BRCA genes: BRCA 1 and BRCA 2

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Summary

BRCA1 and BRCA2 are the genes related with breast and ovarian cancer. They have function in DNA repair processes and thus they are tumor suppressor genes. There are hundreds of mutations identified in these genes. Functional deficiencies due to these mutations impair DNA repair and cause irregularities in the DNA synthesis. The standard method for the laboratory assessment of these BRCA genes includes comprehensive sequencing and testing of broad genomic rearrangements. Members of the families with BRCA mutations have an increased risk for early onset of breast cancer and ovarian cancer occurring at any age. Surveillance of patients with mutations in BRCA 1/2 is done by yearly mammography and breast MRI and by transvaginal ultrasonography and serum CA-125 levels every 6-12 months for ovarian cancer.

Key words: *BRCA, breast cancer, DNA repair, ovarian cancer*

Introduction

BRCA1 and BRCA2, known as breast and ovarian cancer predisposition genes, were discovered in the 1990s. As part of a normal genetic structure, these genes are intrinsic to all human beings, but they are mutated in some individuals increasing the risk for breast and ovarian cancers development [1,2]. BRCA1 is not only expressed in endocrine tissues, but is also detected in other cells such as the neuroepithelial cells in the early stage of cell development. Like BRCA1, BRCA2 is also expressed in a wide variety of tissues and is observed with higher rates in the breast and thymus, and with lower rates in the lung, ovary and spleen [3,4].

More than 400 mutations have been reported for BRCA1 and BRCA2. Despite the numerous mutations identified, such a great quantity does not exhaust the mutations available, and it is likely for an individual to carry a mutation undetected by usual examinations. Therefore, most of the mutations can be considered unique, and each family may present a specific mutation [5,6]. Despite the high variability of familial mutations, certain mutations have been frequently observed at some geographical locations and in some ethnicities. The individuals with mutated BRCA genes are exposed to a significantly higher risk of cancer development compared to the rest of the population. Mutations in these genes are marked risk factors for breast and ovarian cancers in heterozygous carriers and the cumulative risks for such cancers vary depending on age [7].

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Structure of BRCA genes

BRCA1 is located on the chromosome 17q21 and has 24 exons. BRCA1 codes for a protein consisting of 1863 aminoacids (5.6 kb). BRCA2 is located on chromosome 13q12 and consists of 27 exons, with exon 11 being the largest one (4.9 kb). BRCA2 codes for a protein consisting of 3418 aminoacids (10.2 kb). When compared to other gene regions, regions of both genes can be considered large regions. Mutations in exon 13 of BRCA1 and in exon 11 of BRCA2 gene have been associated with ovarian cancer [8,9].

The phosphorylation state and amount of BRCA1 (220 kd) protein depends on the cell cycle. Hyperphosphorylation occurs in the late G1 and S phases and is dephosphorylation occurs immediately after the M phase. The amount of BRCA1 protein is highest in the S phase, which continues to stay high in G2/M phases and falls in early G1 [10-12]. BRCA2 is a large (350-380 kd) protein. It is a nuclear protein expressed especially in the late-G1/early-S phase of the cell cycle in normal cells [8,13].

BRCA1 has a ring region that binds zinc at the N-terminus and contains nuclear localization signals in the central region [14]. DNA damage also induces the nuclear transfer of BRCA1 by a mechanism that requires p53 participation [15,16]. Some studies have also shown that BRCA1 is a nuclear-cytoplasmic transfer protein [16]. Other publications demonstrated that it has nuclear localization in many normal cell types, especially in breast epithelial cells, but it passes to cytoplasmic localization in breast and ovarian cancer cells [17,18]. Moreover, the description of its cytoplasmic localization as a secreted protein has also been demonstrated [18]. This contradictory subcellular localization of BRCA1 is controlled by the nuclear localized signal-mediated nuclear import and export receptor pathways [16-18].

Functions of BRCA genes

BRCA1 and BRCA2 genes normally belong among the DNA repair genes that assume a regulatory function in the cell cycle, coding for the proteins involved in the response to DNA damage, therefore, function as tumor suppressor genes. BRCA genes are involved in the synthesis of multiprotein complexes that ensure transcriptional regulation of DNA synthesis, as well as the recognition and correction particularly of the double-stranded breaks of certain DNA damages. Functional deficiencies due to the mutations in these DNA repair genes impair DNA repair and cause irregularities in the DNA synthesis. These mutations mostly (80%) occur as point mutations or deletion/insertion mutations. As a result of these mutations, the p53-dependent DNA breakdown is activated, which may lead to cell cycle arrest and apoptosis [13,19].

BRCA genes play an important role not only in DNA repair, but also in transcriptional regulation, cell growth control and conservation of genomic integrity [19]. Following the DNA damage, both proteins coexist with RAD51 in the S phase at the subnuclear focus. RAD51 is a key recombinase enzyme that interacts with BRCA2 involved in repairing the double-stranded DNA breaks and in homologous recombination. In addition, exon 11 codes for a structural motif that consists of eight repeats of 'BRC' by which BRCA2 controls RAD51 function. Therefore, the proteins coded by BRCA1/2 tumor suppressor genes play a role in the repair of DNA breaks together with RAD51 protein, thus providing genomic stability [13,15].

Genetic tests for BRCA 1 and 2

Since the BRCA genes are large genes, hundreds of mutations have been identified on them. In general, although it is desirable to perform a genetic analysis to include all frequent and rare mutations in BRCA1 and BRCA2 genes, it is very costly. The standard method for the laboratory assessment of BRCA genes includes comprehensive sequencing and testing of broad genomic rearrangements. If the patient has a relative with a particular mutation, a single-site targeted mutation analysis can also be performed. Today, the use of multi-gene panels including broad genomic rearrangements may be chosen for higher affordability in the risk assessment [20,21].

Recently, many mutations in BRCA 1 and 2 genes have been added to the next generation multi-gene sequencing panels. Clinically, 4-7% of the women tested in large studies with using these multi-gene analyses presented BRCA 1 and BRCA 2 mutations. It is unlikely to detect BRCA 1/2 mutation positivity in a small patient group, which is only possible in expanded multi-gene panels. Therefore, while choosing an appropriate test for a patient, one should take into consideration a series of factors such as cost, laboratory, time to obtain results and reliability [22].

The whole genome sequencing, a method by which all coding and non-coding regions of the DNA are sequenced, is rarely employed in the clinical practice because it is costly and time-consuming method. It has not been accepted as a first-line test for inherited breast/ovary cancers as

well. In addition to the high cost of whole genome sequencing, it has not fully validated. This method may also potentially and incidentally detect irrelevant findings other than the ones subjected to examination. Therefore, whole genome sequencing is not recommended for routine use and only appropriate for a limited number of cases with marked personal and family history and negative results in standard methods [23].

With the next generation DNA sequencing systems, developed following simple and advanced sequencing methods, it is now possible to perform a very quick and highly accurate sequencing. With the help of the next generation DNA sequencing method thousands or even millions of sequences of genes can be evaluated at once. The sequencing by the Whole Genome Sequencing System is based on the principle of sequencing the small DNA fragments and adapter sequences at picolitre volumes and by large-scale parallel sequencing.

In the past, the most commonly employed method for determining the genomic sequence was the Sanger method with the shotgun technique. The shotgun sequencing method is used to sequence long DNA fragments. As part of this method, large genomic DNA is physically divided into small random pieces. Then, captured DNA fragments are sequenced separately, and all readable pieces are unified with the help of various bioinformatics software [24]. With the Sanger method, long enough DNAs do not allow a sequencing analysis at once and are first divided into smaller pieces. Each piece obtained is cloned into a plasmid. The plasmids cloned are then sequenced one by one. By combining these sequences with bioinformatics analyses, the sequence of a long DNA fragment is obtained. The pyrosequencing method has been developed because the Sanger method is time-consuming and laborious to sequence long DNA regions. Pyrosequencing has replaced the conventional Sanger sequencing thanks to a high process volume and affordability. In addition to complex genomes, this method allows simple genomes such as bacteria and viruses to be sequenced within a day [25]. In addition, chain termination sequencing, sequencing by ligation, sequencing by synthesis, ion semiconductor sequencing, and nanopore sequencing are other sequencing methods.

BRCA Syndromes

Only 0.1-0.2% of the general population are carriers of BRCA1 and BRCA2 mutations. BRCA1 and BRCA2 mutations are detected in 2-3% of all breast cancer cases. Families with frequent BRCA mutations are those with early-age breast cancer

cases and ovarian cancers occurring at any age. The penetrance of pathogenic BRCA mutations and age of cancer diagnosis appear to vary both within and among family members. Some populations like Ashkenazi Jews, carry these gene mutations with higher rates [26].

BRCA1-related breast cancers are usually diagnosed with higher histologic grade, proliferative rate and show a predominance of triple negative pathology compared with sporadic tumors. This triple negative phenotype of BRCA1-related breast cancers is further characterized by a basal-like gene expression profile. BRCA-associated cancers also tend to progress directly to invasive disease without the development of a precancerous ductal carcinoma *in situ* component. Therefore, it is less likely to detect the breast cancer early, even by mammographic imaging [27]. Serous adenocarcinoma is the main type of cancer in ovarian cancer patients carrying BRCA1/2 mutations, whereas other cancers such as endometrioid and clear-cell carcinomas occur with a frequency comparable to that of sporadic cases [28]. Many studies have demonstrated that mucinous and borderline ovarian carcinomas are not common in familial ovarian cancer syndromes. Both fallopian tubes and peritoneal primary tumors are with increased frequency in mutation carriers [29].

The risk of developing cancer in BRCA1 and 2 mutation carriers depends on age and the range of breast cancer risk is influenced by the population under study. Breast cancer occurs at an earlier age than the general population in both BRCA1 and BRCA2 families. BRCA2 mutation carriers also present an increased risk of ovarian cancer but the risk is not as high as for the carriers of BRCA1 mutations. Although the carriers of BRCA1 and BRCA2 mutations may develop an ovarian cancer at an early age, an underlying mutation may also be discovered in the ovarian cancers detected at an older age [27,29].

BRCA1 gene mutations were found in 15-20% of families with breast cancer and in 40-50% of families with both breast and ovarian cancer. The risk of breast cancer in a woman with a BRCA1 mutation is 20% after 40 years of age, 51% after 50 years, and 85% after 70 years. The risk of ovarian cancer development is 40-50% after 70 years of age. The risk of breast cancer development in the carriers of BRCA2 mutations is 28% after 50 years of age and 84% after 70 years and the risk in ovarian cancers is 4% after 50 years of age and 27% after 70 years [30,31]. In summary, BRCA1 mutation exposes a woman to the life-long risk of developing breast cancer by 85% and in ovarian cancer by 40-50%, whereas a BRCA2-positive

woman is exposed to these risks by 40-45% and 15-30%, respectively [32,33].

It is also known that prostate cancer and colon cancer risk is increased in the carriers of BRCA1 mutations, and the carriers of BRCA2 mutations are exposed to a higher risk of male breast cancer, pancreatic cancer and prostate cancer. Although rare, the carriers of BRCA2 mutations may also develop malignant melanomas, carcinomas of the fallopian tubes, as well as gallbladder and biliary tract tumors. Studies have indicated that BRCA1/2 gene mutations do not cause a predisposition to the development of borderline neoplasms and are not associated with stromal tumors or malignant germ cell tumors [34].

Surveillance of patients with mutations in BRCA 1/2

The carriers of BRCA 1/2 are recommended to undergo yearly mammography and breast MRI after the age of 25 or 10 years before the earliest age at which the cancer is detected in the family members. Mammography and breast MRI should be performed alternately every six months. A transvaginal ultrasonography (USG) can be performed and serum CA-125 levels can be measured every 6-12 months for ovarian cancer screening starting at the age of 30 or 5-10 year before the earliest age at first diagnosis of ovarian cancer in the family. However, both USG and tumor marker evaluation are less capable of detecting ovarian cancer at an early stage [35,36]. The data for the use of chemopreventive agents, such as tamoxifen and raloxifen, in BRCA carriers is controversial. Risk-reducing salpingo-oophorectomy and mastectomy are protective surgical interventions that can only be recommended for suitable cases in conjunction with genetic counseling. These surgical procedures enable protection from cancer development with rates of 96% for mastectomy and 90% for salpingo-oophorectomy. The breast cancer risk is also reduced by approximately 50% with salpingo-oophorectomy [37-42].

Conflict of interests

The authors declare no conflict of interests.

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