Introduction

The term “hereditary cancer” refers to cancers associated with specific germ-line mutations in highly penetrant genes which are inherited as a Mendelian trait, whether through an oncogene, a tumor suppressor gene, or a DNA repair gene. Since the first association between germ-line mutations and hereditary predisposition for particular cancer types has been found in the mid of 90-ties, investigations pointed out a variety of tumor types with inherited predisposition to a high or moderate/low risk for development of disease (melanoma, gastric cancer, MEN I, MEN II, hereditary nonpolyposis colon cancer, etc) 1–4. Data that hereditary predisposition, recognized by family clustering, has been found for the arising number of cancer types, together with introducing of screening for the mutations in responsible genes, makes different insight into cancer prevention and management of patients with malignant disease.

Breast cancer is the most frequent malignant tumor in females in Serbia. The incidence can be described with more than 4,000 newly diagnosed cases per year 5, 6. Unfortunately, incidence and mortality trends show permanent increment in the few last years. Breast cancer can occur as sporadic, familial and hereditary. The majority of breast cancers are recognized as sporadic in patients with no cancer history in the family. The incidence of sporadic breast cancer rises in women over 50 years old. Minorities of breast and/or ovarian cancer patients (up to 5% to 10%) have a striking family history, suggestive of Mendelian autosomal dominant inheritance. An additional 20% of breast cancer cases are considered as familial describing situation with at least two cancer cases in extended family. In hereditary form of disease one of the two alleles of the gene responsible for the disease is altered by germ-line mutation. The off-spring of the mutation carriers has 50% chance of inheriting a mutant allele from either parent. The most common variant of hereditary breast cancer (HBC) is the appearance of breast as well as ovarian cancer cases in the same family (HBOC). The disease can also occur as site-specific breast or ovarian cancer. Other tumor types such as pancreatic cancer, Fallopian tube carcinoma, melanoma or prostate cancer in men can be commonly present in families with hereditary breast cancer 7–9.

Characteristics and functions of BRCA 1/2 genes

Discovery of the association between breast and ovarian cancer and BRCA1 (in 1994) and BRCA2 (in 1995) genes have made it possible to screen women for genetic predisposition to develop either one or both of these diseases 7. BRCA1 and BRCA2 genes are highly, but not completely penetrant (about 80%). So far, more than 20 genes of low to medium penetrance that can modify the penetrance of BRCA1/2 genes in carriers of mutations (modifier genes), in that way modifying risk for hereditary disease, have been identified 10, 11.

BRCA1 and BRCA2 genes are classified as tumor suppressor genes. Both genes are large – BRCA1 has 22 and BRCA2 26 coding exons. For both genes exon 1 is noncoding, and both have unusually large exon 11. BRCA1 gene is located on chromosome 17q21 while BRCA2 is located on chromosome 13q12. BRCA1 encodes for 1863, while BRCA2 encodes for 3418 amino acid protein product 7.
tations are scattered throughout coding regions of both genes without clustering or “hot spots” resulting in a huge number of mutations detected in each gene – more than 1,600 in BRCA1 and 1,900 in BRCA2 mutations have been reported. The majority of them, but not all of them, are capable of disrupting the function of BRCA protein product in that way affecting the risk for malignant disease. So far, we identified 15 persons affected with BRCA1 (7 types in 11 persons) and BRCA2 (3 types in 4 persons) deleterious mutations in Serbian population. Other BRCA1/2 sequence variants (unclassified and polymorphic) were also found. Among them, 4765del20 in exon 15 of BRCA1 and 4366insTT in exon 11 of BRCA2 gene are new deleterious mutations, firstly reported in our population.

Concerning mutation type, about 70% of detected mutations are frameshift mutations, while nonsense, as well as missense mutations contribute with about 10% each. Besides small changes in DNA structure such as frameshift or point mutations, in BRCA genes are also reported large genomic rearrangements. Somatic BRCA1/2 mutations are rare in sporadic breast cancer, but other mechanisms such as epigenetic inactivation by hypermethylation of BRCA1 promoter sequence were reported. BRCA1/2 somatic mutations are not so rare events in sporadic ovarian cancer.

BRCA1 and BRCA2 mutational spectra are population-specific and different founder mutations are reported in different populations. Data about ethnicity is also important since it has been shown that some ethnically isolated populations such as Ashkenazi Jews or Islanders, due to inbreeding, have limited number of BRCA1/2 mutations – more than 90% of BRCA mutation carriers of Ashkenazi Jewish women can be described with two BRCA1 (185delAG and 5382insC) and one BRCA2 (6174delT) mutations. In Iceland, which is geographically isolated, there is only one founder mutation. This is the consequence of “founder effect” – some of rare mutations in small and isolated ethnic groups may become more frequent in the next generations due to reproductive isolation (founder mutations). All populations have their own founder mutations, but they can not be easily recognized due to the presence of additional BRCA variants that rose in reproductively mixed populations. But, large proportion of BRCA mutations are detected only once – it can be said that the most of families at risk tend to have their own mutation. Besides common, population specific and family specific mutations are detected. It is questionable if all deleterious mutations have the same penetrance. Age-dependent penetrance of different germ-line mutations in BRCA1 genes was recently reported – the authors concluded that different BRCA1 mutations have distinct effects that influence age of onset of breast and ovarian cancer.

BRCA1/2 protein products are implicated in a variety of important cellular processes acting through interaction with other molecules in signalling pathways – DNA repair, transcriptional regulation, cell-cycle regulation and chromatin remodelling. BRCA1 and BRCA2 protein products are included in repair of doublestrand DNA breaks by homologous recombination mechanism. For example, ionizing radiation, as well as cytotoxic agents cause DNA doublestrand breaks.

Mutations in BRCA1/2 genes, especially frameshift mutations, cause the syntheses of proteins truncated at various extent. Such protein product, usually lack some domain crucial for its proper function (such as BRCT or BRC domains in BRCA1 or BRCA2), respectively, leading to genome instability and elevated cancer risk. BRCT domains are crucial for BRCA1 interaction with a variety of proteins, forming complexes and enabling proper function of various important cellular processes. The fact that about 80% of BRCA1 germline mutations result in C ter-

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minus truncation that include BRCT region and lead to increased predisposition for breast and/or ovarian cancer, point out the importance of BRCT domains for BRCA1 protein function. BRCA1 protein has a highly conserved RING domain at its amino terminus and two BRCT domains at the end of its carboxyl terminus. The fact that many clinically important mutations are located in parts of BRCA1 gene that encode these highly conserved domains indicates that they are very important for BRCA1 function. The RING domain of BRCA1 mediates its association with BRCA1-associated RING domain protein 1 (BARD1) 23. BRCA1–BARD1 dimer has been implicated in the maintenance of genomic stability and tumor suppression through its involvement in DNA damage signalling, DNA repair and transcriptional regulation. BRCT domains interact with Rb tumor suppressor gene, as well as with other proteins associated with Rb, that are thought to be included in chromatin remodelling processes 24 that is required before and after DNA repair processes.

The main function of BRCA2 protein is DNA repair. BRCA2 protein in its structure has eight BRC repeats that represent conserved sequence motifs of about 30 amino acids each. BRC repeats are crucial for BRCA2 interaction with RAD51. BRCA2, by interacting with RAD51, plays a key role in DNA repair by homologous recombination. Lack of any of those domains disrupts BRCA2-RAD51 interaction, leading to DNA repair malfunction, elevating cancer risk 7.

**Lifetime risk for the development of cancer in BRCA1/2 mutation carriers**

It must be pointed out that the risk of BRCA-associated breast and ovarian cancer is related only to epithelial malignancies of both organs. As the consequence of limited penetrance of BRCA genes is the fact that all of BRCA1/2 mutation carriers will not develop malignant disease. So far, it is not possible to predict which of BRCA mutation carriers will develop disease, although genetic or environmental factors affecting penetrability of BRCA genes are intensively investigated 25-27. Identification of BRCA1 and BRCA2 mutation can be used only for the risk estimation. But, it is obvious that the majority of women with inherited mutation in BRCA1 or BRCA2 gene will develop breast and/or ovarian cancer. Earlier estimates of lifetime risk were higher, especially if they were derived from families with a strong positive family history. Estimates of lifetime risk vary considerably depending on the group studied (for instance, if cases are selected for family history or not), type of mutations included in study, age selected etc. For instance, for Ashkenazi Jewish BRCA1 mutation 5382insC, which is also present in Serbian population, lifetime risk by the age of 70 is 67% and for ovarian cancer is 33% 28. Generally, reported BRCA1 - and BRCA2 - related risk estimates by the age of 70 for breast cancer range from 45% to up to 87% – it can be said that the risk is elevated about 5 to 8 times in comparison to the risk for sporadic breast cancer. The risk of ovarian cancer by the age of 70 in BRCA1 mutation carriers range from 28% to 44%, while for BRCA2 carriers estimates are lower (from 11 to 27%) 29. The risk for the development of ovarian cancer is elevated about 20 times in BRCA1/2 mutation carriers 29. Characteristic of BRCA-related cancer is that hereditary breast cancer occurs at an earlier age than the sporadic form of disease. Women with BRCA1/BRCA2 mutation have a 33% to 50% chance to develop breast cancer before the age of 50 in comparison with general population with the chance of 2% only 30.

The presence of BRCA1/2 mutation elevates the risk for bilateral breast cancer. BRCA1 mutation elevates the risk for contralateral breast cancer up to 64% by the age of 70, while BRCA2 mutations elevates this risk to about 50% 31. Besides this, elevated lifetime risk (up to 7%) for male breast and prostate cancer (up to 20%) is mostly related to BRCA2 mutation 32.

**Hereditary predisposition identifying**

Now it is clear that with BRCA1/2 alterations all hereditary predisposition for breast/ovarian cancer can not be covered. It was shown that BRCA mutations caused hereditary form of the disease in families with both tumor types clustering (breast/ovarian) (75%). In site-specific breast cancer the percent is lower – about 60% of female breast cancer families are caused by BRCA1/BRCA2 mutations 8. This data depends on population studied. It is clear that besides contribution of alterations of some other genes such as p53 (Li-Fraumeni syndrome), PTEN (Cowden syndrome), ATM ataxia–telangietasia 1 (mutated protein) etc., which rarely influence hereditary predisposition to breast/ovarian cancer, some now undefined genes will be discovered.

As major clinical benefits may occur through identifying clinical risk indicators of the hereditary cancer phenotype, it is necessary that attention be paid to 33: positive family history of breast cancer, especially with early age of onset (at least two cases of breast cancer before the age of 50 from the same side of family tree - about 50% of breast cancer cases are diagnosed by age of 41); breast cancer before the age of 35, without positive family history; bilateral breast cancer; ovarian cancer at any age associated with positive family history of breast and/or ovarian cancer; multiple cancers in the same person; male breast cancer at any age; relatives of a BRCA mutation carrier; Ashkenazi Jewish ancestry.

**Genetic susceptibility testing**

Hereditary breast and/or ovarian cancer belong to the group of hereditary syndromes with a high probability of linkage to known cancer susceptibility genes (BRCA1/BRCA2). It is accepted that identification of mutation carriers carry medical benefit, although due to limited penetrance of BRCA genes it can be only used for the risk estimation for malignant disease. BRCA1/2 genetic tests were among the first genetic tests to become widely available, due to high incidence and high mortality of breast cancer in Western countries. Genetic testing is performed in order to determine the molecular basis of the disease.

**References**

BRCA testing is performed on a peripheral blood sample due to the fact that we are searching for germ-line mutation present in all cells in organism. The most appropriate test for identification of BRCA1/BRCA2 mutations is a complete sequence analysis of the entire coding sequence and this remains the “gold standard” for mutation screening. An ideal situation is the possibility to test a patient with breast/ovarian cancer as the first in the family with clustering of disease. In addition to the whole BRCA genes sequencing, other options are possible in certain situations: for BRCA testing of relatives, when a specific type of mutation is confirmed in family member; for limited number of founder mutations in some populations such as Ashkenazi Jewish women; in 2%–12% of high-risk patients BRCA alterations are consisted of large genomic rearrangements and specific techniques such as multiplex ligation-dependent probe amplification (MLPA) are indicated.

BRCA1 and BRCA2 testing can result in the finding of deleterious mutation, with known clinical impact. The presence of benign polymorphisms, but also of unclassified variants in both genes was reported. Special problem in determining genetic susceptibility to breast and/or ovarian cancer are unclassified variants in BRCA genes. These variants are missense mutations with uncertain influence on structure and function of BRCA1/2 protein products that result in their unknown clinical impact.

Bioethical principles of BRCA testing

Awareness of one’s susceptibility to disease without an actual possibility of intervention can lead to an unacceptable use of such information (discrimination or social instrumentalization), or might have psychological impact on the person involved. The question that must be first asked is: Are the risks connected with the knowledge of susceptibility to genetic disease proportional to the benefits that such knowledge may provide? This problem is vast and involves medical, psychological, social and ethical dilemmas. These dilemmas are common to all predictive medicine, but the most evident in predictive DNA testing in hereditary breast cancer due to high risk for breast/ovarian cancer in healthy BRCA mutation carriers. All available preventive measures (follow-up, chemoprevention, bilateral prophylactic mastectomy or oophorectomy) are important but not definitive. The choice of whether to pursue DNA testing belongs to the individual. Respect for the individual’s autonomy is ensured by obtaining informed consent from that person. It is essential to offer pre-test counseling to evaluate individual capacity for autonomous decision-making. Pre-test counseling also provides view of the risks and benefits, potential treatment as well as social and ethical implications involved. Genetic information has implications not only for the patients but also for their biological kin. Another big problem is to confront confidential setting between a patient and the physician and the importance of forwarding that information to biological kin who can easily be carrier of the same gene alteration. Appropriate pre-test counseling is finished with a person’s signing the consent form, where the persons are asked to state that they fully understand the terms and have had adequate opportunity to ask questions – our informed consent has been approved by the Ethics Committee of the Institute. It is imperative that counseling must be nondirective, allowing a patient full autonomy in deciding whether to be tested. Pre-test education should include the following information: description of the patient’s risk status; explanation of what it means to have inherited susceptibility to cancer; information about testing outcomes – results may be positive, negative or uninformative; appraisal of the risks, benefits and limitations of genetic testing; discussion on cancer surveillance and limitations of anticancer therapies; information about the risk of passing a mutation to children; review of psychological issues related to genetic testing; explanation of alternative to genetic testing.

In post-test counseling the counselor have to help patients to understand the results. A patient has also the right to decide not to be told about test results. Post-test counseling for mutation carriers must include a full explanation of a positive result accompanied by a description of surveillance and options for clinical management. It must be performed by a genetic counseling team, composed at least of a physician, genetic counselor, psychologist and registered nurse. Moral problem lies mostly in concerns how to make meaningful use of the available genetic information and it is necessary to weigh the risks against the harms in concrete cases.

Pathobiology of hereditary breast cancer

Breast cancer is heterogeneous disease in regard to pathobiological characteristics, prognosis of disease and predicting response to specific anticancer treatment. Growing data about different pathobiological characteristics among BRCA1- and BRCA2-related hereditary breast cancers, as well as especially BRCA1-related cancer compared to sporadic breast cancer with a consequent influence on the course of the disease, enforce the need for hereditary breast cancer characterization. Gene expression profiles of breast cancer have defined specific molecular sub-types with clinical, biological and therapeutic implications. According to pathobiological characteristics, the majority of BRCA1-related cancers can be classified in the group of „triple-negative breast cancer” (TNBC) since they are characterized with the lack of expression of steroid receptors (estrogen and progesterone) and lack of Her-2 receptor overexpression. BRCA1-associated breast cancers are mostly pure differentiated carcinomas with ductal histology. About 10% of BRCA1-associated tumors show atypical medular histology. These cancers show also high mitotic index, pushing margins and the presence of necrosis. p53 mutations are more frequent in BRCA1-related than in sporadic breast cancer. Although TNBC represents almost the exclusive phenotype in BRCA1-related breast cancer, it was recently reported that approximately 10 to 36% of BRCA1-associated breast cancers can be ER- positive. It seems that TNBC BRCA1-related cancer is associated with younger age of onset (≤50), while ER+ BRCA1-related cancer occurs in older mutation carriers. It is suggested that ER+ BRCA1-related cancer is
pathologically intermediate between BRCA1-related ER+ breast cancer and ER+ sporadic breast cancer with the possibility that some of ER+ breast cancers in BRCA mutation carriers may be incidental. Our previous investigation in Serbian population showed the presence of new BRCA1 mutation, previously not reported, in a patient with ER+ breast cancer. It was found in older BRCA mutation carrier with strong family predisposition supporting an idea about ER+ BRCA1-related breast cancer as distinct entity of hereditary cancer rather than possibility that ER+ breast cancer in BRCA1 mutation carriers is not the consequence of BRCA1 dysfunction and can be considered as sporadic one. BRCA2-associated breast tumors are more similar to sporadic breast cancer, with predominant ductal histology, frequent carcinoma in situ and expression of ER (about 75%) in BRCA1/2 mutations are common in high-grade serous papillary ovarian carcinomas.

The main question related to pathobiological characteristics of BRCA-associated cancer is if hereditary form of disease may have different course of disease in comparison to sporadic cancer, i.e. be more aggressive. Due to its characteristics, BRCA1-associated breast cancer has more aggressive phenotype and women with BRCA1-associated breast cancer seem to have worse prognosis of disease than women with sporadic cancer, while BRCA2-associated breast cancer is more similar to sporadic one.

Treatment options for hereditary breast/ovarian cancer

So far, BRCA-related cancer has been treated as sporadic cancer – in accordance to classic prognostic parameters (TNM, grade, histology etc) and breast cancer biomarkers (steroid and Her-2 receptors, Ki67 etc). BRCA1/2 status as predictor of various chemotherapy regimens was discussed in the literature. BRCA deficient tumors was shown to be more sensitive on platinum derivates regimens. But, it seems that real revolution will be made with targeted therapy (PARP inhibitors) for patients with BRCA mutations. Poly(ADP-ribose) polymerase is nuclear enzyme involved in base excision repair of single-stranded DNA breaks. When activity of PARP is disrupted, DNA replication can be stopped causing double-strand DNA breaks. Tumor cells with BRCA1/2 mutations are very sensitive to the lack of single-stranded breaks repair by PARP inhibition. By mechanisms of synthetic lethality which confers situation when there is lethal synergy between two originally non-lethal cellular events such as inhibition of PARP mediated repair of single-strand breaks, inducing double-strand breaks as well, in combination of loss-off-function of BRCA1/2 mediated homologous recombination, new targeted therapy for BRCA1/2 deficient tumors is constructed. According to the results of phase II clinical trials with PARP inhibitor Olaparib (Astra-Zeneca), this approach is very promising for the treatment of BRCA1/2 mutated breast and ovarian cancer – complete or partial response was shown in more than 40% of BRCA1/2 deficient patients.

What can be now recommended for healthy BRCA1/2 mutation carriers? Two main approaches are, so far recommended: clinical surveillance and prophylactic surgery. Chemoprevention, as the method also recommended for medical management of BRCA1/2 mutation carriers, was shown to be mostly effective for the prevention of contralateral breast cancer in affected BRCA mutation carriers.

In surveillance for breast cancer, breast self-exam, clinical breast exam as well as radiological exams including magnetic resonance imaging in certain time-periods is recommended for healthy BRCA1/2 mutation carriers under the age of 18. In surveillance for ovarian cancer pelvic exams, transvaginal ultrasound and CA-125 detection are included. However, prophylactic surgery is so far, the only approach with benefit in risk reduction for hereditary disease. Bilateral prophylactic mastectomy with breast cancer risk reduction greater than 90%, as well as bilateral salpingo-oophorectomy at the age of 35 or after childbearing is complete with nearly 100% risk reduction for ovarian cancer, but also up to 68% risk reduction for breast cancer is recommended for healthy female BRCA1/2 mutation carriers.

Conclusion

Genetic testing for BRCA1/2 mutations is not a screening procedure for general population and is addressed to a selected part of population eligible according to including criteria. BRCA testing, since the presence of BRCA1/2 mutation is one of the best characterized genetic risk factor for disease, can give reliable result that help in risk estimation for development of breast/ovarian cancer in healthy individuals. Unfortunately, interpretation of BRCA testing results may be complex, especially due to possible presence of unclassified variants in both genes. Furthermore, only invasive prevention strategies such as profilactic surgery demonstrate risk reduction in healthy BRCA1/2 mutation carriers. Recent data that BRCA deficient tumors are target for treatment with PARP inhibitor rise possibility of targeted therapy in the treatment of BRCA-related cancers, but it is possible that this approach would be in future exploited for cancer prevention in healthy BRCA carriers.

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