. The percentage of consent is calculated on the basis of the number of users scheduled/attending the counselling session.

testing but decided not to know the genetic test result, or who died (T3); and (c) subjects who, although they were informed by the proband that they belonged to an at-risk family, decided not to undergo genetic counselling (subjects who opted for surveillance measures only).

Discussion

Here we report the experience of five centres of the Italian Network for the Study of Hereditary Breast Cancer in applying a new model of cancer genetic counselling. This counselling service was available to cancer patients and to healthy subjects with a family history of cancer [15].

There is now general consensus that primary breast cancer exists in distinct forms: hereditary, familial and sporadic. The development of cancer genetics has led to a need for medical services, including cancer genetic counselling, for affected individuals and their families. In this regard and considering the complexities of cancer genetic counselling and the time required for the process, the oncologist involved in general oncology practice would be well advised to refer patients to an established onco-genetic service, if available [12, 25]. The oncologist counsellor is able to cover the whole spectrum of cancer genetic counselling, from verification of cases, risk assessment, genetic counselling and testing, and follow-up of at-risk subjects. The oncologist can refer users to other professional figures that can address the psychosocial needs of family members or that are involved in the educational and clinical management process.

It is difficult to compare our model of genetic counselling with others being applied nationally and internationally, particularly because, to our knowledge, data on adherence to the various

models are lacking. The multistep counselling model described herein is based on the concept that information-giving is a dynamic process occurring over time because the individual needs time to assimilate new information and to adapt to a new reality. In particular, users must come to terms with the fear evoked by cancer, loss of functioning, and the possibility of transmitting cancer to progeny. Because the proband must give written informed consent at each crucial step of cancer genetic counselling, and because he/she has ample time to assimilate the contents of the previous counselling step, consent is not merely informed but is an aware consent. With the awareness resulting from this step-by-step counselling, users probably have a correct perception of their risk.

An interesting bi-model profile emerged from the consent results (Figure 2). In fact, sessions from T0 to T2, which cover information-giving and risk communication, were characterised by a high level of consent, after which consent decreased. Interestingly, the crucial point occurred when the question of genetic testing became a reality, i.e. when the user must decide whether or not to take the test, and when it comes to deciding whether or not to know the test result. Consent decreased even further when the user had to decide whether or not to inform relatives that they belonged to a family bearing a predisposing cancer mutation. These results demonstrate that the users felt completely free to reconsider their decision at any time during the counselling process.

The model aims at identifying at-risk subjects (i.e. defining the risk as hereditary, familial or individual when the subject referred to counselling is disease-free), and directing subjects to surveillance [17–19] and prevention [25–29]. In fact, immediately after pedigree analysis, subjects are referred to surveillance and prevention as necessary regardless of consent or not to subsequent

counselling sessions. In the multistep model, through information-giving and the implication-counselling discussion, users probably become more aware of their risk, and are thus more likely to adhere to surveillance and prevention regimes. In fact, users were informed that effective preventive measures can significantly reduce the risk of breast and/or ovarian cancer in individuals at increased risk, and that surveillance modalities favour the early diagnosis of cancer so that the vast majority of patients diagnosed with early-stage breast cancer die from causes other than cancer [29]. This is important also in the light of the recent widespread advertising campaign for genetic testing in the USA, which may be open to criticism on the grounds that it is a predictive test for a condition for which there is no cure, namely predisposition to cancer. Our educational model of cancer genetic counselling is aimed not only at genetic testing, but also at surveillance and preventive measures not only in the proband but also in relatives at risk of both hereditary and familial forms of cancer, irrespective of the identification of the predisposing mutation in the family.

In accordance with the recent American Society of Clinical Oncology Policy Statement Update: Genetic Testing for Cancer Susceptibility [12], our model is based on the fact that 'many of the management decisions surrounding the care of cancer patients with inherited cancer-predisposing mutations require a level of clinical expertise that is most likely within the purview of the oncology practitioner or a multidisciplinary team of specialists.' Our model also incorporates other main features recommended by ASCO: educational opportunities, requirement for informed consent, and integration of cancer risk assessment and management into oncology practice and prevention. The costs of this type of cancer genetic counselling will probably be offset by a decrease in cancer patients because more patients and relatives are taking early surveillance and preventive measures.

Given the high rate of consent throughout the counselling process, we believe that this multistep model might represent one of the strategies for the management of subjects at risk of hereditary and familial breast and/or ovarian cancers.

Acknowledgements

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The Italian Multi-Centre Project on Evaluation of MRI and other Imaging Modalities in Early Detection of Breast Cancer in Subjects at High Genetic Risk[1]

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This report presents the preliminary results of the first phase (21 months) of a multi-centre, non-randomised, prospective study, aimed at evaluating the effectiveness of contrast-enhanced magnetic resonance imaging (MRI), X-ray mammography (XM) and ultrasound (US) in early diagnosis of breast cancer (BC) in subjects at high genetic risk. This Italian national trial (coordinated by the Istituto Superiore di Sanità, Rome) so far recruited 105 women (mean age 46.0 years; median age 51.0; age range 25-77 years), who were either proven BRCA1 or BRCA2 mutation carriers or had a 1 in 2 probability of being carriers (40/105 with a previous personal history of BC). Eight cases of breast carcinomas were detected in the trial (mean age 55.3 years, median age 52.5; age range 35-70 years; five with previous personal history of BC). All trial-detected BC cases (8/8) were identified by MRI, while XM and US correctly classified only one. MRI had one false positive case, XM and US none. Seven "MRI-only" detected cancers (4 invasive, 3 in situ) occurred in both pre- (n = 2) and post-menopausal (n = 5) women. With respect to the current XM screening programmes addressed to women in the age range 50-69 years, the global incidence of BC in the trial (7.6%) was over ten-fold higher. The cost per "MRI-only" detected cancer in this particular category of subjects at high genetic risk was substantially lower than that of an XM-detected cancer in the general women population. These preliminary results confirmed that MRI is a very useful tool to screen subjects at high genetic risk for breast carcinoma, not only in pre-, but also in post-menopausal age, with a low probability of false positive cases.

Key Words: BRCA1 gene mutations, BRCA2 gene mutations, Hereditary breast cancer, Magnetic Resonance Imaging, X-ray mammography, Ultrasound, Surveillance, Screening

Although correctly classified as a sporadic disease, breast cancer (BC) presents a substantial component of genetic, multi-factorial transmission, referred to hereditary forms of autosomal dominant type (1-3). It is estimated that about 5% of all BC cases are likely due to primary genetic causes (3), while as many as 5-15 % show familial clustering (1).

Pathogenetic mutations of two genes, BRCA1 (4-6) and BRCA2 (7,8) are today held responsible for at least 50% of hereditary BC cases, the remaining ones being likely due to still unknown gene mutations (9-11). In BRCA1 and BRCA2 mutation carriers the cumulative life time risk for BC may reach values between 60 and 85% (10,12).

Besides the vertical transmission and aggregation of cases of carcinomas in the family (occurring in breast or in other organs such as ovary, prostate and colon-rectum), hereditary BC has a high probability of early onset, more than 50% of women at high genetic risk being affected by the disease before the age of 50 years (13-17). With respect to BRCA1, BRCA2 mutation carriers present a risk profile shifted to more advanced ages (18). Hereditary BC may develop under the form of multifocal or multicentric lesions, often caused by highly proliferating, poorly differentiated and hormone-receptor negative tumour cells. Moreover, the risk of developing a second cancer in the contralateral breast or an ovary cancer within five years from a previous neoplastic event is estimated to be between 30 and 60% (19,20).

No specific surveillance programmes have been as yet activated at the national level for early diagnosis of breast carcinoma in subjects with hereditary predisposition to this disease.

Current risk reduction strategies propose (besides information, counselling and some changes in lifestyle) the participation in chemoprevention trials (21-24), prophylactic surgery - i.e. preventive bilateral mastectomy (25) and/or oophorectomy (26) - or secondary prevention by adoption of specific recommendations, early diagnosis by screening and follow-up care (27,28). It is reported that a large proportion (40-80%) of asymptomatic carriers of BRCA1/2 mutation are more inclined to surveillance rather than to preventive mastectomy or chemoprevention (29-31).

With respect to a BC screening programme addressed to the general women population (50-69 years), the screening of subjects at high genetic risk requires earlier and closer controls and the use of diagnostic techniques which combine maximum diagnostic sensitivity with high predictive value and independence from breast density. In fact, the sensitivity of X-ray mammography (XM), which is at present the modality of choice for BC screening, may be severely reduced in case of dense breast, not only in young women, but also at ages over 50 years (32-38). Moreover, some concern has been expressed regarding repetitive exposure to ionising radiation of BRCA1/2 gene mutation carriers, especially at young ages, in view of a suspected higher tissue vulnerability to a DNA-damage producing agent (39-49), as also indicated by studies on model systems (50).

In the light of the benefits expected from the adoption of a more effective surveillance programme for subjects at high genetic risk, even the application of more expensive examinations than those adopted for the general women population might be justified. This view is further supported by the predicted reduction in total health care and social costs deriving from an early diagnosis of hereditary BC, a disease characterised by early onset and fast progression.

Following its first introduction in the 80s (51), dynamic contrast-enhanced magnetic resonance imaging (MRI) progressively developed to become the most sensitive modality today available for BC diagnosis (52-56). As reviewed in other papers of these Proceedings, specific indications to MRI in the area of breast oncology are: multicentric/multifocal disease; assessment of recurrence even in the presence of severe scarring or prostheses; occult tumour (CUP syndrome); monitoring the response to therapy; differential diagnosis of special cases. On the other hand, among drawbacks and limitations of MRI with respect to conventional mammography, are the use of intravenous contrast agents, the longer examination time, a higher dependence on the menstrual cycle, higher costs, and general contraindications to MRI (pace-maker, ferromagnetic vascular clips, claustrophobia, etc.).

Regarding the possible use of MRI in screening subjects at high genetic risk of BC, this technique combines the advantage of being independent from breast density with that of not using ionising radiation. Additional benefits derive from the peculiar feature of MRI of providing *in vivo* measurements of tissue parameters like microvascular permeability and extracellular volume fraction, related to neo-angiogenesis and tumour progression (57-62).

A number of research projects and study groups have been recently activated in Europe and in North America, with the aim of assessing to which extent the combined use of MRI and conventional mammography may enhance the diagnostic accuracy and therefore the effectiveness of a screening programme specifically directed to subjects at high genetic risk of BC (63-68).

In Italy, a network of highly specialised Centres has been activated in 1998 by the Istituto Superiore di

Sanità, Rome, within a research project aimed at evaluating the effectiveness of combining MRI with conventional imaging examinations for the early diagnosis of BC in subjects at high genetic risk. The network presently comprises twelve institutions (five Institutes of Cancer Research and Treatment and seven University General Hospitals). A clinical trial has then been activated in 2000, in the frame of this project. The trial is currently conducted by nine functional units (active in Aviano, Chieti, Genova, Milano, Modena, Napoli, Padova/Udine, Pisa and Torino), each endowed of integrated services of clinical oncology, medical genetics, psycho-oncology counselling, molecular genetics laboratories, breast MRI, XM and high-frequency ultrasound (US).

This report presents a preliminary analysis of the data obtained in the first phase of this trial (June 2000-March 2002).

Study design

This prospective, non-randomised and comparative study is carried out in different Italian Centres, on the basis of common recruitment criteria and diagnostic protocols.

Eligibility. Subjects at very high risk for breast cancer were selected according to one of the following criteria: a) proven carriers of germ line, pathogenetic BRCA1 or BRCA2 mutation; b) first-degree relative with proven BRCA1/2 mutation (but unknown personal mutation status). One woman belonging to a family at very high risk of BC likely associated with a non-BRCA1/2 mutation and one woman belonging to a family at very high incidence of breast cancer, were also entered into the study.

Women could be recruited starting from the age of 25 years, and men (BRCA2^{+}) from 50. Women with personal history of unilateral BC were offered to enter the study, provided that at least one breast had not been removed. Bilateral breast screening was performed as a rule (i.e. also on the breast previously submitted to conserving surgery); for those who had undergone unilateral mastectomy, only the contralateral screening was performed.

Enrolment was offered to eligible subjects and to their eligible relatives, in the context of genetic counselling (and, if necessary, psychological assistance), following informed written consent. Preventive approval by the institutional review board had to be requested locally. In case of hormonal replacement therapy, diagnostic examinations started at least three months after its interruption. Exclusion criteria were: pregnancy, breast-feeding, current chemotherapy, terminal illness or specific contra-indications to MR examinations. The screening protocol consisted of two annual diagnostic packages including XM, US and MRI. For pre-menopausal women, MRI was performed within the second week of the menstrual cycle.

Techniques

X-ray mammography. Examinations were performed on conventional high frequency generator units with rotating anode; focus 0.3-0.1 mm; focus-film distance ≥55 cm; homogeneous breast compression; mobile grid; automatic exposure control, dedicated film-screen system (day-light treatment). Regular (daily and 6-month) quality controls of the system performance were carried out together with controls on exposure dose (<12 mGy/45 mm Plexiglas). Standard medio-lateral oblique and cranio-caudal projections were obtained for each breast. Further views were taken when necessary. Mammographic findings were reported by using the BI-RADS (American College of Radiologists) 5-score system: 1) negative; 2) benign finding; 3) probably benign finding; 4) suspicious abnormality; 5) highly suggestive of malignancy.

Ultrasound. Breast US examinations were performed at a frequency ≥7.5 MHz, axial resolution of 0.5 mm and latero/transverse resolution of 1 mm; optimal contrast variable focussing. Regular periodic quality controls were carried out using an appropriate phantom.

MRI. Requirements for the MRI equipment were an operative static magnetic field Bo ≥1.0 T; actively shielded gradients ≥15 mT/m; dedicated, bilateral, synchronous breast coil.

MR image acquisition. Dynamic contrast-enhanced MR acquisitions were obtained using a spoiled gradient-echo sequence (e.g. FLASH, SPGR or FFE (52)). Three-dimensional (3D), T1-weighted images were acquired in coronal or axial planes (slice thickness 3 mm; no gap; FOV 350 mm; matrix 128 x 256 for coronal planes; rectangular FOV adapted to the patient for axial planes; number of partitions per breast sufficient to cover the entire mammary tissue (i.e. 40-48); phase encoding axis vertical for coronal planes, horizontal for axial planes; TR and flip angle selected according to the available sequence; TE value selected to avoid fat-water signal opposition).

MRI exam comprised one pre-contrast and five post-

contrast acquisitions. The contrast agent, a two-compartment Gd-chelate (0.1 mmol/kg) was injected as intravenous fast bolus (about 2 ml/s), followed by 20 ml saline solution (NaCl 0.9%) flush. The temporal resolution of post-contrast images was 90 s. The first post-contrast image acquisition started at the same time as the contrast agent injection.

Post-processing and data storage. The pre-contrast 3D images were subtracted from the first, second and fifth contrast-enhanced images and the Maximum Intensity Projection (MIP) algorithm was applied to the subtracted images. The curves representing the temporal dependence of signal intensity [SI(t)] and/or that of the percent SI enhancement, [SI(t)-SI(0)]/SI(0) ˇ 100], were then determined in selected small regions of interest (ROI, 3x3 pixel). The acquired, subtracted and MIP-reconstructed images, together with the ROI-based curves, were stored on dedicated magnetic support.

Lesion classification. The scoring system adopted for classifying MRI-detected lesions was based upon a combination of morphological features and enhancement kinetics parameters (69), as reported in Table I. Lesions with scores 0-2 were classified as benign; score 3 suggested uncertain lesion; scores 4-8 indicated malignancy. In case of non-benign (scores 3-8) lesion detected only by MRI, the latter was repeated after 1-2 months. If the lesion was confirmed, a US-guided (second look) fine needle aspirate (FNA) cytology or core-biopsy or a MRI-guided biopsy was performed. In these cases, the final diagnosis was established by cytology of FNA, or pathologic exam of core-biopsy or mastectomy specimens.

True negative cases were defined as those for which no suspicion was raised at a given diagnostic examination, nor BCs were detected during follow-up.

Clinical and imaging follow-up was scheduled for at least two years for subjects whose imaging examinations gave negative results in the two-round study.

Results

In the period June 2000 - March 2002, 105 patients (mean age 46.0 years, median age 51.00, age range 25-77) were enrolled in the first annual round, while 14 of them also underwent a second round. Out of the 105 recruited women, forty (38%) had a previous personal history of BC. Seven patients were found to be affected by BC at the first round and one at the second round, eight in total, for an overall global incidence of 7.6% (8/105). As summarised in Table II, these eight patients had a median age of 52.5 years (mean 55.3, range 35-70). Five of them (63%) had a previous personal history of BC, giving a ratio of with/without previous BC history of 1.7 (5/3) for the patients presently affected versus 0.62 (40/65) for the screened women. Pathology demonstrated: 2 invasive ductal carcinoma (IDC), 2 invasive lobular carcinoma (ILC), 1 IDC+ILC, 2 multifocal DC in situ (DCIS), and 1 DCIS+LCIS. Out of the eight cancers, 7 (88%) were detected only by MRI, 4 invasive and 3 in situ, both in pre- (n=2) and post-menopausal (n=5) women. Only one cancer was detected also by XM and US. MRI had one false positive case, XM and US none. Table II also shows the genetic status of each patient.

Discussion

The preliminary results of this trial indicated that Gd-enhanced MRI is a very useful tool to screen subjects at high genetic risk of BC, not only in pre-menopausal, but also in post-menopausal age, with a low probability of false positive cases. Previous personal history of BC was associated with higher probability of BC detection during the screening. Although the trial is still at a too early

Table I - Scoring system for the classification of MRI lesions (according to ref. 69)

Lesion feature	Score		
	0	**1**	**2**
shape	round, oval, lobular	linear, dendritic, stellate	——
margin	well defined	ill-defined	——
enhancement pattern	homogeneous	heterogeneous	rim sign
initial SI enhancement	low (<50%)	moderate (50-100%)	high (>100%)
SI time dependence	continuous increase	plateau	wash-out

[1] Total score: 0-2, benign; 3, uncertain; 4-8, malignant.

Table II - Breast cancers detected in the Italian ISS trial in the period June 2000-March 2002

Patient	age(years)	genetic mutation	previous BC	trial-detected BC
Mo1	69	BRCA1[+]	yes	DCIS, multifocal, high grade. Imaging finding: positive to MRI only. Diameter of the largest focus, 3 mm (PA). MRI-guided FNAC; mastectomy.
Mi1	35	BRCA2[+]	no	Invasive lobular, T2N0, G2. Imaging finding: positive to MRI only. Lesion dimension, 23x27 mm. FNAC positive before surgery.
Mi2	61	unknown [1]	yes	Invasive lobular and ductal, G1. Imaging finding: positive to MRI only. Lesion dimension: 8 mm (MRI), 5 mm (PA).
Mi3	53	BRCA1[+]	yes	Invasive ductal, G2 (slightly differentiated). Imaging finding: positive to MRI only. Lesion dimension: 7-8 mm (MRI), 6 mm (PA).
Mi4	61	FHx	yes	Invasive ductal; bifocal, G1 and G2 (moderately differentiated), recurrent cancer adjacent to surgical scar. Imaging finding: positive to MRI only. Lesions' dimensions: 10 and 6 mm (MRI); 6 and 3 mm (PA).
Av1	70	BRCA2[+]	yes	DCIS (micropapillary), surrounded by a few LCIS foci. Imaging finding: positive to MRI only. Lesion dimension, 4 mm.
Av2[2]	53	BRCA2[+]	no	Invasive lobular, multifocal, associated with intraductal carcinoma foci, PT1cNo, G1. Imaging findings: positive to XM, US and MRI. Lesion dimension: 1.5 cm.
Pi1 before	41	BRCA1 [1:2]	no	DCIS, multifocal; scarcely differentiated (G3), with no angio-invasion. Imaging findings:at MRI, suspicious ductal morphology; at retrospective XM evaluation, asymmetric hyperdensity; at second US look, unspecific hypoechogenic area. Echo-guided FNAC mastectomy.
8 cases	mean 55.3 median 52.5 range:(35-70)		5/8 previous BC	5/8 invasive

[1] For further details, see ref. 70. [2] This case was the only one detected by all diagnostic modalities.

Abbreviations: BC, breast cancer; BRCA1^{+}, proven BRCA1 mutation carrier; BRCA2^{+}, proven BRCA2 mutation carrier; BRCA1 [1:2], 1 in 2 probability of being BRCA1 mutation carrier (i.e. first relative with proven BRCA1 mutation; personal mutation status unknown); FHx, family history indicative of high genetic risk of breast cancer (untested mutation); FNAC, fine needle aspirate cytology; nr, not reported; PA, pathological analysis.

Table III - Comparison of MRI, XM and XM+US results reported by non-randomised studies on women at high genetic risk of breast carcinoma

Trial	Nijmegen 1994-2001 (ref. 63)	Rotterdam 1995-1998 (ref. 64)	Bonn 1996-1998 (ref. 65)	Toronto 1997-2000 (ref. 66)	Italy (ISS) June 2000- March 2002	Total
enrolled subjects	179	109[1]	192[2]	196	105	781
age range	21-71	22-68	18-65	26-59	25-77	18-77
Previous BC	no	nr	58	55	40	≥ 153
Trial-detected BC cases						
biopsy-proven	13	3	9	7	8	40
invasive	10/13	2/3	7/9	6/7	5/8	30/40
age of patients[3] (years)	30,30,31,35, 40,42,44,44, 46,47,49,50,50	29,42,53	28,34,36,38, 44,47,48,53,57	33,46,49,50, 52,52,.53	35,41,53,53, 61,61,69,70	mean 45.6 median 49.0 range 28-70
previous BC	no	nr	nr	4/7	5/8	$\geq 9/15$
genetic status	BRCA1+ or BRCA2+ or FHx (LTR>15%)	3FHx (LTR: one 40%, two 25%)	6 BRCA1+ 1 BRCA2+ 2 FHx	4 BRCA1+ 2 BRCA2+ 1 FHx	2 BRCA1+ 1 BRCA1 [1:2] 3 BRCA2+ 1 unknown mutation 1 FHx	
BC diagnosis by different methods						
MRI	13/13	3/3	9/9	6/7	8/8	39/40
XM	6/13	0/3	3/9	3/7	1/8	13/40
XM+US		nr[4]	4/9	4/7	1/8	
detected by MRI only	7/13	3/3	5/9	2/7	7/8	24/40
False positive cases						
MRI	17	6	5	17	1	46
XM	10	nr	7	1	0	≥ 18

[1] Breast density $\geq 50\%$. [2] Six symptomatic subjects (ages 25, 35, 44, 48, 53 and 55) at high genetic risk of breast cancer on the basis of family history, were also analysed: out of these six breast cancer cases, six were detected by MRI, four by XM and four by (XM+US). [3] Age of the patient at the time of breast cancer diagnosis during the trial. [4] MRI-guided US identified suspect lesions in two out of three cases; results of US examinations before MRI, not reported.

Abbreviations: BC, breast cancer; FHx, family history indicative of high genetic risk of breast cancer (untested mutation); BRCA1+, proven BRCA1 mutation carrier; BRCA2+, proven BRCA2 mutation carrier; BRCA1 [1:2], 1 in 2 probability of being BRCA1 mutation carrier (i.e. first relative with proven BRCA1 mutation, personal mutation status unknown); LTR, lifetime risk; nr, not reported.

stage to allow calculation of the diagnostic indices for each used modality (validation of negative findings is, according to the protocol, still under way), a first comparison can already be made with the results of similar non-randomised studies, conducted in other Countries (Table III).

A retrospective study carried out by Stoutjesdijk et al. (Nijmegen, The Netherlands, November 1944 - February 2001) was aimed at determining whether MRI could play a role in the early detection of BC in women with hereditary risk of the disease (63). To this end, a retrospective group of 179 women (age range 21-71 years) was assembled, in which all subjects had received, besides biannual palpation, annual imaging by MRI, XM or both (258 images and 262 mammograms). Out of the 179 women, 75 had received both MRI and XM examination within a

4-month period. Inclusion criteria were: lifetime risk of BC ≥15%, according to Claus et al. (71), based on family history of breast or ovarian cancer or on the presence of a germ line mutation in the BRCA1 or BRCA2 gene. In this study no patients with personal history of BC were included. In the group of 179 women 13 cancers were detected (7.3%), all revealed by MRI, while only six were identified by XM. MRI was therefore found to be more accurate than XM in the annual BC surveillance of women with hereditary risk of BC, justifying the activation of larger prospective studies to evaluate the role of MRI in dedicated screening programmes.

First experiences in screening women at high risk of BC were reported by Tilanus-Linthorst et al. (64). The study (Rotterdam, The Netherlands, September 1995-April 1998) was aimed at investigating whether MRI, in addition to the normal surveillance, could detect cancers otherwise missed in a group of women (n = 109, mean age 41.5 years, range 22-68) with over 25% risk of BC and more than 50% breast density at mammography. MRI detected three cancers (2.8%) occult at mammography and did not give any false negative; it was false positive in 6 women (resulting in two benign local excisions because FNA cytology confirmed suspicion) and recognized 4 true benign cases.

In the first thirty months of a 5-year study carried out in Bonn, Germany (March 1996 - October 1998), Kuhl et al. (65) identified nine BCs in a group of 192 women who, on the basis of personal or family history or genetic analysis, were suspected or proved to carry a BC susceptibility gene. In the absence of genetic tests, the inclusion criteria followed in this pilot study were: women with personal history or history of a relative corresponding to at least one of the following conditions: BC diagnosed at or before the age of 35 years; ovarian cancer diagnosed at or before the age of 40; bilateral BC; both breast and ovarian cancer; at least two relatives with breast and/or ovarian cancer, one of whom diagnosed at or before 50 years. Men were included in case of personal history of BC or a history of a male relative with BC. The mean age ±SD of the study participants was 39±9 years, the median age 38 and the age range 18-65. Out of nine biopsy-proven cancers (4.7% in the group of 192 subjects) all nine were detected by MRI, three by XM and four by combined XM and US. Five carcinomas were therefore detected only by MRI. Regarding false positive cases, five were due to MRI and seven to XM.

Comparison of breast MRI, XM and US for surveillance of women at high risk of hereditary BC was reported by Warner et al. (66). The study (Toronto, Canada, November 1997 - May 2000) was conducted on 196 women (mean age 43.3 years, age range 26-59). Inclusion criteria were : 1) a germ line BRCA1 or BRCA2 mutation or a first-degree relative with a BRCA1 or BRCA2 mutation (but unknown personal status); or 2) a strong family history of breast or ovarian cancer, i.e. three or more relatives on the same side of the family with cancer diagnosed before the age of 50 years or ovarian cancer. A woman with a past history of BC could be included, provided that her contralateral breast had not been removed. In this case, she could be counted in the number of affected relatives in the reconstruction of the family history. Seven cancers were diagnosed in the screening (3.6%), six detected by MRI, three by XM (four by XM plus US). One case of DCIS was detected only by XM. Out of 23 women who had a result that was suspicious by some of the adopted modalities, MRI was false positive in 17, XM in 1 and US in 13.

The screening studies so far carried out on women at high genetic risk of BC led to the conclusions that MRI was not only an effective modality in the detection of occult cancers (64), but was also more sensitive and significantly more accurate than conventional imaging (65,66).

The preliminary results of our prospective study substantiate these conclusions. The rate of tumour detection (7.6%) was similar to that of the retrospective study carried out in Nijmegen (63), although in the Italian study the monitoring time was as yet much shorter (21 vs. 64 months) and the two populations were not identical. In fact, our study so far enrolled subjects belonging to the highest category of risk of BC and did not exclude women with previous BC.

On the basis of the data so far obtained by the Italian study, higher sensitivity and accuracy can be predicted for MRI with respect to both XM and a combination of XM and US, in agreement with Kuhl et al (65) and Warner et al (66). In addition, the results of our study point to the need of including in a special surveillance programme also women with previous history of BC. In fact, out of eight cancers detected in the trial, five (62%) were identified by MRI (but not by XM or US) in women who had a previous history of the disease.

Concerning the additional costs associated with the introduction of MRI in a screening programme specifically designed for subjects at high genetic risk, an analysis reported by Tilanus et al. (64) on the basis of the results of the Rotterdam trial, showed that the extra cost of breast MRI (in addition to XM and physical examination) was € 13,930 per detected cancer, as compared to the cost of € 9,000 for the diagnosis of one BC patient in the Dutch general screening programme. In consideration of the higher rate of breast cancer detection in the Italian with respect to the Rotterdam trial (7.6% vs

2.7%), and the lower number of MR false positives (1 vs 6, over groups of similar size), the total cost per detected BC case in our study was about € 6,200 (computed from the costs of 119 MRI, 119 XM and 119 US examinations, plus that of one excisional biopsy due to the false positive MRI examination). The extra costs associated with the addition of MRI to XM and US in our special trial devoted to high-risk subjects, were estimated to be about € 41,000 (computed from 119 MRI and one excisional biopsy). This additional expenditure was however very cost-effective, since it allowed the detection of seven BC cases which would have been missed by the other imaging modalities (XM plus US), with an average cost of about € 6,000 per "MRI-only" detected cancer (i.e. about 2/3 the cost afforded by a general XM screening programme for detecting one BC in the general population of women between 50 and 69 years). Obviously, only a very high incidence/prevalence of breast cancer cases in a restricted population of subjects at high genetic risk makes the extra-costs of the MRI-screening affordable and reasonable.

Furthermore, six out of eight cases of BC detected in our trial were diagnosed in women above 50 years. Out of these six cases, five were only detected by MRI, indicating that 83% of these cases would have been missed in the general XM screening programme (50-69 years).

Data of Table III point to the potential of accruing information from different studies which used similar, standardised technical procedures. In spite of some differences in the design of individual trials, some consistent conclusions seem to emerge from the overall body of information so far provided by five studies (Nijmegen, Rotterdam, Bonn, Toronto and Italy) which adopted a combination of MRI and conventional imaging procedures for the screening of women at high genetic risk:

• over a total group of 781 women, 40 biopsy-proven cases of BC were detected (average detection rate 5.1 %, ranging from 2.7% to 7.6%), 30/40 (75%) of these being invasive lesions and 25/40 (62%) diagnosed at ages below 50;

• regarding the sensitivity of individual modalities in BC detection during these trials, 39/40 cases (97.5 %) were identified by MRI and 3/40 (32.5%) by XM; 60% of all trial-detected BC cases were therefore diagnosed by MRI but not by conventional imaging modalities;

• in the total group of 781 women there were 46 false positive cases due to MRI and at least 18 due to XM;

• the average ratio of false positive to true positive cases in MRI was 1.18 (46/39), but there was a large variability among trials (from 0.12 to 2.83).

The preliminary results of this comparative analysis of different studies, point to the need of more extensive, multi-centre and multi-national trials on the evaluation of benefits and costs associated with the introduction of MRI into appropriate screening programmes specifically addressed to subjects at high genetic risk of BC. These efforts should allow the collection of a sufficient body of data to define to which extent breast MRI could be integrated with XM and US for an effective surveillance of these subjects. This is a non-negligible point in the general problem of the correct use of MR technique in breast imaging, since subjects at high genetic risk of BC represent the only one population for which MRI can be proposed as a screening method.

Additional, important questions should also be asked in the future, in relation to this particular category of subjects. Could an annual single-view (medio-lateral-oblique) XM be enough to exclude microcalcifications (and therefore avoid possible MRI false negative cases, more frequently associated with in situ cancers)? Or do we still need the usually proposed annual two-view XM? Should US be performed after and not before MRI, with the result of immediately increasing the diagnostic sensitivity to the level of that now obtained with the second look examination (that is to say with an MRI-based breast US examination), and therefore changing the diagnostic flow-chart? And last, but not least, what level of familial history of BC will make of MRI a specific screening method for women who refuse genetic tests?

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HUMAN GENE MUTATIONS

Gene Symbol: BRCA1

Disease: Breast/Ovarian Cancer

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Small insertions (< 21 bp)

Accession Number	Codon Number	Insertion	Disease state
H972119	460	AGACAAA^ATAaTTTGGGAAAA	Breast/Ovarian Cancer

Gene Symbol: RET

Disease: Hirschsprung's Disease

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Splicing mutations (single base-pair substitutions)

Accession Number	IVS	Donor/ Acceptor	Relative location	Substitution	Disease state
H972168	10	as	-2	A-G	Hirschsprung's Disease
H972169	19	as	-9	C-T	Hirschsprung's Disease

Nucleotide substitutions (missense/nonsense)

Accession Number	Codon Number	Nucleotide Substitution	Amino acid Substitution	Disease state
H972170	202	cGTG – ATG	Val – Met	Hirschsprung's Disease
H972171	480	cGAA – AAA	Glu – Lys	Hirschsprung's Disease
H972172	771	aGAC – AAC	Asp – Asn	Hirschsprung's Disease