



Hereditary breast and ovarian cancers: genetic testing and its clinical implications

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Abstract

Breast and ovarian cancers are the most commonly encountered cancers in women, although only 5% or less of these arise from hereditary predisposition. Mutations in two of the cancer susceptibility genes, BRCA1 or BRCA2, explain breast and ovarian cancers in a majority of these hereditary cancers. With the advent of gene testing and dissemination of information over the public domains, clinicians are often confronted by patients about the options and benefits of these tests. Genetic counseling and risk estimation for breast or ovarian cancer along with BRCA mutation analysis has been initiated by our group at the Tata Memorial Hospital and ACTREC. The purpose of this review is to a) provide basic information about the mutations in BRCA1 and BRCA2 genes b) highlight the relevance of population based data on the occurrence of mutations in these genes c) evaluate various methods of genetic testing and d) discuss clinical aspects for the management of disease in mutation carriers.

Introduction

Seminal work by Lynch and his co-workers led to the identification of a large number of hereditary and familial cancer syndromes¹. Breast and ovarian cancers represent the most frequently occurring malignancies in a variety of familial cancer syndromes with seemingly autosomal dominant pattern of inheritance of the disease. Confirmation of the

contribution of inherited mutations in cancer susceptibility gene to familial syndromes in Retinoblastoma families² provided a boost to the hunt for breast and ovarian cancer susceptibility genes. Subsequently, two high penetrance breast cancer genes, called BRCA1 and BRCA2 were identified which could explain hereditary breast as well as hereditary breast-ovarian cancer (HBC / HBOC) syndromes in many of the families where these cancers were inherited in an autosomal dominant manner^{3,4}. Typical features of such families are presented in **Table 1 and Figures 1 and 2**.

BRCA1 and BRCA2 genes

The BRCA1 gene is located on chromosome 17q21. It is made up of 24 exons of which 22 exons actually code for the protein. The BRCA1 protein is made of 1863 amino acids. These are organized into multiple functional domains spanned over the entire length of the molecule (**Figure 3**). The two distinct domains in these proteins are a zinc binding RING finger motif near the N terminus and two BRCT (BRCA1 C – terminal) domains in tandem. The RING finger motif has been identified in several transcription factors and cofactors involved in both DNA and protein binding, suggesting a role for BRCA1 in regulation of expression of various target proteins. The BRCT motifs have been found in a number of proteins involved in cell cycle control and DNA repair. In agreement with these considerations, BRCA1 protein has been found to be involved in a variety of protein-protein and protein-DNA interactions associated with regulation of gene expression, DNA repair and homologous recombination.

The BRCA2 gene is located on chromosome 13q12-13. It comprises of 26 exons with exon 1 forming part of the 5' untranslated region. The BRCA2 protein is made up of 3418

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Table 1. Features of Hereditary Breast-Ovarian Cancer (HBOC) Syndrome

1.	Early age of onset of breast cancer (often before age 50).
2.	History of breast and / or ovarian cancers, in two or more consecutive generations.
3.	Higher frequency of bilateral cancers (in either breasts or ovaries) or an individual developing both breast and ovarian cancers.
4.	Autosomal dominant pattern of inheritance (vertical transmission through either the mother or father's side of the family) with an increased incidence of tumors of other specific organs, such as prostate (involving mutations in <i>BRCA1</i> and <i>BRCA2</i>), pancreas, larynx, stomach cancers or melanomas (<i>BRCA2</i>).
5.	Family history of male breast cancer (more often associated with <i>BRCA2</i> mutation).

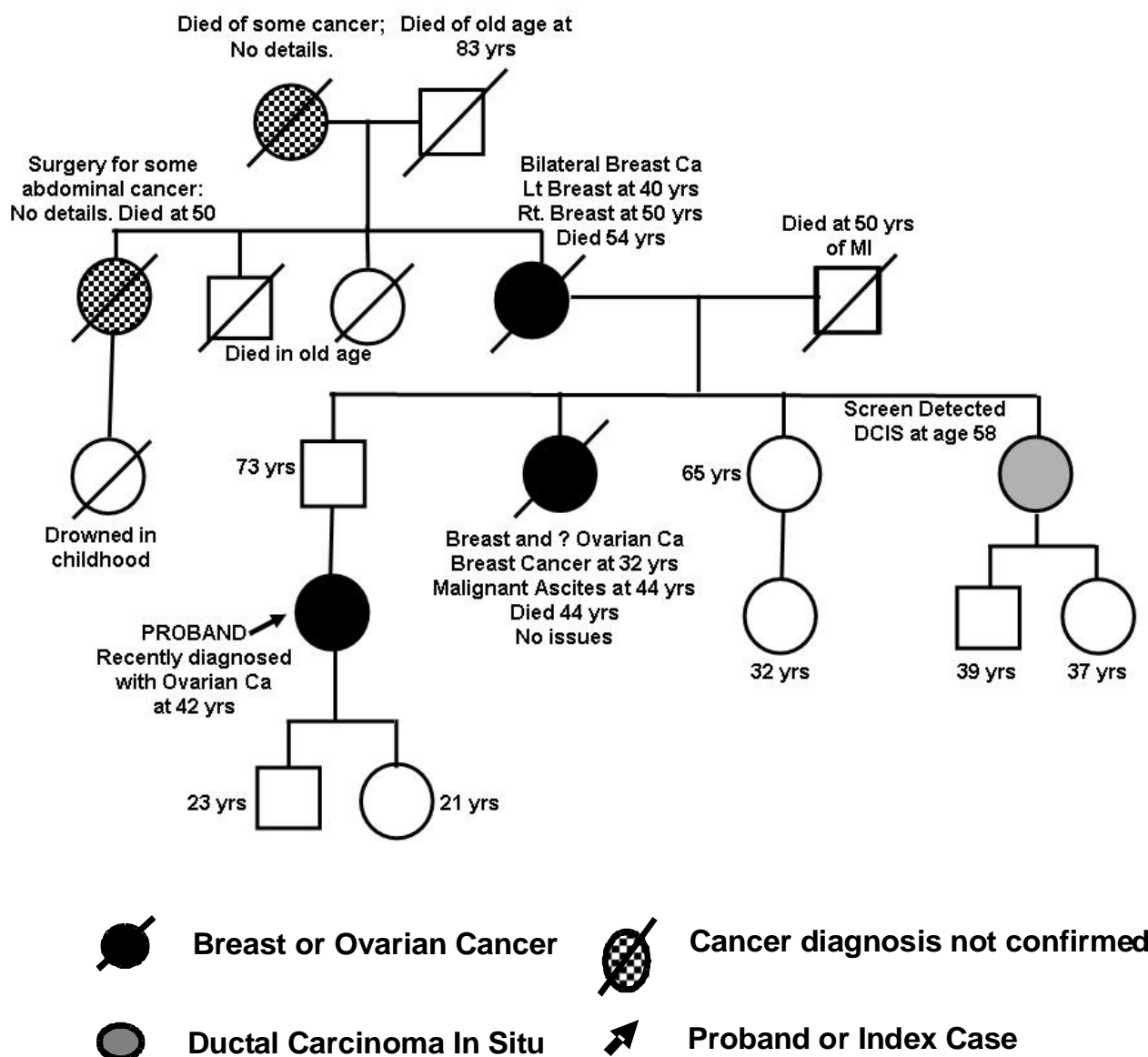
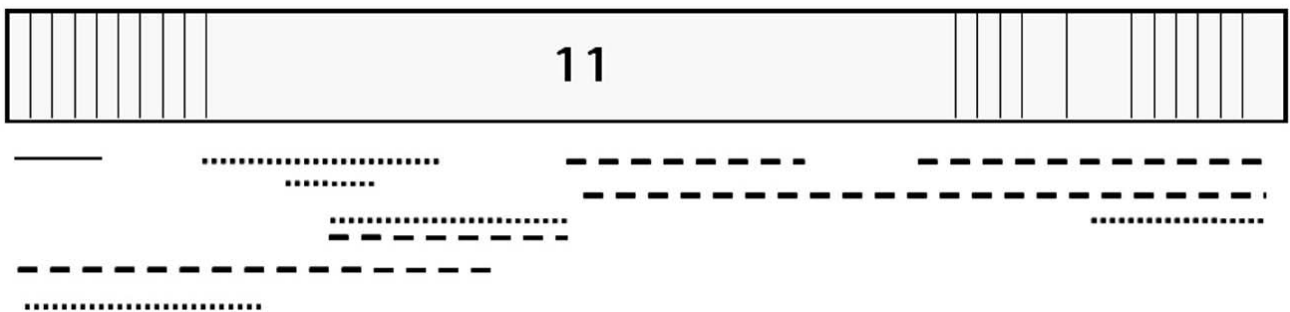
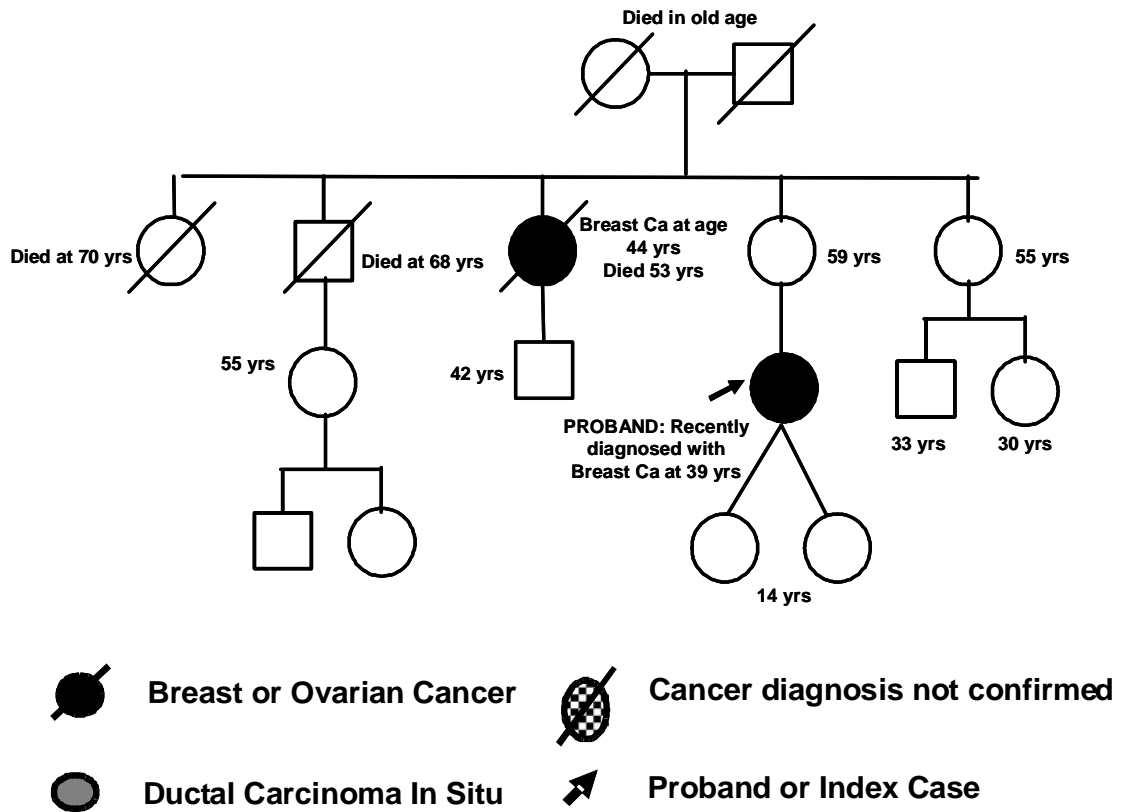


Figure 1. Characteristic history in a hereditary breast ovarian cancer family



(a) The organization of exons in *BRCA1* gene.

(b) Stretches of sequences associated with various functions of the *BRCA1* protein.

Sequences involved in interactions related to the control of cell cycle.

..... Sequences involved in the regulation of expression of target genes.

- - - Sequences involved in the DNA-repair related interactions.

Figure 2. BRACA 1 gene - exons and distribution of functional domains

amino acids. Like BRCA1, BRCA2 also has a large exon 11. Several copies of BRC repeats are found in this gene. BRCA2 shares a number of functional features with BRCA1. Both these proteins thus not only act as the ‘guardians’ or ‘caretakers’ of the genome but also as ‘gatekeepers’ controlling transition across different phases of growth cycle⁵. The “breast and ovary” specific effect of the mutations in these genes remains poorly understood.

Types of mutations

Different types of alterations in the sequence of DNA coding for BRCA1 and BRCA2 constitute spectrum of mutations that have pathogenic consequences. The message in the DNA sequence is read as triplets of nucleotides (codons) each representing a particular amino acid. Mutations involving an insertion or deletion of one or more nucleotides can lead to an altered reading of codons such that codons after the mutation represent different amino acids from those in the original sequence. Such mutations lead to extensive change in the amino acid sequence of the protein and may also result in production of a shorter protein. These ‘frame-shift’ mutations constitute nearly 50% and 30% of all the mutations reported in BRCA1 and BRCA2, respectively⁶. On the other hand, single nucleotide changes (point mutations) that alter a single triplet codon thereby changing a single amino acid in the whole protein may have variable effects. Such ‘point mutations’ causing substitution of a functionally and/or structurally different amino acid are termed as ‘mis-sense’ mutations. Impact of such mutation on the function of the protein depends on the site as well as the nature of the amino acid introduced. Similar single nucleotide base pair changes that result in the generation of a ‘stop codon’ (amino acid chain termination signals), termed as ‘nonsense mutations’, lead to the production of a truncated protein. The site of these mutations would determine their influence on the function of the generated protein. Such variations in the influence of point mutations highlight the relevance of study of phenotype-genotype relationships for various mutations⁷. Missense and nonsense mutations constitute nearly 25% and 10% of reported mutations in BRCA1, respectively⁶. Missense mutations have been found to be more common in BRCA2 (~50%). These various types of mutations have been detected along the entire length of both the genes.

Population genetics of mutations in BRCA1 and BRCA2 genes

There are wide variations in the population frequency of mutations in BRCA1 and BRCA2 in different geo-ethnic groups. The estimated frequency for mutations in general population (Caucasians as well as African-Americans) for BRCA1 has been reported to be in the range between 0.04 to 0.3% while prevalence of mutations in BRCA2 is estimated

to range between 0.4 to 0.7%⁸. Ashkenazi Jewish population has one of the highest frequencies of carriers of BRCA1 / BRCA2 mutations (2.5%) and correspondingly higher incidence of breast / ovarian cancers⁹. Determination of the frequency of mutations in these genes in general population across the Indian subcontinent would be of interest since the incidence of breast cancer is 3-5 folds lower in Indian women (one in 30-40 women) compared to that in Caucasian women (one in 7-12). Literature from the west indicates that mutations in these genes are seen in 2-5% of all breast cancers⁹, and approximately 5-13% of ovarian cancers^{8,11}.

It is important to note that the mutations in BRCA1 account for 60% of the total deleterious mutations in hereditary breast-/ovarian cancer families. A lower frequency of mutations in BRCA2 compared to BRCA1 reflects a significantly greater role of BRCA1 in breast or ovarian cancers than BRCA2¹². This is especially true in families with history of ovarian cancers along with breast cancers while mutations in BRCA2 are dominant in male breast cancer patients^{13,14}. Overall, depending on the family history, carriers of germline mutations in BRCA1 or BRCA2 thus have a 50-80% life time risk of for developing breast cancer and 10-50% for developing ovarian cancer.

Importance of family history is substantiated by the fact that the incidence of cancers in women with positive family history is higher than that in women from the general population with comparable age¹⁴. The contribution of family history in determining the risk associated with the carrier status can also be interpreted as involvement of other genes which may modulate risk alone or in conjunction with additional genes. Mutations in such ‘other’ risk modulating genes may be relevant in a significant fraction of familial breast cancers¹⁵ that do not have mutations in the BRCA1 or BRCA2 genes.

A large number of reports, primarily in the Caucasian patients are available that describe the percent of families in which the disease is explained on the basis of mutation in BRCA1 or BRCA2. The trends seen in these studies are very similar to those reported by the Myriad Genetic Laboratories that describes analysis of BRCA1 and BRCA2 gene mutations in 10,000 subjects, one of the largest cohorts studied so far. The findings demonstrate that the mutations in BRCA1 and BRCA2 account for cancers in 60-80% of patients with family history of breast and ovarian cancers but only in 15-30% of patients with history of breast cancers alone¹⁴. In this context, Hedau et al¹⁴ detected mutations in BRCA1 in 25% (4/16) of the patients with family history from New Delhi region. On the other hand, another study from the same region¹⁷ reported detection of rare variations in only 11.7% (4/34) familial

patients. Similar differences in the detection of mutations in BRCA1 / BRCA2 are apparent in the two studies of familial breast cancer patients from Southern Indian region. While a study of 21 patients by Rajkumar et al¹⁸ found presence of two pathogenic mutations in BRCA1 and one in BRCA2 in 22 patients (3/22; ~14%), Kumar et al, reported that three of 14 (21%) breast cancer patients with a family history of breast cancer carried mutations in BRCA1 (19). The findings suggest an overall frequency of about 17% which is close to that reported for high risk women (712/4379; 16%) by Myriad Genetic Laboratories¹⁴.

Importance of understanding the frequency and types of mutations in BRCA1 and BRCA2 genes at population level is highlighted by the fact that to date, in BRCA1 gene alone, more than 1600 distinct alleles, (sequence variations) that represent mutations, polymorphisms and variants have been documented⁶. Several recent studies in oriental and middle-eastern populations have reported detection of novel variants and the list is expected to grow with data coming from larger study cohorts and diverse communities. Nearly 50% of the reported alleles have been encountered only once and clinical significance of about 35% remains ill defined⁶. Further, different domains would have variable contribution to the growth controlling and DNA repair related functions critical for tumor suppressor activity. This may explain the variation in the level of penetrance of individual mutations. Similar considerations would apply for study mutations in BRCA2, in the context of modulation of risk for breast as well as ovarian cancer.

The population structure, and geographic and cultural isolation are important considerations with regards to the spectrum and frequency of individual mutations detected in BRCA1 or BRCA2. Specific mutations found in multiple unrelated families of a given population are designated as founder mutations. Three founder mutations (185delAG and 5382insC in BRCA1 and 6174delT in BRCA2) are present in 2.5% of the Ashkenazi Jewish population and account for majority of breast cancers in this community. These are also the most commonly encountered mutations among Caucasians and Russian populations¹². Similarly, a single founder mutation in BRCA2 accounts for 7-8% of breast cancers in women and 40% of male breast cancers from Iceland²⁰. The history of occupation by English, Portuguese and Dutch as well as trade with populations from Chinese and African continents may be reflected in the BRCA1 / BRCA2 mutations detected in Indian population²¹. Presence of an Ashkenazi Jew specific founder BRCA1 mutation in a family from Goa may indeed be an indication of such a possibility¹⁹.

Methods of genetic testing

Detection of variations (polymorphisms) and pathogenic

alterations (mutations) in the ordered sequence of human genes has become the basis for analysis of hereditary disorders including hereditary cancers. A number of tests for screening cancer predisposition genes are now available. Most of these qualify as genetic screening tests because of their high degree of specificity and sensitivity. The BRCA1 and BRCA2 can be comprehensively screened using various methodologies like Denaturing High Performance Liquid Chromatography (dHPLC), Conformation Sensitive Gel Electrophoresis (CSGE), Single Strand Conformational Polymorphism (SSCP), Protein Truncation Test (PTT) and DNA sequencing which are briefly described below.

The most common screening tests to detect the presence of a mutation, namely dHPLC, CSGE and SSCP, exploit the differences in the thermodynamic properties of DNA conformation in its single or double stranded form. An individual with a germline mutation in BRCA1 has a mutant allele of this gene derived from one parent and a normal allele (termed as wild type) from the other parent. The first step in genetic testing is extraction of the genomic DNA from the peripheral blood lymphocytes followed by amplification of different coding regions (exons) of the gene using polymerase chain reaction (PCR). The amplified segments of DNA through PCR are termed as amplicons and are subject to strand separation (denaturation) followed by re-annealing under appropriate conditions. In a patient with a change in the DNA sequence a mixed population of homoduplex and heteroduplex is created following denaturing and reannealing of the wild type and mutant allele. The variations and mutations detected by these screening methods are confirmed and characterized by sequencing.

The dHPLC identifies differences in homo- and hetero- duplex DNA on subjecting the PCR product to a special type of chromatography that employs a unique DNA separation matrix under partially denaturing conditions. The heteroduplexes are retained for a lesser time than their corresponding homoduplexes and hence elute out of the matrix faster. dHPLC is used to analyze amplicons of 50-800 bp in size²².

In CSGE, the conformational differences between the homoduplexes and heteroduplexes are detected by changes in their electrophoretic mobilities. During electrophoresis of the PCR product under conditions (mild denaturing) that enhance conformational differences, the heteroduplexes show a decreased mobility as compared to the homoduplexes. CSGE has been recommended for mutation screening of AT-rich genes that have multiple exons²³.

The SSCP uses the conformational differences between single stranded DNA. The double stranded DNA in the PCR product is denatured (by heating) and cooled to keep the two strands

separate. Differences in mobilities caused by even a single nucleotide difference can be detected on electrophoresis. SSCP is better suited for fragments of size less than 300 bp²⁴.

Comparison of the important features of the three methods is presented in Table 2.

Table 2. Sensitivity and specificity of methods used for genetic screening

Method	DHPLC	CSGE	SSCP
Sensitivity	>98%	97%	50-95%
Specificity	100%	100%	>95%
Base pair	150-800	200-800	<300

^a Markeff et al 23²³, Gross et al 24²⁴.

The PTT detects premature termination of translation of mRNA to protein, due to introduction of a stop codon by a nonsense or a frame shift mutation. The premature termination results in a truncated or smaller sized protein. In this test RNA is reverse transcribed to cDNA which is amplified with PCR. The PCR product serves as a template for in vitro translation in the presence of radioactively labeled amino acids to generate peptide fragments. The peptide fragments are analyzed by gel electrophoresis for estimation of peptide size²⁵. Missense mutations are not detected by PTT. The PTT has been most commonly employed for the detection of mutations in exon 11 of BRCA1 and BRCA2, as exon 11 covers 60% of the coding sequence of these genes²⁵.

DNA sequencing: This method for detection of mutations in BRCA1 and BRCA2 genes is considered the “gold standard”. The sequencing of various exons of these genes is generally carried out by a method wherein the dideoxy (ddNTPs) analogues of deoxyribonucleotide triphosphate (dNTPs) are used that do not allow further addition of nucleotides leading to chain termination of the nucleotides. Incorporation of these analogs results in a chain termination during DNA strand extension. In a typical sequencing reaction the template DNA (PCR product / gene) is copied into a new strand using a primer, enzyme DNA polymerase and dNTPs. The ddNTPs present in the mix also get incorporated into the strand being newly synthesized, leading to termination of the growing chain, as the ddNTPs lack a 3' hydroxyl (-OH) group required for formation of new phosphodiester bond. The size of the products of sequencing reaction with a given dideoxy analog indicate the position of the corresponding nucleotide in the gene.

Though direct sequencing of the entire coding region of a gene has highest sensitivity and specificity, it is not a very

cost effective method, especially for large genes such as BRCA1 and BRCA2²⁶.

Sequencing results could be interpreted as:

- A) Negative for pathogenic mutations
- B) Positive for pathogenic mutations
- C) Genetic variant

The genetic variants can be divided into three classes as: a) favour polymorphism b) suspected deleterious and c) uncertain clinical significance.

The details of the sequencing analysis can be accessed at the Myriad Laboratories technical website (www.myriadtests.com/provider/doc/tech_specs_brac.pdf).

Genetic counseling

The decision to recommend genetic testing to examine presence of mutation in BRCA1 or BRCA2 in a particular individual largely depends upon the family and medical history of the person. The risk assessment, based on the analysis of the genogram (family tree) and the available information, performed by the counselor can significantly influence the decision to offer genetic testing. Thus genetic counseling plays a pivotal role in this decision making process.

A detailed history of the number of cases affected with cancer in the family, their age at diagnosis, their relationship with the proband, the site and histology of their cancer is a prerequisite. Specific, pointed questions related to chemotherapy and ascites need to be asked pertaining to ovarian cancer which is commonly misinterpreted as ‘some abdominal cancer’ especially in our set-up where most patients do not have all the details of the medical illness in the family members due to the prevailing socio-economic factors and health care set up. Both paternal and maternal histories are to be taken. The person taking such history must have a good understanding of the heterogeneity of hereditary breast or ovarian cancer and knowledge of those facets of its natural history that can expedite in making the correct diagnosis of a syndrome.

The genetic testing has to be carefully implemented keeping in mind the psycho-social impact of the results of the tests. Therefore, counseling prior and following the test is an integral part of the genetic testing. For the individual to be competent to make the decision he or she must be educated about the diagnosis, etiology, history and prognosis of the condition. Further, it is important that the subject is made aware of the genetic, medical and technical information about the testing especially of the possible limitations of results of the test being offered. Finally, information related to the various options

for treatment or management of the condition vis-à-vis test results allows the subject to decide if they wish to undergo genetic testing. Thus the doctor or counselor is expected to help in the process of making an informed decision in a non-directive manner.

Population based information of the mutations in BRCA1 and BRCA2 can be important in the nature of tests that may be performed. Knowledge of a specific mutation in a family or in a community (founder mutations) helps in screening for these specific mutations in other members, obviating the need for screening / sequencing the entire gene.

BRCA1 and BRCA2 genetic testing in India

While few small research studies using diverse screening techniques have reported results of BRCA mutation analysis in Indian families with variable genetic selection criteria¹⁶⁻¹⁹, presently there are no published reports of clinically validated BRCA mutation analysis in large cohort of high risk Indian families. The most widely used, reliable and comprehensive commercial genetic testing for BRCA1 and BRCA2 is available with the Myriad Genetics at Salt Lake City, USA. While such test can be performed on peripheral blood samples sent in EDTA vacutainer tubes to Myriad Genetics from India and reported within 6 weeks, the cost of US\$ 3000 is prohibitive for most Indian families. We provide clinical evaluation, pedigree analysis and risk estimation for inherited predisposition for breast or ovarian cancer to a large number of such high risk families at the Tata Memorial Hospital, Mumbai and have initiated mutation analysis for these two genes at ACTREC, Navi Mumbai using dHPLC followed by full sequencing.

Clinical Management

There are several expert group recommendations available for screening women from such high-risk families. There is greater consensus with better defined recommendations for breast cancer as opposed to ovarian cancer. The usually followed regime is monthly breast self examination and a six-monthly clinical breast examination by trained oncologist, surgeon or gynecologist. This should be complimented with annual radiological screening using either a mammography, MRI or ultrasonography, as appropriate for woman's age and breast density. MRI has higher sensitivity for detecting breast cancer among young women with a BRCA1 or BRCA2 mutations than does mammography, clinical breast examination, or ultrasonography²⁷. Annual mammography may miss aggressive cancer in BRCA mutation carriers, especially in younger women with dense breasts²⁸. Reports of interval cancers, occurring between mammograms, are known but one has to make a judicious decision regarding the type and frequency of these radiological investigations

taking into account the yield, psychological impact of equivocal results or unnecessary biopsies and the risks of exposure to radiation. In the west, physicians are liable for malpractice actions and ultimately penalties emanating from the legal proceedings if they fail to take advantage of the genetic knowledge and its translation into the clinical practice setting²⁹. Malpractice claims may relate to failure to recommend appropriate imaging or genetic counseling or genetic clinic referral at an earlier age and/or at more frequent intervals for women at very high risk for developing breast or ovarian cancer than is recommended for the general population.

The guidelines for ovarian cancer screening are not that clear. Present recommendations, includes transvaginal ultrasound and CA-125 measurements once or twice a year, starting from the age of 25 to 30 years with consideration of prophylactic oophorectomy after completion of childbearing or at age of 35 years³⁰. The impact of such screening is uncertain with the available evidence showing no definite advantage³¹.

Understanding of the role of estrogens and progesterone in the genesis of breast cancer has led to development of preventive strategies in high risk population which vary from non-invasive chemoprevention with anti-estrogens to invasive prophylactic oophorectomy. Chemoprevention using selective estrogen receptor modulators has shown to decrease the incidence of estrogen-receptor positive tumors to a great extent but this benefit has to be weighed against the risk of exposing the subject to serious adverse effects like thrombo-embolism and endometrial cancer³²⁻³⁵. It is also important to keep in mind the fact that most breast cancer associated with BRCA1 mutation are often estrogen receptor negative³⁶⁻³⁷.

An alternative, yet a drastic method used to prevent breast cancer development is prophylactic surgical intervention. Prophylactic bilateral mastectomy with immediate reconstruction has been shown to reduce the risk for breast cancer by 85-100% as seen in four observational studies and a meta-analysis³⁸⁻⁴¹. The PROSE study, which evaluated 483 women with BRCA1 or BRCA2 mutation showed a 90% reduction in risk of breast cancer, which increased to 95% with prior or concurrent bilateral prophylactic oophorectomy⁴⁰. Despite such high prevention rates, subjects are reluctant to undergo such treatment because of the very severity of the intervention involved. Subcutaneous mastectomy as against total mastectomy is another option as cosmesis is a major concern, especially for younger women. It involves leaving residual tissue immediately beneath the nipple and areola, making further reconstruction easier and cosmetically more satisfying⁴²⁻⁴⁴. Prophylactic oophorectomy reduces the

risk of ovarian cancer by 85-100% and that of breast cancer by 53-68%. The risk reduction is greater in BRCA-1 carriers⁴⁵. It is important to discuss these treatment and prophylaxis options with all high risk patients who can then take an informed decision in their best interest.

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