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Original Article

STUDY OF THE CARRIER STATE FOR FIVE BRCA1/BRCA2 DELETERIOUS MUTATIONS IN BULGARIAN WOMEN WITH BREAST CANCER

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Summary

Genetic testing for BRCA1/2 mutation is a well recognized medical management tool. Identification of healthy carriers of such mutations allows effective risk reduction procedures to be performed. There is no data reported on the founder mutations in the Bulgarian population. To evaluate the contribution of genetic factors to breast cancer (BC), we investigated the carrier state of Bulgarian women with BC for five common (according to BIC database) deleterious BRCA1/2 mutations. The list of patients diagnosed with BC between January 2011 and April 2012 was obtained from the Cancer Registry of University Hospital, Pleven. Eighty-two women with BC were interviewed and a pedigree was constructed of each of them. The patients were classified into seven categories, according to personal, disease and family history. Based on the preliminary prepared selection criteria and the personal family history, we defined a target group of 33 Bulgarian women with BC. They were screened for five deleterious mutations: 5382insC in BRCA1 and 6174delT, 6079del4, 8138del5, 5946delCT in BRCA2, by DNA sequencing. The genetic analysis detected none of the tested mutations. Two polymorphic variants were found in BRCA2 gene: c.5744C>T (rs4987117, SNP database) in exon11E in one patient and c.7806-14T>C (rs9534262, SNP database) in exon17 in 22 patients. In conclusion, without basic information on the founder mutations in the population, the genetic screening for the specific mutations in a small group of tested patients is ineffective.

Key words: BRCA1, BRCA2, Breast cancer, Bulgarian population, polymorphism

Introduction

A woman born in Bulgaria has a 7% average lifetime risk of being diagnosed with breast cancer (BC) [1]. This risk of BC is determined by both genetic and lifestyle factors. The first significant report concerning the genetics of BC was made by Broca in 1866, who traced the cause of death of 38 members of his wife's family through five generations. He found an increased frequency of BC in female relatives of BC probands. Since then, familial clustering has been verified repeatedly in other studies [2].

Breast cancer is approximately twice as common

in women with an affected first degree relative; the risk increases with the number of affected relatives and is greater for women with relatives affected at a young age [3]. Many multiple-case families have been investigated to identify high-risk susceptibility genes, using linkage analysis to identify markers that cosegregate with the disease. This approach has led to the demonstration of linkage to chromosome 17q and to chromosome 13q in a subset of BC families. Subsequent positional cloning has resulted in identifying the BRCA1 and BRCA2 genes with mutations in families linked to these gene regions [4]. Germline BRCA1 or BRCA2 mutations account for 20-40% of BC that cluster in families and less than 5% of BC overall [5-9]. Hereditary BC is actually the inheritance of susceptibility to cancer rather than the cancer itself.

Many studies report the typical tumour characteristics of BRCA1-associated BC, such as lack of expression of estrogen receptor (ER), progesterone receptor (PR), and HER2 (defined as a triple-negative breast cancer - TNBC), and high histological grade. Nearly 75% of BRCA1-associated BC is either TNBC, basal-like or both [10, 11]. Although fewer data are available on BRCA2-associated BC, the phenotype appears to be partly similar to that of BRCA1 with respect to young age at diagnosis, increased risk of contralateral BC and high histological grade [12].

The Breast Cancer Information Core (BIC) database has recorded 1810 distinct germline BRCA1 mutations and 2037 for BRCA2 mutations [13]. Of these, 996 BRCA1 mutations (55%) and 1094 BRCA2 mutations (54%) have been reported just once. Mutations appear to be equally distributed across the coding sequences, with no obvious "mutation hot-spot" [4, 14, 15].

Most of the reported disease-associated alleles of BRCA1/2 have been attributed to frameshift, nonsense or missense mutations, large rearrangements or splice alterations. They usually lead to truncation of BRCA1 or BRCA2 protein or affect aminoacids that are critical for the protein structure or function. However, a large number of sequence variants (particularly missense variations) cannot be distinguished with certainty as either disease-causing (deleterious) mutations or benign polymorphisms (clinically not significant). They are classified as variants of unknown clinical significance [16]. At present, no reliable functional assay exists to determine whether such a variant is likely to be deleterious. Only epidemiological evidence on the frequency of the variant in BC cases and controls, and on cosegregation of the variant with disease in families can be regarded as definitive. Unfortunately, there is no evidence for most of the variants [4, 14, 15].

A healthy individual could inherit a germline mutation in one allele of BRCA1/BRCA2 while the other one is normal. However, a somatic mutation may arise in the second allele in one's lifetime. The inheritance pattern is of an autosomal dominant type, with a relatively high penetrance. Therefore, a female carrier of BRCA1/BRCA2 germline mutation could have more than 60% lifetime risk of being diagnosed with BC. By identifying a female carrier of such a germline mutation, appropriate prevention measures (mastectomy, hormonal therapy) can be used to decrease the risk [17].

Mutations found more often in certain ethnic groups are known as founder mutations. In the Bulgarian population (7 364 570 people), an average of 3,400 new BC cases annually have been reported over the last 10 years (with a moderate tendency of increase), and 1,200 women die of BC each year [1, 18]. There is no available data of prevalence and type of BRCA1/2 mutation for the Bulgarian population.

Without any reported data concerning the founder mutations in the Bulgarian population and in order to appreciate the contribution of genetic factors to BC, we used the BIC data to select five of the most common deleterious BRCA1/2 mutations and investigate the carrier state of Bulgarian women with BC for those mutations.

Material and Methods

Patients

The list of the patients diagnosed with BC for the period between January 2011 and April 2012 and the information regarding their clinical history were provided by the Cancer Registry of University Hospital, Pleven.

Letters with information about the aim of the study and an invitation to participate were sent to all live female patients. The study was approved by the hospital's ethics and research committees.

The patients who responded to the invitation visited the medical genetics unit at the Medical University, Pleven. A questionnaire was prepared beforehand and then filled in by a genetic counselor during the interview with each woman. The questionnaire aimed to provide

information about the patient's age, menarche and menstrual history, history of childbearing, breastfeeding, menopause, intake of oral contraceptives, hormonal therapy for menopause, diet, intake of alcohol and smoking, previous benign breast disease or some other type of cancer, and family history of BC or other cancer. A pedigree, including at least three generations of relatives, was constructed for each patient. A patient with BC was defined as a proband (individual from whom the family history was traced).

The probands were classified into seven categories, according to family and personal disease history: (1) probands with familial BC – the occurrence of two or more first degree relatives in the pedigree including the proband; (2) probands with no family history but with early-onset BC (< 50 years); (3) probands with no family history but with bilateral BC; (4) probands with no family history but with both BC and ovarian cancer (OC); (5) probands with no family history but with TNBC; (6) probands without family history of BC but with family history of other associated cancers (ovarian, pancreatic, gastric and prostatic cancers); (7) probands without any of the criteria mentioned above.

Based on the literature data, we defined the criteria for selection of patients in our target group: patients who were more likely to carry germline mutation in BRCA1/2 gene. The target group included all women from groups 1, 2, 3, 4 and 5. They were referred for genetic testing after signing an informed consent form. The genetic screening for five of the most commonly reported [13] point mutations in BRCA1 and BRCA2 genes (5382insC in BRCA1 and 6174delT, 6079del4, 8138del5, 5946delCT in BRCA2) was performed at the Molecular Medicine Center, Medical University – Sofia.

Screening for BRCA1 and BRCA2 germline mutation

Genomic DNA was isolated from peripheral blood using standard protocols (CHEMAGEN® Magnetic Separation Station). To amplify exon 20 of BRCA1 and exons 11n and 17 of BRCA2 and exons boundaries, primer pairs were used. Primer selection was made from BIC database at http://research.nhgri.nih.gov/projects/bic/Member/brca1_mutation_database.shtml and http://research.nhgri.nih.gov/projects/bic/Member/brca2_mutation_database.shtml. Genomic DNA was amplified by the Polimerase Chain Reaction.

Mutation analysis of PCR products was performed by direct DNA sequencing. Sequencing was done using Big Dye® Terminator kit v3.1, (Appilied Biosystems), according to manufacturer's instructions using ABI Prism 3130 xl (Applied Biosystems) sequencer. The results were compared with the reference DNA sequences using SeqScape, Sequencing analysis and FinchTV software, and then reviewed manually. All mutations and sequence variants were named according to HGVS (nomenclature guidelines for cDNA sequence) and dbSNP .

Results

For the study period, there were 258 patients with BC recorded in the Cancer Registry of University Hospital, Pleven. Of all recorded patients, 5 were males and 13 dead females and they were initially excluded from the study. An invitation to take part in our study was sent to the rest of 240 live females of whom 77 (32%) agreed to participate in the study at the Section of Medical genetics. Five more patients were referred from the Department of Surgical Oncology, University hospital, Pleven. The final total number of patients included in the study was 82, with average age at BC diagnosis 57 years. All these patients were interviewed and pedigrees were constructed. On the basis of the selection criteria prepared beforehand and the family history, the patients were subdivided into the following groups: with familial BC - 12 (14.6%) women; with early age of onset (< 50 years) - 19 (23.2%); with bilateral BC - 3 women (3.7%), with BC and OC - one (1.2%) woman; with TNBC - 6 (7.3%). The rest of the patients – 41 (50%) were without any specific characteristics of BC and were excluded from the study. The characteristics of all 82 probands, according to the selection criteria, are presented in detail on Table 1.

The final target group included 41 women who met the selection criteria. They were referred for genetic testing. As three patients with familial BC and five with early-onset BC refused to be tested for mutations, a total of 33 women were screened for mutation. Nine of them had family histories of BC, 14 women were with early-onset BC, 3 with bilateral BC, one of them had BC and OC. Six other patients were with TNBC. The genetic testing for one mutation in BRCA1 (5382insC) and four in BRCA2 (6174delT, 6079del4, 8138del5, 5946delCT)

gene was performed. None of these five deleterious mutations was detected in the patients screened. The DNA sequencing revealed two polymorphic variants in BRCA2 gene: c.5744C>T (rs4987117, SNP database) in exon11E in one patient and c.7806-14T>C (rs9534262, SNP database) in exon17 in 22 patients. Detailed information about the type and frequency of sequence variants detected is shown on Table 2.

Table 1. Characteristics of probands

Characteristics	**Number of probands** (total number=82)	**%**
Familial BC	12	14.6
With one close relative with BC	8	9.9
With one close relative with BC	1	1.2
With one close relative with BC	2	2.5
Early age of onset (< 50)	19	23.1
< 40 years	5	6.1
40-50 years	14	17
Bilateral BC	3	3.7
BC and Ovarian cancer	1	1.2
Triple-negative BC	6	7.3
With other cancer in family	15	18.3
Without any high risk features	26	31.7

Table 2. Allele frequencies of the polymorphic variants: Comparison of frequency in the European populations published in HAPMAP and the frequency found in our study

SNP	**SNP frequency (according to CSHL-HAPMAP:HapMap-CEU)**			**SNP frequency in probands of our study**		
Genotype	Wild-type	Heterozygous	Homozygous	Wild-type	Heterozygous	Homozygous
rs9534262	TT-26.5%	TC-39.8%	CC-33.6%	TT-33% (11/33)	TC-36.6% (12/33)	CC-30.4% (10/33)
rs4987117	CC-93.6%	CT-6.4%	TT-1.1%	CC-96.9% (32/33)	CT-3.1% (1/33)	TT-0% (0/33)

Discussion

It is known that female BC incidence is strongly related to age, with the highest overall incidence rates in older women, supporting a link with hormonal status. In the UK between 2008 and 2010, an average of 20% of women with BC was diagnosed before the age of 50 [19]. In our study, the average age at diagnosis of BC was 57 years and about 23% of the patients were younger than 50 years.

The average rate of familial BC estimated to be 10-20% of all BC cases [20]. We detected similar incidence of familial BC - 14.7% and, in most of these cases (66.7%), the proband had only one close relative with BC.

According to current estimates, TNBC accounts for an average of 10-17% of BC, depending on the thresholds used to define ER and PR positivity and HER2 over expression [21]. In different studies and patient populations, TNBC may range from 6% to 28% of BC [22-25]. The incidence of TNBC that we found in our target group was 7.3%. The relatively low incidence established could be explained with the small number of the patients studied and the strict criteria used to define a negative steroid receptor status.

Bilateral BC is rare and the reported incidence varies from 1% to 14% [26-28]. This type of BC is more frequent among women aged under 50 at first diagnosis [29]. Young age at primary BC

diagnosis is associated with an increased susceptibility to bilateral BC, mainly due to the likelihood of living long enough to develop contralateral BC. In our study, three (3.7%) of all 82 BC patients had bilateral BC and two of them (66%) were with early (<50 years of age) onset of the disease.

An unaffected woman who is a carrier of mutation in BRCA1/2 genes has between 56% and 87% lifetime risk to develop BC by age 70. The genetic testing for BRCA1/2 mutation is a well recognized and useful medical management tool. The identification of healthy carriers of such mutations allows effective risk reduction procedures (prophylactic mastectomy) and screening measures (breast MRI) to be performed [17].

In our study, the genetic testing of five deleterious mutations in BRCA1 and BRCA2 gene, preliminary selected based on the BIC data, did not reveal any of these specific defects in the investigated Bulgarian population of women with BC. It might be due to: (1) The small number of the tested patients in our final target group (with expected low prevalence of BRCA1/2 mutations - about 5% of all BC cases); (2) BRCA1 and BRCA2 genes together consist of approximately 20 000 nucleotides and the mutations are spread in the entire sequences with no obvious "mutation hot-spot" [15]. Obviously, genetic screening for specific mutations, without basic information concerning the founder mutations in the population, is ineffective; (3) Although the most common BRCA mutations are point mutations and usually detected by sequencing analysis, the large genomic rearrangements comprise a significant component of identifiable mutations in BRCA1 and BRCA2 genes. These rearrangements cannot not be identified using a PCR-based method. An increase in the rate of mutation detection could be achieved by including Multiplex ligation-dependent probe application (MLPA) and highly sensitive DNA-based quantitative techniques of mutation screening [30].

Concerning the polymorphisms detected in our study, one of our patients was with heterozygous state for polymorphism c.5744C>T in BRCA2 gene. This woman was diagnosed with TNBC at the age of 39 years. This polymorphism is a missense mutation that causes the change of amino acid sequence in BRCA2 from threonine to methionine at codon 1915. It is one of the missense BRCA2 mutations with a relatively high penetrance and unknown clinical significance. Studies carried out on the Polish population have concluded that the heterozygote state (CT) for this mutation is associated with early onset BC and decreases the risk for the disease (above 40 years of age); and that the homozygote state (TT) is associated with a later age of cancer (above 40 years of age) [31, 32].

The second polymorphic variant c.7806-14T>C in BRCA2 was found in 22 of our patients. This is an intronic sequence variant with unknown clinical significance. We did not find any published data on the association between this variant and increased risk of BC for any population.

Conclusion

The incidence rate of familial BC (about 15%) in the Bulgarian population is similar to that in other European populations. Genetic screening for the specific point mutations in a small group of tested patients, without basic information concerning the founder mutations in the population is ineffective. In a future investigation, in order to increase the rate of mutation detection, genetic screening should combine methods for identification of both point mutations and large genomic rearrangements.

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