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ORIGINAL ARTICLE

BRCA1 and BRCA1 Genes and Inherited Breast and/or Ovarian Cancer: Benefits of Genetic Testing

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Abstract The breast cancer associated genes BRCA1 and BRCA2 were discovered in 1994 and 1995 respectively. Since then in addition to our understanding how these proteins function in particular reference to DNA repair, enormous amount of knowledge has been gained regarding genetic epidemiology of inherited breast and ovarian cancer, mutation prevalence among different ethnic groups, presence of founder mutations, varying penetrance, genetic testing and potential management options of mutation carriers. This review will focus on the status of understanding of the role of BRCA1 and BRAC2 mutations among Indian women, structure and biology of these two genes, different methods used for mutation detection and different management options available for BRCA1 and BRCA2 mutation carriers.

Keywords BRCA1 · BRCA2 · Familial breast and/or ovarian cancer · Inherited breast cancer

Abbreviations

BRCA1	Breast Cancer Gene 1
BRCA2	Breast Cancer Gene 2
HBOC	Hereditary breast and ovarian cancer syndrome

Introduction

The discovery of two genes BRCA1 and BRCA2 were reported in the year 1994 and 1995 respectively [1–3]. Mutations in these two genes were found to be responsible for the hereditary breast and ovarian cancer (HBOC) syndrome. Women who carry mutations in BRAC1 or BRCA2 gene mutations have a cumulative lifetime risk of breast cancer of approximately 60% to 80% [4–6]. Similarly, the lifetime risk of ovarian cancer in women with BRCA1 or BRCA2 mutations is found to 40% to 50% and 10% to 20% respectively [4–6].

BRCA1 and BRCA2 Proteins: Structure and Functions

The first breast cancer susceptibility gene (BRCA1) was found on chromosome 17q12–21 in humans and encodes an 1,863 amino acid polypeptide (Fig. 1) [1]. BRCA1 is a large and complex gene about 100 kb long with a transcript size of 7.8 kb.

BRCA1 contains several important functional domains that interact with a range of proteins. The different functional domains are 1) RING-finger domain, 2) the nuclear localization signals (NLSs), 3) the DNA-binding domain, 3) SQ sequences (clusters of serine and threonine sequences), known as SQ-cluster domains (SCDs) and 4) two regions at the carboxyl terminus—known as the BRCT domains. Each of these domains binds to specific cellular proteins in carrying out functions like transcription and DNA repair [23]. The second breast cancer susceptibility genes (BRCA2) was discovered in the year 1995 and found on the chromosome 13q12.3 in human [3]. BRCA2, which consists of 3,418 amino acids, contains two known functional domains (Fig. 1). There are eight BRC-repeat motifs, which are essential for its function in DNA repair, in the middle region of the protein. The carboxy-terminal region of BRCA2 contains two nuclear localization domains [23]. The functions carried out but BRCA proteins

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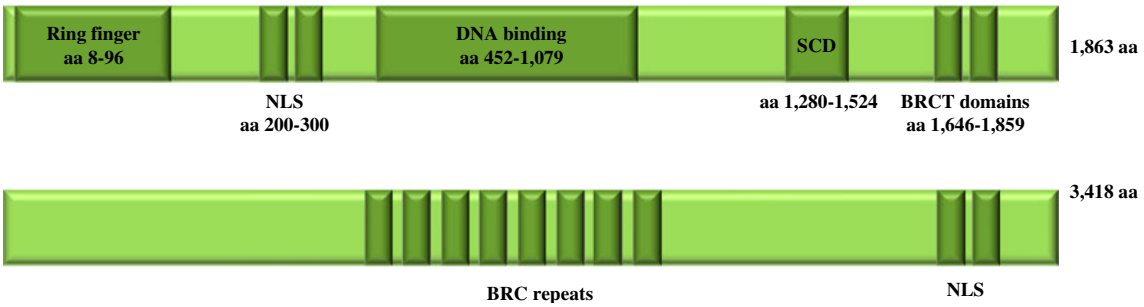

Fig. 1 The structure of BRCA1 and BRCA2 proteins

rather appear to be very complex. Some of the known functions of BRCA1 are role in transcription, DNA repair, cell cycle check-point, protein ubiquitylation and chromatin remodeling [13, 23]. The well demonstrated function of BRCA2 is its involvement in homologous recombination [23].

BRCA1 and BRAC2 Mutation Spectrum World Wide and India

Reports worldwide suggest that mutations frequency of BRCA1 and BRCA2 genes differ greatly among populations from different geographic regions and ethnicities. Among families with inherited breast and/or ovarian cancer syndrome, it has been estimated that BRCA1 accounts for 0.7–29% mutations while BRAC2 accounts for 1.5 to 25% of mutations [18–20, 26]. However, based on several publications emerged from studies on different popualtions world wide, the germline mutations in known breast cancer genes account for approximately 20% of breast cancer associated with family history [21, 22]. It is therefore very important to understand the contribution of BRAC1 and BRCA2 in any given population.

In india, there are only few reports of BRCA1 and BRCA2 mutation screening among Indian women (Table 1). However, these sudies were done on a smaller number of breast cancer families suggestuing a need to screen large number of patients in multiple centers in different parts of India. The first report by Kumar et al., used CSGE based scanning method followed by DNA sequencing, found BRAC1 mutation prevalence as 21% [8]. Saxena et al. even though screend 204 families, this study group contained 105 early onset cases, 65 late onset cases and 34 familial cases [11]. Since early onset is a feature of inherited breast cancer involving *BRCA1* and *BRCA2* mutations, families with an early onset of breast cancer are generally considered for *BRCA1* and *BRCA2* mutation screening programmes. However, it may not ideal to include such families in *BRCA1* and *BRCA2* mutation screening in India because of the average age of breast cancer patients in various population based registries in India has been reported to be 50–53 years compared with 61 years among American women [24, 25]. This is a very important point, considering the fact that approximately 90% of breast cancer is of the sporadic type. Therefore, including patients with early-onset breast cancer in mutation screening studies may undermine estimations of the frequency of *BRCA1* and *BRCA2* mutations. In fact, the study by Saxena et al., which included 50 % families with early onset as criteria for selection, reported a very low combined prevalence rate of BRCA1/BRCA2 mutations [11]. Recently, a study from our laboratory in collaboration with Kidwai Memorial Institute of Oncology screened 61 breast cancer patients that belong to breast and/or ovarian cancer families from south India and reported the overall BRCA1 and BRCA2 mutation frequency to be 28.00% [26]. While the prevalence of BRCA1 mutation was found to be 24.6%, BRCA2 gene mutation was found in only 3.28% of the families.

Methods Used for Mutation Detection

Although direct sequencing allows direct identification of specific sequence alteration and is considered to the gold standard, it is time consuming and costly. Hence there is a need to develop a reliable alternate faster and less expensive method(s) for routine BRCA1 and BRCA2 mutation screening programme. What is needed is a cost effective scanning technique to identify regions containing genetic alterations, which can be subjected to DNA sequencing subsequently. Some of the techniques which are commonly used are single-strand conformation polymorphism (SSCP), restriction endonuclease fingerprinting (REF)-SSCP, conformation-sensitive gel electrophoresis (CSGE), fluorescence-based conformation-sensitive gel electrophoresis (F-CSGE), two dimensional gene scanning (TDGS), protein truncation test (PTT), and denaturing high performance liquid chromatography (DHPLC). Gerhardus et al., provides a

Table 1 Prevalence of BRAC1 and BRCA2 mutataions among Indian women

S. No	Reference	Method used	Family Selection criteria	No. of families/ Individuals studied	BRCA1 mutation prevalence	BRCA2 mutation prevalence	BRCA1 and BRCA2 mutation prevalence
1	[8]	Conformation sensitive gel electrophoresis followed by DNA sequencing	Breast and or ovarian cancer inherited families	14	21% (3/14)	NA	NA
2	[12]	Heteroduplex analysis followed by DNA sequencing	Breast and or ovarian cancer inherited families and individuals with early onset breast cancer (≤35 years)	20	10% (2/10)	None[a]	10%
3	[10]	Heteroduplex analysis by denaturing high peformance liquid chromatography (DHPLC) followed by DNA sequencing	Breast and or ovarian cancer inherited families and individuals with early onset breast cancer	22	10% (2/20)	10% (1/20)	30% (3/20)
4	[14]	Combination of polymerase chain reaction-mediated site directed mutagenesis (PSM), polymerase chain reaction-single stranded conformation polymorphism assay (PCR-SSCP) and direct DNA sequencing of PCR products (DS).	Breast and or ovarian cancer inherited families	16	19% (3/16)	19% (3/16)	38% (6/16)
5	[7]	Single stranded DNA conformation polymorphism (SSCP) followed by DNA sequencing	Breast cancer inherited families	24	25% (6/24)	NA	NA
6	[11]	Heteroduplex analysis followed by DNA sequencing	Breast and or ovarian cancer inherited families and individuals with early onset breast cancer (≤35 years)	204[b]	1.46% (3/205)	1.46% (3/205)	2.93 (6/205)
7	[26]	Conformation sensitive gel electrophoresis followed by DNA sequencing	Breast and or ovarian cancer inherited families	61	24.6% (15/61)	3.28% (2/61)	28.00% (17/61)

[a] Two BRCA2 missense variations each seen in two patients were concluded as polymorphisms

[b] 204 includes 105 early onset cases, 65 late onset patients and 34 familial cases

NA not available

systematic study of analyzing the accuracy of different scanning methods using for BRCA1 and BRCA2 mutation screening [16]. In our laboratory as a part of a recently completed study, we used CSGE as a method of scanning to identify the potential exons where the mutations are likely to occur, followed by DNA sequencing to locate and find out he nature of mutations [9, 26]. We carry out a total of 34 PCR reactions to cover 24 exons of BRCA1 and 52 PCR reactions to cover 27 exons of BRCA2. The sequence for primers for each exon is chosen such that they are located at least 50 bp away from exon-intron boundaries in order to detect the splice junction mutations. The non-coding exons 1 and 4 of BRCA1 and exon 1 of BRCA2 are usually not included for the study.

Who Should Undergo BRCA1 and BRCA2 Mutation Testing?

Since the prevalence of BRCA mutation in general population is estimated to be between 1/800 and 1/1,000, screening of general population for BRCA mutations is not required. As per the literature, it appears varying criteria were used for choosing patients for BRCA mutation screening. As per European Society of Medical Oncology (ESMO) recommendations, families where three or more breast and/or ovarian cancer cases, at least one aged <50 years; two breast cancer cases aged <40 years; male breast cancer and early onset female breast cancer; and breast and ovarian cancer in the same patient [15]. However, the genetic testing criteria needs

to formulated based on the various factors specific to a country or specific ethnic population as the mutation frequency appear to vary in different communities.

Utility of BRCA1 and BRCA2 Mutation Screening

BRCA1 and BRCA2 mutation carriers have several risk reduction options. As per the ESMO guidelines, the following is recommended [15]. The risk reduction procedures include non-surgical preventive options and prophylactic surgical options. The non-surgical preventive options include surveillance and chemoprevention. Under surveillance, BRCA1 mutation carriers may undergo monthly self-examinations, clinical breast examination once or twice a year and yearly mammograms and magnetic resonance imaging of breast starting at age 25–30. Under chemoprevention, tamoxifen has been shown to reduce the risk of contralateral breast cancer in affected BACA mutation carriers. However, the use of tamoxifen in the prevention of primary breast cancer has not been established [15].

Under prophylactic surgical options, bilateral mastectomy has been recommended as the most effective strategy for risk reduction of BRCA mutation carriers. However, no benefit in survival has been demonstrated and is not acceptable to many women for cosmetic reasons. BRCA mutation carriers with early breast cancer and unilateral mastectomy, contralateral mastectomy is recommended. To decrease the risk of of developing ovarian cancer, BRCA mutation carriers are recommended to undergo bilateral salpingo-oopherectomy after age 35 and when childbearing decisions are complete.

With regard to surgical treatment of BRCA mutation carriers with breast cancer, ESMO recommends the same parameters as that of sporadic cancer [17]. However, the high risk of contralateral breast cancer should be considered while making decisions.

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