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MOLECULAR DIAGNOSTICS OF HEREDITARY BREAST CANCER

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Summary

Mutations in BRCA1 and BRCA2 genes are associated with the family predisposition to breast and ovarian cancer. The main purpose of genetic testing is an early detection of the predisposition, because even early detection increases the effectiveness of treatment.

Keywords: breast cancer; hereditary breast cancer; BRCA1 and BRCA2 genes; mutations.

INTRODUCTION

Breast cancer is a leading cancer in female population worlwide. Epidemiological data indicate 5-10 % of all breast and /or ovarian cancer cases are hereditary, and germline mutations in BRCA genes account for the majority of hereditary breast nad ovarian cancers [1]. By today, those two genes Breast Cancer Gene 1 (BRCA1) and Breast Cancer Gene 2 (BRCA2) are only known breast cancer predisposing genes, whose mutations can be first sign of familial setting, before cancer occurs. Those genes are tumor suppressors, involved in DNA repair processes, and are the major breast and ovarian cancer susceptibility genes.

The penetrance of deleterious BRCA mutations has been variably estimated; a recent combined analysis of different reports [2] estimates the average cumulative risk (by the age of 70) in BRCA1 mutation cariers to be about 70 % for breast cancer and 40 % for ovarian cancer, whereas the corresponding risk for BRCA2 are 45 % and 11 %. So, person with inherited mutation in

BRCA1 and BRCA2 genes have 45-85% probability of developing breast cancer, and 11-62% probability of developing ovarian cancer by age 70, comparing to general population risk of 10%.

In familial setting, breast cancer occurs at young age and risk of bilateral breast and ovarian cancer is increased. Age of onset in subsequent generations of BRCA mutations carrying families is lower by 7,9 years [3]. Also, carriers of BRCA1 and BRCA2 mutations are at increased risk for other cancers: uterine, cervical, prostate, pancreatic, male breast, bile duct, stomac cancer and melanoma [4]. Breast cancer can occur in men as well, but the risk is 100 times lower than in women.

In general population the overall prevalence of BRCA1 and BRCA2 mutation carriers is estimated to be from 1 in 400 to1 in 800, the quite variable ratio among etnic groups and by geographic region.

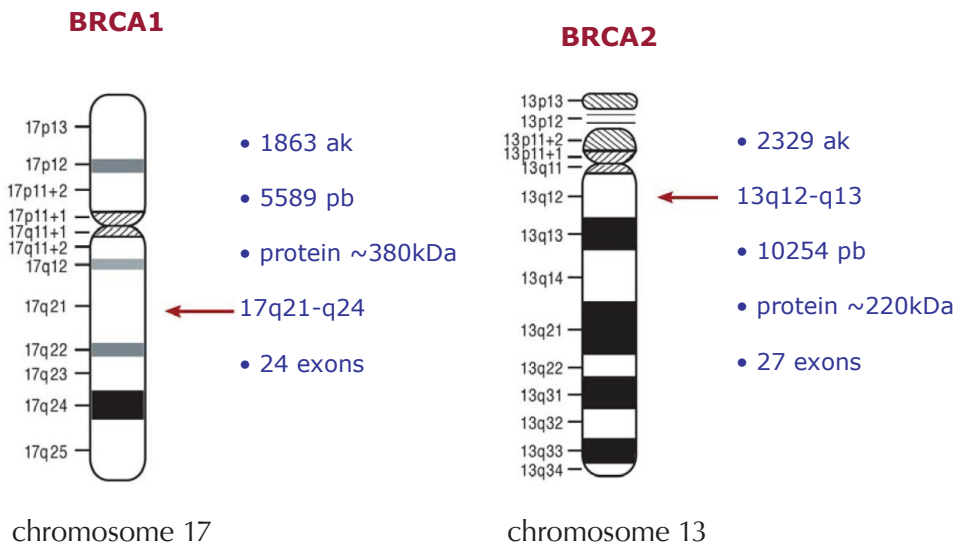

Figure 1. Shematic presentation of the location of BRCA 1 gene mapped on chromosome 17 and BRCA 2 gene on chromosome 13. There are listed also few data about size of the gene, exon number and protein size they code.

Most reported disease-associated alleles of BRCA1 and BRCA2 genes (Figure 1) have been attributed to frameshifts, nonsense or missense mutations, large rearrangements and splice alterations. They usually lead to truncated BRCA1 or BRCA2 protein or affect critical aminoacids for its structure or function. However, a large number of sequence variants, particularly missense variants, routinely encountered in clinical and research laboratories, cannot be readily distinguished as either disease-causing (deleterious) mutations or benign polymorphisms (clinically not significant) and thus are classified as variants of unknown clinical significance.

To date, more than 3600 BRCA1 and BRCA2 variants have been reported in locus-specific databases like Breast Cancer Infromation Core (BIC) - http://research.nhgri.nih.gov/bic/, than Universal Mutation Database (UMD) - http://www.umd.be/BRCA1/ and http://www.umd.be/BRCA2/, Leiden Open Variation Database - http://chromium.liacs.nl/LOVD2/cancer/home.php and kConFab - http://www.kconfab.org/Progress/Mutations.aspx.

Considering these numbers, genetic testing represents invaluable step forward in defynig these malignant disease. The testing is especially important in predicting the risk of developing ovarian cancer, as this kind of cancer is difficult to detect until late, when the chances of cure are less efficient.

Unfortunately, every year in Croatia 2500 women develop breast cancer and 450 develop ovarian cancer, and more than 900 die of breast cancer and almost 400 deaths of ovarian cancer. According to statistical estimates based on 4,3 million Croatian population, at least two hundred breast cancers have familial predisposition, and those estimates follow general worldwide statistics (data from Central Bureau of Statistics of Republic of Croatia and Croatian National Institute of Public Health, 2006). No data on BRCA variants in affected population of Croatia have been gathered so far.

EXPERIENCE WITH GENETIC TESTING IN CROATIA

In Croatia few years ago conditions were appropriate for providing genetic testing of inherited predisposition for breast and ovarian cancer . It was organized in Laboratory for Hereditary Cancer at Rudjer Boskovic Institute thanking to funds of Terry Fox donation and support by Croatian League against Cancer and Europa Donna Croatia.

The screening was performed by high-resolution melting approach, direct sequencing and semi-quantitative multiplex PCR method and provides

a comprehensive analysis on the type and distribution of BRCA1 and BRCA2 mutations and allelic variants [5].

The test entails very complex molecular-genetic methods that have been used in most developed countries for decades. The Laboratory is a member of EMQN (European Molecular Genetics Quality Network), international organization focused on standardizing protocols for molecular genetics diagnostics and on quality assurance of its members. Therefore, the methods used are at the European level of quality.

The main purpose of this testing was early detection of predisposition, because it lowers the cost of treatment as well as declines the need for long hospitalizations, chemotherapy, radiation and multiple surgeries. Even early detection increases the effectiveness of treatment. Targeted and preventive therapy, as well as the lifestyle changes can significantly decrease the mortality.

CANDIDATES FOR TESTING

Each candidate has to undergo genetic counseling before and after testing. The test is mainly for breast and ovarian cancer prone family members and is not informative for sporadic cases of breast and ovarian cancer. BRCA1 and BRCA2 mutations are inheritable with the same probability from either parent.

Potential candidates for BRCA1 and BRCA2 mutation testing are:

- persons with two or more relatives with breast cancer
- persons with early (before age of 50) breast cancer in the family
- persons with breast cancer in the family in more than one generation
- persons with bilateral breast cancer in the family
- persons with multiple ovarian cancer in the family
- persons with early (before age of 40) bilateral ovarian cancer
- persons with one or more relatives with BRCA1 and/or BRCA2 gene mutation in the family.

FIRST STUDY IN CROATIA

The results of BRCA1 and BRCA2 analysis in a group of 168 candidates from Croatia were selected for increased risk of breast and ovarian cancer on the basis of their personal or family history of the diseases. Candidates were

volunteers from several national associations and support groups for breast cancer patients, selected according to the set criteria.

In this study, 168 women from 147 families with positive family or personal history of breast and ovarian cancer were screened for mutations in BRCA1 and BRCA2 genes. All candidates gave their informed consent to perform DNA analysis on their blood samples before the samples were taken. The study was conducted according to the Declaration of Helsinki Principles.

The candidates were recruited from mostly all locations in Croatia (Kaštel Novi, Rijeka, Ploče, Šibenik, Osijek, Zagreb, Dubrovnik, Zadar, Čakovec, Karlobag, Gospić) and were classified into nine categories according to family or personal history of disease. Table 1. shows the specific characteristics of the candidates according to the inclusion criteria.

Table 1. Inclusion criteria for the candidates included in this study (from 6).

Group	Inclusion criteria	Cases (%)
A	affected individual with breast or ovarian cancer before 35 years of age	9 (5.3)
B	affected individual with breast cancer before the age of 35 with at least one case of breast or ovarian cancer in the family	4 (2.4)
C	affected individual with breast or ovarian cancer between the age of 35 and 55 with at least one case of breast cancer in the family	14 (8.3)
D	affected individual with breast or ovarian cancer between the age of 35 and 55 with at least one case of breast and at least one case of ovarian cancer in the family	7 (4.2)
E	unaffected individual with at least two cases of breast cancer in the family	65 (38.7)
F	unaffected individual with at least one case of breast cancer in the family before 50 years of age	39 (23.2)
G	unaffected individual with at least one case of breast and at least one case of ovarian cancer in the family	18 (10.7)
H	unaffected individual with at least one ovarian cancer in the family	8 (4.8)
I	unaffected individual with at least one case of breast cancer and male breast cancer in the family	4 (2.4)
Total		168 (100)

Candidates in group A are considered early onset breast cancer patients, as the usual cut off for early onset is 35 years of age.

From the 168 candidates which participated in this study, 32 (19%, age range 25-58, median 41) were affected with breast cancer, 2 (1.2%, ages 31 and 53) with ovarian cancer, and one with both breast and ovarian cancer (0.6%, ovarian cancer in 51 and breast cancer in 54). The remaining candidates were unaffected (133) but with positive family history (groups E-I).

Mutations in BRCA1 and BRCA2 genes detected

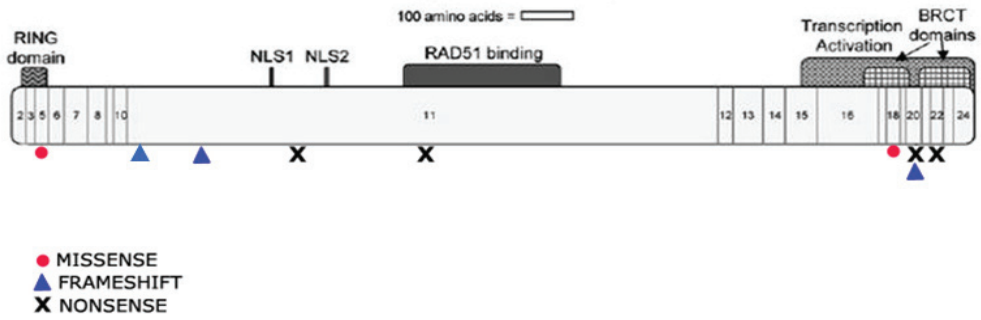

Figure 2. BRCA1 gene shematic presentation with marked type and position of detected mutations.

BRCA2

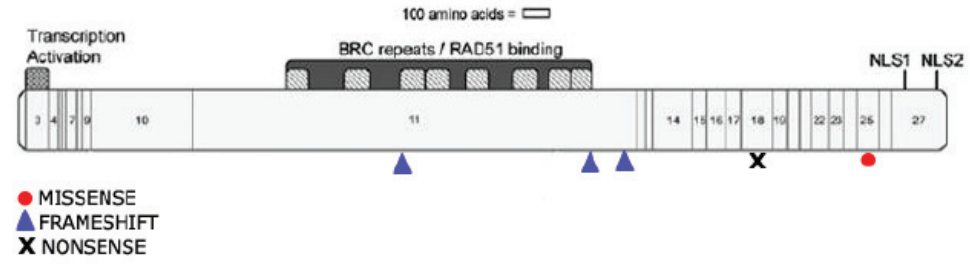

Figure 3. BRCA2 gene shematic presentation with marked type and position of detected mutations.

Al together (in 10 %) genetic testing identified 14 pathogenic mutations in17 candidates, 9 in BRCA1 and 5 in BRCA2 (Figures 1 and 2). Of those, 11 have been previously described and 3 were novel [6].

Nine pathogenic mutations in BRCA1 and five in BRCA2 were detected [6]. Croatia shares most of the mutations with Slovenia and Germany . No founder mutations were detected in Croatia, although one is a candidate. Further analyses on a larger number of samples is necessary.

The benefit of this testing is clear, since mutations were detected in young unaffected women who will be closely monitored.

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Sažetak

Molekularna dijagnostika nasljednog raka dojke

Mutacije u genima BRCA1 i BRCA2 povezuju se sa nasljeđenom sklonošću za nastanak raka dojke i jajnika. Svrha genetičkog testiranja je utvrditi dovoljno rano postoji li predispozicija, čime bi se dovoljno rano mogle poduzeti učinkovite mjere prevencije.

Ključne riječi: rak dojke; nasljedni rak dojke; geni BRCA1 i BRCA2; mutacije gena.

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