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Identifying and testing for hereditary susceptibility to breast/ ovarian cancer in Serbia: Where are we now?

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ABSTRACT

About 90% of all breast cancers can be considered as sporadic, without inherited gene alteration. The rest of breast cancers (about 5 to 10%) are considered hereditary, most commonly caused by alterations of BRCA1/2 tumor suppressor genes. Lifetime risks for breast and ovarian cancers are increased among BRCA1/2 mutation carriers – 4 to 8 and 10 to 20 fold higher respectively. Due to the small proportion of hereditary form of disease, as well as to the high cost, BRCA testing is not screening test for general population. It is addressed to selected part of population that fit to recommended criteria. Full coding region sequencing of both genes is "gold standard" for detection of BRCA mutation. Concerning BRCA testing in Serbia, complete or partial sequencing of BRCA1/2 coding region was performed in 60 samples. The presence of 4 BRCA1 known mutations, previously detected elsewhere, has been shown: 185delAG, C61G, 3447del4 and 5382insC (detected twice). In BRCA1 gene, exon 16, an unclassified variant M1652I was found. Polymorphic variants in BRCA1 (8 polymorphisms) and BRCA2 (5 polymorphisms) genes were also detected. The majority of found BRCA1 and BRCA2 polymorphic variants are the missense ones and their influence on breast/ovarian cancer risk in our population has to be proved. Identification of BRCA mutations carriers and establishment of spectra and frequency of BRCA mutations should enable introduction of BRCA1/2 testing into the clinical practice of Serbia.

KEY WORDS: *Breast Neoplasms; Ovarian Neoplasms; Genes, BRCA2; Genes, BRCA1; Mutation; Tumor Markers, Biological; Genetic Predisposition to Diseases; Non MeSH Serbia*

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In Serbia, similarly to Western countries, breast cancer is the most frequent malignancy and the leading cause of cancer-related death in females with mortality rate 16.9 (ASR_W) in 2000 year (1). The incidence of breast cancer in Serbia can be described with about 3700 newly diagnosed cases per year. About 1200 of them are newly diagnosed at the Institute for Oncology and Radiology of Serbia.

About 90% of all breast cancers can be considered as sporadic, without inherited gene alteration. The rest of breast cancers (about 5 to 10%) are considered to be hereditary, most commonly caused by alterations of tumor suppressor genes BReast CAncer gene 1 and 2 (BRCA1 and BRCA2). Up to 25%-40% of breast cancer cases under the age 35 years harbors deleterious BRCA1/2 mutation. BRCA1 and BRCA2 genes have been implicated in about 50% of all hereditary breast cancers. The frequency of BRCA mutations is 1 in 250 women. Offspring of BRCA mutation carriers have 50% chance to inherit mutated gene allele from one of the parents.

The most common variant of hereditary breast cancer (HBC) is hereditary breast and ovarian cancer (HBOC) syndrome. Besides that, HBC can occur as site-specific one. Contribution of BRCA mutations to site specific hereditary breast cancer is the following: about 65% of female breast cancer families are associated with BRCA1/BRCA2 mutations, whereas up to 35% site-specific breast cancer is associated with undefined gene mutations. Concerning families with HBOC, even up to 95% of cases are related to BRCA1/BRCA2 mutations (2). Alterations of some other genes such as p53 (Li-Fraumeni syndrome), PTEN (Cowden syndrome), STK11 (Peutz-Jeghers syndrome) can also influence development of hereditary breast cancer. Hereditary predisposition related to these genes is very rare (about 1% for each of them) and they do not significantly contribute to inherited breast cancer – together they account for less than 5% of hereditary breast cancer cases.

Concerning undefined genes, it is obvious that new breast cancer predisposing gene will be discovered. Various candidates for BRCA3 gene are investigated. It has recently been shown that CHK2 gene mutations harbor intermediate risk for breast cancer, especially in Northern Europe. Alterations of ATM gene that controls development of ataxia telangiectasia – bialleleic mutations in ATM gene results in autosomal disease - are also related to hereditary form of breast cancer. Heterozygosity for ATM variants may confer an increased risk for breast cancer - it seems that only mutations that are involved in the development of ataxia telangiectasia are implicated in hereditary predisposition for breast cancer (3). However, due to limited spread in the world and lack of high penetrability, ATM and CHK2 are not real BRCA3 candidates (4).

BRCA GENE ALTERATIONS

BRCA1 and BRCA2 genes were identified in the middle of 1990s. BRCA1 gene is comprised of 24 exons and located on chromosome 17q21, while BRCA2 gene is even larger than BRCA1, with 27 exons mapped on chromosome 13q12. Both genes exhibit similar structure with noncoding exon 1 and very large exon 11. Mutation spectra of both genes are also very similar, with more than 1300 mutations detected in each gene. BRCA mutations are scattered throughout these large genes without clustering. Concerning mutation type, about 70% of detected mutations are frameshift mutations (consequence is frameshift in open reading region), while nonsense (consequence is cessation of protein synthesis), as well as

missense mutations (consequence is synthesis of altered protein) contribute with 10% each. More than 50% of all mutations are registered only once, meaning that majority of families with hereditary predisposition tends to have their own mutation. Somatic BRCA mutations have not yet been found in sporadic breast cancers and are very rare in sporadic ovarian cancers – mechanism of inactivation of BRCA genes in sporadic cancer is dissimilar to that in hereditary ones. Hypermethylation of regulatory region of BRCA1 gene with consequent inhibition of transcription and lack of BRCA1 protein expression was reported (5).

BRCA1 and BRCA2 are tumor suppressor genes. They are involved in the processes of DNA repair, rather as sensor than as effectors. Cells harboring BRCA mutations exhibit higher degree of spontaneous and induced chromosome aberrations, they are more sensitive on DNA damaging agents and have higher mutation rate. Both protein products are involved in homologous recombinant repair, but for BRCA1 protein product, multiple additional functions are suggested. With the number of other proteins, BRCA1 is involved in nonhomologous end-joining of double-strand breaks, as well as in nucleotide excision repair. Further, BRCA1 protein is involved in X chromosome silencing and chromosome remodeling (6). Cells with BRCA mutations show defect in DNA repair by homologous recombination. In the same time, BRCA1 and BRCA2 mutations lead to hereditary predisposition for breast/ovarian cancer, which implicates the role of homologous recombinant repair pathway in protecting individuals against carcinogenesis (4).

According to classification of genes involved in carcinogenesis on "caretakers" and "gatekeepers", BRCA genes can be considered "caretakers" – genes that are guardians of genome, indicating their indirect role in the development of malignant phenotype (7). When BRCA genes are inactivated, tumor occurs as the consequence of genome instability resulting with high number of mutations in some other "gatekeepers" genes that regulate balance between cell proliferation and cell apoptosis.

After BRCA genes mapping, epidemiological studies aimed to characterize BRCA mutations in different ethnic groups started. It was noticed that in some of the ethnicities particular BRCA mutations were more frequent than it was expected. For example, among Ashkenazi Jews' hereditary breast/ovarian predisposed families, two BRCA1 (185delAG and 5382C) and one BRCA2 (6174delT) mutations are present with frequency higher than 90% (8, 9). This phenomena is associated with "founder" effect (effect of founder mutation), mostly observed in geographically or reproductive isolated populations. Individual from relatively small and isolated ethnic group may raise or harbor germ-cell mutation that is rare in this population, but it will become more frequent in the next generations due to reproductive isolation. "Founder" mutations occur in other populations as well, but they can not be easily registered because of the presence of additional BRCA variants that rose in the meantime in reproductively mixed populations.

Lifetime risk for breast/ovarian cancer in BRCA mutation carriers

BRCA genes are genes with high, but not absolute penetrability (about 80%). Limited penetrability complicates BRCA counseling - not all BRCA mutation carriers will develop malignant disease. Identification of BRCA mutation can only be used for the estimation of breast/ovarian cancer risk. Lifetime risk for breast cancer among BRCA1/2 mutation carriers ranges between 40-85% (10). Since lifetime risk for breast cancer in general population is about 10%, this risk is high. BRCA1 associated breast cancer usually occurs at younger age – about 50% of all BRCA1 associated breast cancers are diagnosed under age 41 (11). BRCA1 mutations cause bilateral breast cancer (lifetime risk is up to 60%). BRCA1 associated ovarian cancer develops with lifetime risk up to 40% (10), while ovarian cancer risk in general female population is about 2%. With occurrence of BRCA1 mutations, appearance of some other malignant tumors such as colon carcinoma, prostate cancer etc is associated. In BRCA2 mutation carriers, lifetime risk for female breast cancer is similar to those characteristic for BRCA1 mutation carriers (40 to 85%). Besides this, elevated lifetime risk for male breast cancer is related to BRCA2 mutation (lifetime risk is 6%). Ovarian cancer risk in BRCA2 mutation carriers is about 20% (10). Hereditary prostate cancer is mostly associated with BRCA2 mutations. It is likely that different BRCA mutations are associated with different risk for the development of the disease (12).

Identification of individuals as potential BRCA1/2 carriers

Identification of BRCA1/2 mutation carriers in families with positive family history of breast/ovarian cancer is important, since it is now possible to recommend them options for life style, surveillance or risk reduction. However, due to the small proportion of hereditary form of disease, as well as to the high cost, BRCA testing is not screening test for general population. It is addressed to selected part of population that fit to recommended criteria. Genetic testing is multi-step process, which includes identification of individuals at risk, pre-test counseling, informed consent giving, laboratory test performing, disclosing of result and post-test counseling. First step in genetic testing process is identification of the individuals that probably carry BRCA1/2 mutations. Family clustering of breast and/or ovarian cancer, bilateral breast cancer and male breast cancer indicate the presence of BRCA mutations (Table 1). Ethnicity can also point out the need for BRCA testing – for example, Ashkenazi Jews have higher incidence of BRCA1/2 mutations than other ethnical groups.

Table 1. Identification of individuals at risk for BRCA1/2 associated breast cancer

Multiple cases of breast cancer from the same side of family tree (especially early onset, under 50 years old)
Ovarian cancer (with family history with breast/ovarian cancer)
Breast and ovarian cancer occurrence in the same person
Bilateral breast cancer
Male breast cancer
Ashkenazi Jewish origin

Genetic testing must be performed in specialized medical institution, with highly trained multidisciplinary team.

Since we are looking for germ-line mutation, BRCA testing is performed in the sample of peripheral blood. Full coding region sequencing (determination of DNA purine and pyrimidine bases order in gene region) of both genes is "gold standard" for detection of BRCA mutation. When in one family BRCA1 or BRCA2 mutation is detected, other members of the family can be tested only for this mutation. Results of BRCA testing can be positive (presence of mutation), negative (absence of mutation) or uninformative (presence of unclassified variant). Negative result of BRCA testing for the member of family with previously detected family mutation is real negative result. When family mutation is not yet detected, negative result of BRCA testing must be considered carefully – maybe risk for disease is related to some unidentified mutation (13). In addition, the result of testing can be the presence of unclassified variant for which it is not yet clear, due to the lack of data, if it is deleterious mutation or benign polymorphic variant.

The particular problem of BRCA testing is the presence of polymorphic variants. In fact, synonymous mutations can be considered as true polymorphisms because they do not cause amino acid change in protein product. On the other hand, classification of missense variants, which lead to the altered amino acid sequence in the protein product, is not simple. Altered protein structure and function has to be analyzed to determine whether amino acid change alters the folding of the polypeptide or the function of the protein. If the folding is not interrupted, it remains to be established whether changed amino acid resides in the part of the protein important for its function and, if so, is the protein function altered due to this amino acid change. Literature data suggest that the majority of polymorphic variants, regardless of synonymous or missense type, harbor low or moderate breast/ovarian cancer risk (14). It is likely that some of BRCA1/2 polymorphisms can alter breast /ovarian cancer risk throughout modifying penetrance of deleterious BRCA1 mutations in BRCA1 mutation carriers. It has been reported that BRCA1 wild-type polymorphic allele (Gly1038/E1038) can increase risk for ovarian cancer in BRCA1 mutation carriers (15). Otherwise, recent data show HH genotype of the amino acid substitution polymorphism N372H in the BRCA2 do not elevate the risk of breast and ovarian cancer in BRCA1 mutation carriers (16).

Individuals with confirmed BRCA mutations, particularly demand genetic counseling, since they are faced with serious dilemmas concerning surveillance or risk reduction (17), as well as with the consequence on their offspring.

Pathobiological characteristics of BRCA1/2 associated breast/ovarian cancer

BRCA1 and BRCA2 associated breast tumors exhibit certain morphological and immunohistochemical characteristics. BRCA1 associated breast tumors are often pure differentiated invasive ductal carcinomas. Medullary histology is also more common. Concerning histological grade, BRCA1 breast carcinomas are predominantly high grade tumors (up to 84% of them). In the majority of cases (up to 90%), they are estrogen independent, since they do not express estrogen as well as progesterone receptors. Up to 95% of BRCA1 and BRCA2 tumors do not express Her-2 receptors. Histology of BRCA2 breast tumors is more similar to sporadic breast cancer (18).

BRCA1 and BRCA2 associated ovarian cancer is exclusively of epithelial origin, with high grade and predominantly serous histology.

BRCA testing in Serbia

Investigations on BRCA genes in Serbia started in the end of nineties in collaboration of the Institute for Oncology and Radiology of Serbia with National Institute of Oncology of Hungary, Budapest (19). After the pause, it continued throughout joint research programs with National Centre for Scientific Research "*Demokritos"*, Athens, Greece, 2001 – 2003 and 2004 – 2006 (20). Both of collaborations, especially the one with Greece, which included possibility of more than one working visit, strengthened orientation toward BRCA testing at the Institute for Oncology and Radiology of Serbia. During 2004, the Institute, with financial support of Ministry of Science of Serbia, purchased refurbished ABI PRISM 310 genetic analyzer that enabled complete BRCA testing in our institution.

Firstly, it was necessary to make the conditions for blood and tissue samples, as well as DNA storage. Since we have already been equipped with liquid nitrogen containers, tissue and cell samples, as well as DNA samples for long maintenance, are stored in liquid nitrogen on -196°C.

Besides storage conditions, the most important step towards BRCA testing is the establishment of Registry of hereditary breast/ovarian cancer. In Laboratory of Molecular Genetics, the Registry is under construction, mainly based on medical records from Institute for Oncology and Radiology of Serbia.

Genomic DNA bank is consisted of about 220 blood samples from the individuals at risk for hereditary breast and/or ovarian cancer. The individuals are included in the process of blood and data collection according to established criteria. From 2004, subjects are signing inform consent, approved by Ethics Committee of the Institute.

About 80% of included individuals are breast/ovarian cancer patients. The majority of families included in our bank are represented by one member only. Generally, relatives of breast/ovarian cancer patients refuse to be tested – in part because of insufficient awareness of genetic testing and in part because of lack of appropriate procedures for risk reduction (only invasive prevention strategies such as prophylactic surgery demonstrates relevant results in risk reduction).

So far, complete or partial sequencing of BRCA1/2 coding region was performed in 60 samples. 5 BRCA1 mutations were detected in the examined group (Figure 1). The presence of 4 known mutations, previously detected elsewhere, has been shown: 185delAG (exon 2), C61G (exon 5) 3447del4 (exon 11) and 5382insC (exon 20) which was detected twice. All identified mutations except one missense mutation (C61G) were frameshift mutations.

Three mutations were detected in site-specific breast cancer, one in breast/ovarian cancer family and another one in family with ovarian cancer only.

Concerning the age of onset, mutations were detected at extremely young age (22 years) in one proband with ovarian cancer whose mother developed breast cancer at age 30.

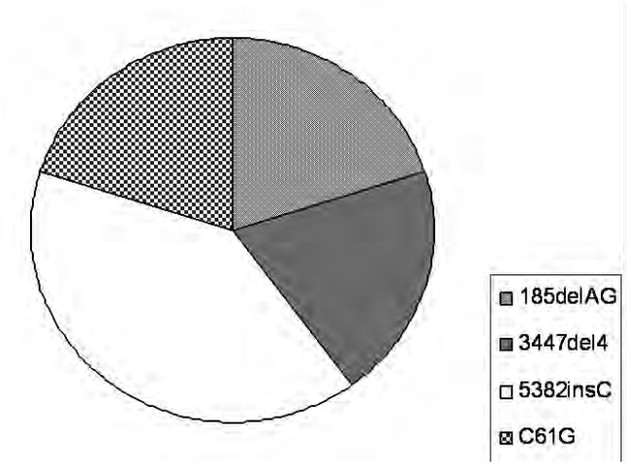

Figure 1. Proportion of each of mutation type among detected BRCA1 mutations

Besides deleterious mutations, in BRCA1 gene, exon 16, we detected an unclassified variant M1652I. This variant has been detected in young female who developed breast cancer at age 20. Six months later she developed Hodgkin disease. The influence of this BRCA1 variant on breast cancer risk is unclear. Further analysis concerning its position in BRCA1 gene, as well as the impact on developing malignant disease has to be conducted.

We also detected polymorphic variants in BRCA1 (8 polymorphisms, Table 2) and BRCA2 (5 polymorphisms, Table 3) genes. Among them, in BRCA2 gene are two variants that have not yet been registered in BIC base. According to mutation type both are missense with consequent amino acid change in BRCA2 protein. Since both of them have been detected in probands with breast or ovarian cancer, it is possible that their influence on breast/ovarian cancer risk will be of significance. They can be classified as unclassified variants whose importance has to be further clarified.

The majority of BRCA1 (5/8) as well as BRCA2 (3/5) polymorphic variants are, according to mutation type, missense ones (Figures 2a and 2b).

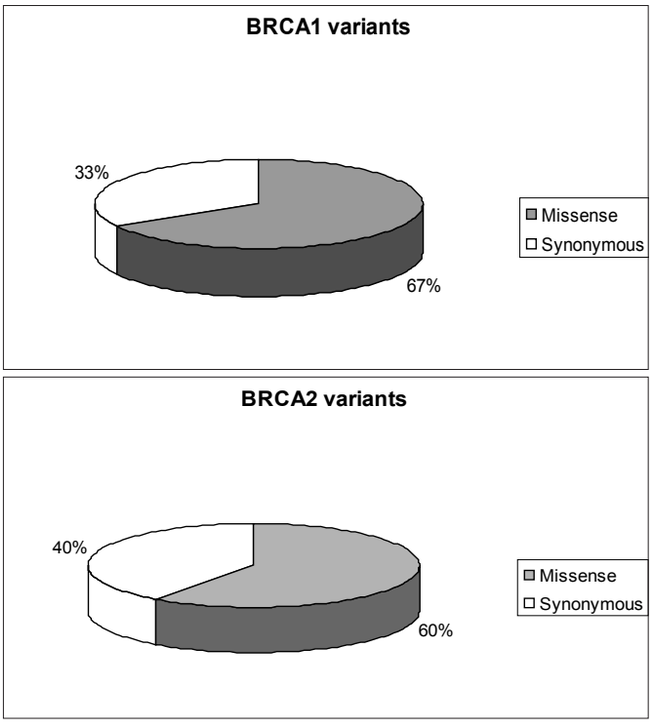

Figure 2. a. Proportion of missense and synonymous variants found in BRCA1 gene; b. Proportion of missense and synonymous variants found in BRCA2 gene

Table 2. Detected BRCA1 variants

EXON	MUTATION	AA CHANGE	MUTATION TYPE	MUTATION EFFECT*
11	2430T>C (L771)	Leu>Leu	Synonymous	Polymorphism
11	2731C>T (P871L)	Pro>Leu	Missense	Polymorphism
11	1186A>G (Q356R)	Gln>Arg	Missense	Polymorphism
11	2201C>T (S694S)	Ser>Ser	Synonymous	Polymorphism
11	3232A>G (E1038G)	Glu>Gly	Missense	Polymorphism
11	3667A>G (K1183R)	Lys>Arg	Missense	Polymorphism
13	4427T>C (S1436S)	Ser>Ser	Synonymous	Polymorphism
16	4956A>G (S1613G)	Ser>Gly	Missense	Polymorphism
16	5075G>A (M1652I)	Met>Ile	Missense	Unclassified variant

*According to Breast Cancer Information Core (http://research.nhgri.nih.gov/bic)

Table 3. Detected BRCA2 variants

EXON	MUTATION	AA CHANGE	MUTATION TYPE	MUTATION EFFECT*
10	1342C>A (H372N)	His>Asn	Missense	Polymorphism
10	2024T>C (S599F)	Ser>Phe	Missense	Not yet registered
11	4035T>C (V1269V)	Val>Val	Synonymous	Polymorphism
11	3624A>G (K1132K)	Lys>Lys	Synonymous	Polymorphism
11	3941T>C (V1238A)	Val>Ala	Missense	Not yet registered

*According to Breast Cancer Information Core (http://research.nhgri.nih.gov/bic)

Particular question is if the risk for breast/ovarian cancer associated with polymorphic variants in the cases with more than one of them in the same individual, can be simply added to each other, staying in the range of low/moderate risk, or their combination can significantly alter the risk to be rather moderate or even high. The obtained results support this dilemma. For example, all found BRCA1 polymorphic variants were detected in one proband - young female who developed breast cancer at age 20 and six months later she developed Hodgkin disease.

FUTURE DIRECTIONS

BRCA1/2 testing is of value in the estimation of familial risk for breast and ovarian cancer. It has been proved that BRCA associated, especially BRCA1 associated carcinomas, have special pathological characteristics, which can be relevant for treatment and prognosis. Clarifying the role of BRCA1/2 genes in maintenance of genome stability throughout DNA repair systems is beginning to suggest potential therapeutic use, since BRCA1/2 mutated tumors may be more sensitive to DNA cross-linking agents.

Recommended whole gene analysis, on the contrary to countries with identified founder mutations, besides expensive and to small audience directed BRCA testing, makes difficult population-based screening. That raises dilemma in the country with limited resources - what is better to do: to recognize our recurrent mutations even if they are not as highly recurrent as in Israel, Poland etc. and to wide-spread BRCA testing, or to do sequencing of the entire coding region of the gene, that is more precise and covers almost all mutations, but it is directed to the small audience? For our country, this problem is pointed out, due to the lack of population-based studies of hereditary breast and ovarian cancer and missing data with follow-up of BRCA mutation carriers (21).

Identification of BRCA mutations carriers, establishment of spectra and frequency of BRCA mutations, especially for those with developed breast cancer, together with long-term follow-up of mutations carriers, should allow the research on the factors affecting BRCA genes penetrability. Systemic BRCA analysis will enable introduction of genetic testing process into the clinical practice of Serbia, resulting with the real benefit for mutation carriers that are highly vulnerable part of population.

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